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Research Article

Antifungal Activity of Three Desert Plants of *Artemisia herb alba* (Asso.), *Pulicaria undulata L.* and *Gymnocarpos decandrus* Against Phytopathogenic Fungi

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Abstract
The current study evaluated the antifungal potential of three desert plant extracts, i.e., *Artemisia herb alba*, *Pulicaria undulata*, and *Gymnocarpos decandrus* against soil-borne phytopathogenic fungi including *Fusarium oxysporum*, *Fusarium solani*, and *Rhizoctonia solani*. The study employed solvent extraction, MIC/MFC determination, enzyme activity assays, TEM analysis, and HPLC-based phytochemical profiling. Results demonstrated significant antifungal activity of tested extracts against tested fungi, particularly from *A. herb alba*, with MICs as low as 0.75 mg/mL. *A. herb alba* extract effected on secretion of *F. oxysporum* extracellular enzymes (protease and catalase) and caused malformations in morphology of *F. oxysporum* conidia and hyphae. Phytochemical studies indicated detection of phenolics, flavonoids, saponins, tannins, alkaloids and high concentration of benzoic acids such as caffeic acid and ferulic acid in tested plants. The current work highlights the role of phenolic acids and flavonoids in antifungal mechanisms and suggests these plants as eco-friendly fungicides.

Keywords: antifungal Activity, *Artemisia herb alba*, *Gymnocarpos decandrus*, *Pulicaria undulata*, phytopathogenic fungi

1. INTRODUCTION

Phytopathogenic fungi include a diverse group of microorganisms that can cause significant disease in plants, leading to substantial economic losses in agriculture sector. Recent report estimates that plant diseases caused by pathogens, including fungi, are responsible for loss of 10–15% of the world's major crops of total agriculture production annually [1]. Control of phytopathogenic fungi relies on chemical and biological methods. Application of biological fungicides proved to be one of the most effective strategies in managing fungal diseases. Chemical fungicides as systemic and contact fungicides play a critical role in managing fungal growth and reproduction; however the effectiveness of such chemicals is often tempered by environmental issues such as persistence in the environment, potential effect on non-target organisms and soil pollution. In light of these concerns, there is a growing demand in alternative strategies, including botanical fungicides. Botanical fungicides derived from plant extracts have gained attention due to their lower environmental impact. Botanical fungicides are safe alternatives to chemical one with potentially less risk to humans and environment. They could be effective, selective, biodegradable and less toxic to the environment. Preliminary studies highlighted the potential of botanical fungicides like Expel as effective substitutes to chemical fungicide for controlling branch canker pathogen in tea plants [2].

Plants are incredible chemical factories that have been utilized for various purposes, including medicinal and agricultural applications. According to some estimates, at least nearly 100,000 secondary metabolites are now known to occur in 50,000 plant species and about 4,000 new secondary metabolites are being discovered every year from a variety of plant species [3]. Egyptian flora is rich with plant species that could be a promising source for new chemicals especially in the search for antimicrobial and fungicidal compounds. *Artemisia herb alba*, *Pulicaria undulata* and *Gymnocarpos decandrus* are perennial plants with a geographical distribution in arid regions of North Africa. *A. herb alba* (f. Asteracea) is wormwood plant with high content of secondary metabolites. Various extracts of *A. herb alba* showed different antimicrobial activities

Table 1. Extraction of *A. herb alba*, *P. undulata* and *G. decandrus* with polar solvents.

Plant	Solvent	Color	Consistency	Yield (mg/g)
	Methanol	Yellowish Green	Slight sticky	80.04
A. herb alba	Ethanol	Yellowish Green	Slight sticky	45.21
	Acetone	Green	Slight sticky	78.99
	Methanol	Green	Slight sticky	100.70
P. undulata	Ethanol	Green	Slight sticky	93.12
	Acetone	Dark green	Slight sticky	75.84
	Methanol	Yellowish Green	Slight sticky	88.46
G. decandrus	Ethanol	Yellowish Green	Slight sticky	66.95
	Acetone	Yellowish Green	Slight sticky	47.39

against several human and animal pathogens [4][5]. *P. undulata* L. (f. Asteracea) is low perennial shrub, widely distributed in Egypt and most tropical areas of Asia and Africa. This plant is used traditionally to treat diabetes, cardiac disorders, inflammations and as insect repellent [6]. *G. decandrus* (f. Caryophyllaceae) is a perennial shrub widely distributed in the Mediterranean coastal strip, Egypt. Limited studies tested the antifungal potential of *G. decandrus* extracts [7]. Early investigations explored the polyphenolics content, biological activities and antioxidant activity of *A. herb alba, P. undulata* and *G. decandrus* extracts [8][9]. However; there is a limited data on their antifungal potential against phytopathogenic fungi. In these regard the current study aimed to test the antifungal activity of *A. herb alba*, *P. undulata* and *G. decandrus* against phytopathogenic fungi (*Fusarium oxysporum, Fusarium solani* and *Rhizoctonia solani*).

2. MATERIALS AND METHODS

2.1. Plant Collection and Extraction

Fresh specimens of *A. herb-alba*, *P. undulata,* and *G. decandrus* were collected from non-reclaimed area in Sadat city, Menoufiya governorate, Egypt at coordinates (30.37895 N/30.50768 E, 30.38137 N/30.51234 E and 30.38137 N/30.51234E) respectively. Aerial parts were washed with tap water to remove dust particles and shade dried at room temperature followed by grinding to a fine powder using electrical blender. As much as 50 g of each plant powders mixed with solvents (methanol, ethanol and acetone) individually with (1:10 ratio) for 7 d at room temperature Then, extracts were shaken for 24 h with continuous agitation at 150 rpm using Heidolph unimax shaker. After filtration with Whatman no. 1, the solvents were removed using rotary vacuum evaporator with water path at 40 ºC. The crude extracts have left to dry at room temperature for 3 d. The yield percentages were determined by dividing the weight of extract by the weight of the sample multiplied by 100. The extracts were stored at 4 °C for further use.

2.2. Antifungal Assay

Fungal species of *F. oxysporum*, *F. solani* and *R. solani* isolated early from *Vicia faba* roots were prepared from 6–10 old day cultures grown in potato dextrose agar medium (PDA). Petri dishes were flooded with 8–10 mL sterile distilled water and spores were scraped using a sterile spatula. The spore density of each fungus was adjusted to obtain final concentration approximately 10^5 spores/mL using hemocytometer.

Antifungal activity of solvent extracts was evaluated using well plate method. Fungal inoculates of *F. oxysporum, F. solani* and *R. solani* were spread over PDA plates by spread plate technique. Then, aseptically wells (15 mm) was made into each plate with the help of a sterile cork borer and 100 µL of methanol, ethanol and acetone extracts (100 mg/mL) were added to separate wells. Wells containing the corresponding solvents served as negative control while fungicide Mancozeb: 64% Manganese ethylene_bis (dithiocarbamate), (polymeric) complex with zinc salt and 28%

inactive matter as 72% wettable powder was used as positive control. The plates were incubated at 25 °C for 48–72 h. Each experiment was repeated three times and the mean of inhibition zone around the wells was calculated [10].

2.3. Minimum Inhibition Concentrations (MICs) and Minimum Fungicidal Concentration MFC)

Extracts were dissolved in acetone to a concentration of 20 (mg/mL). Then, 100 µL of plant extracts were serially diluted up to 50% with water in 96 well micro-liter plates. A 100 µL of fungal inoculates transferred into fresh potato dextrose broth was added to each well and appropriate solvents were included as blank. As an indicator of fungal growth, 40 µL of 0.2 mg/mL *p*-iodonitrotetrazolium violet (Sigma®) dissolved in water was added to each of the microplate wells. The covered microplates were incubated for 2 d at 25 °C. The MIC was recorded as the lowest concentration of the extract that inhibited fungal growth after 24 h [11]. The MFCs were determined by inoculating 20 µL from the wells with concentration equal and higher than minimum inhibition concentration on PDA and incubated over night at 25 °C. MFCs values were determined as the concentration that totally inhibited the growth of fungal colonies [12].

2.4. Mode of Action

Seven old day culture of *F. oxysporum* was inoculated into glucose nitrate media amended with plant extracts of *A. herb alba, P. undulata* and *G. decandrus* individually and kept for 10 d at 25 °C for incubation. Then, the culture filtrate was obtained using filter paper (whatman No.1) and used for the following tests [13].

2.4.1. Test for Amylase

Two ml of (0.1%) starch solution was added to culture filtrate containing plant extracts in test tube and kept for 4 h at 25°C. The presence or absence of starch was treated by adding drops of I_2-KI solution to each test tube.

2.4.2. Test for Protease

Two ml of culture filtrate was added to 2 mL of 1% gelatin solution in a test tube and incubated at 25 °C for 24 h. Then 2 mL of 6 M NaOH and 0.5 mL of $CuSO_4$ solution was added. Observation of deep violet to blue coloration indicates the presence of proteins while light blue color indicated that enzyme protease exerted by fungal cell has hydrolyzed protein partially.

2.4.3. Test for Catalase

One mL of diluted H_2O_2 (30:1) with water was added to a test tube containing 4 mL culture filtrate of *F. oxysporum*. Evolution of oxygen bubbles

Table 2. Inhibition zone diameters of *A. herb alba, P. undulata* and *G. decandrus* extracts against *F. oxysporm.*

Treatment	IZ diameter (mm) M±SD		
	ME	EE	AE
Artemisia herb alba	35.00 ± 0.40 [a]	34.20 ± 0.49[b]	28.60 ± 0.41[c]
Pulicaria undulate	32.33 ± 0.20[d]	33.30 ± 0.20[e]	27.30 ± 0.25[f]
Gymnocarpos decandrus	32.50 ± 0.84[d]	34.00 ± 0.36[b]	25.30 ± 0.42[g]
Solvent	21.60 ± 0.21[h]	21.60 ± 0.26[h]	16.63 ± 0.04[h]
Fungicide	29.30 ± 0.20 [c]		

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	940.425	12	78.369	385.231	< 0.001
Within Groups	5.289	26	.0203		
Total	945.714	38			

IZ: inhibition zone, ME: methanol extract, EE: ethanol extract, AE: acetone extract, M: methanol, E: ethanol, A: acetone, df: degree of freedom; F: F value. Values are expressed as ,ean ± SD, analysis was performed with one-way ANOVA. Mean values represented by different letters are significantly different at $p < 0.05$.

Table 3. Inhibition zone diameters of *A. herb alba, P. undulata* and *G. decandrus* extracts against *F. solani.*

Treatment	IZ diameter (mm) M±SD		
	ME	EE	AE
Artemisia herb alba	24.00 ± 0.04^{A}	27.00 ± 0.29^{B}	20.00 ± 1.20^{C}
Pulicaria undulate	27.00 ± 0.62^{B}	33.33 ± 0.42^{D}	23.36 ± 0.91^{E}
Gymnocarpos decandrus	25.30 ± 1.05^{F}	39.00 ± 0.29^{G}	22.50 ± 0.23^{E}
Solvent	16.00 ± 0.09^{H}	20.00 ± 0.23^{C}	15.50 ± 0.18^{H}
Fungicide	33.30 ± 0.12^{D}		

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1745.059	12	145.422	292.600	< 0.001
Within Groups	12.922	26	.0497		
Total	1757.981	38			

IZ: inhibition zone, ME: methanol extract, EE: ethanol extract, AE: acetone extract, M: methanol, E: ethanol, A: acetone. Values are expressed as mean ± SD, analysis was performed with one-way ANOVA. Mean values represented by different letters are significantly different at $p < 0.05$.

indicates the presence of enzyme.

2.5. Effect of Plant Extracts on Fungal Cell

F. oxysporum was treated with 2× concentration of MIC of *Artemisia herb alba* extract and incubated overnight at 25 °C. Sections of fungal growth were prepared according to the previous method [14] and ultrathin sections were observed at 160 kV using a JEOL JEM-2100 at Electron Microscope Unit, Mansoura University, Egypt.

2.6. Preliminary Phytochemical Screening of Plant Extract

All extracts were subjected to qualitative phytochemical screening to determine the presence of alkaloids, flavonoids, saponins, phenols, steroids, and tannins by standard methods [15]-[17].

2.7. Quantification of Phenolic Acids and Flavonoids in Plant Extracts using High Performance Liquid Chromatography Technique (HPLC)

HPLC analysis for methanolic extracts of *A. herb alba*, *P. undulata* and *G. decandrus* were performed using agilent 1260 infinity HPLC series (agilent, USA) equipped with quaternary pump, akinetex 5 µm EVO C18 (4.6 mm × 100 mm) (phenomenex, USA), and operated at temperature 30 °C. The separation was achieved using linear elution gradient with mobile phase (A) water 0.2% H_3PO_4 (V/V), (B) methanol, (C) acetonitrile, the injection volume was 20 µL and UV detection was affected at 284 nm.

2.8. Statistical Analysis

All experiments were conducted in completely randomized design with three repetitions for each treatment. All values of antifungal effect were expressed as the mean ± standard deviation (SD) of the mean. The statistical analysis of results was conducted by analysis of variance (ANOVA) using computer SPSS 15 software package. Differences on statistical analysis of data were considered significant at $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Plant Extraction

Organic solvents (methanol, ethanol and acetone) were used to extract the bioactive compounds from *A. herb alba, P. undulata* and *G. decandrus*. Table 1 describes the different obtained extracts with their yield, color and consistency.

3.2. Antifungal Assay

The antifungal activities of *A. herb alba, P. undulata* and *G. decandrus* extracts were determined against different fungal strains and recorded as inhibition zone diameters. Table 2 indicates the inhibitory effects of plant extracts against *F. oxysporum* strain compared with control plates with inhibition zone diameters ranged between 25.30–35.00 mm. Methanol extract of *A. herb alba* employed the highest inhibition activity against *F. oxysporum* strain of 35 ± 0.40 mm and acetone extracts of *G. decandrus* showed the lowest inhibition activity of 25.30 ± 0.42 mm.

Table 3 indicates the high inhibition activity of *G. decandrus* ethanol extract against *F. solani* compared with all tested extracts and control with inhibition zone diameter of 39.00 ± 0.29 mm.

In case of *R. solani* inhibition zone diameters caused by plant extracts ranged between 42.60 ± 0.41 mm for acetone extract of *A. herb alba* and 16.33 ± 0.70 mm for acetone extract of *G. decandrus*. Table 4 indicates the high antifungal potential of *A. herb alba* extracts against tested fungi compared with control plates.

3.3. MIC and MFC Determination

Determination of MIC involved a semi-quantitative test by dilution method. Solvent extracts exhibited different MICs and MFCs values on different fungal species. Results of MICs and MFCs are listed in Table 5. The MICs ranged between 0.75–20.00 mg/mL while MFCs ranged between 2.5–20.0 mg/mL. Extracts of *A. herb alba* and *P. undulata* showed fungicidal effects on tested fungi.

3.4. Mode of Action of Plant Extracts

Fungi produce extracellular hydrolases as a resistance mechanism against pathogenic invasion to obtain nutrition from the host. Effect of plant extracts on secretion of amylase, protease and catalase enzymes by *F. oxysporum* are indicated in Table 6. Results indicated absence of protease and catalase enzymes in growth media containing *A. herb alba* extract and weak activity of both enzymes in the presence of *P. undulata* extract while activity of amylase enzyme not affected by all plant extracts.

3.5. Effect of Plant Extracts on Fungal Cell of F. oxysporum

Electron photomicrographs of *F. oxysporum* conidia and hypha under treatment with *A. herb alba* extract illustrate variability in the ultrastructure of the fungi compared with control. As indicated in Figure 1, variations include vaculation in the cytoplasm, decrease in number of lipid bodies, irregular thin cell wall and damaged cytoplasm in the conidia of treated plates. Furthermore, Figure 2 shows a decrease in lipid bodies, thin cell wall, and vaculation in cytoplasm and partially damaged nucleus in treated hyphae of *F. oxysporum*.

3.6. Phytochemical Screening

Phytochemical screening of different extracts revealed the presence of flavonoids, saponins and phenolic acids in fractions of all plant and absence of steroids in all fractions of *A. herb alba*. Moreover; alkaloids were absent in all fractions of *G. decandrus*, as listed in Table 7.

Table 4. Inhibition zone diameters of *A. herb alba, P. undulata* and *G. decandrus* extracts against *R. solani*.

Treatment	IZ diameter (mm) M±SD		
	ME	EE	AE
Artemisia herb alba	28.60 ± 0.16a	32.00 ± 0.18b	42.60 ± 0.41c
Pulicaria undulate	29.60 ± 0.32ad	27.30 ± 0.32ae	23.30 ± 0.41f
Gymnocarpos decandrus	31.60 ± 0.22b	29.00 ± 0.94ade	16.33 ± 0.70g
Solvent	22.00 ± 0.96f	23.30 ± 0.57f	18.00 ± 0.17g
Fungicide	41.00 ± 0.47c		

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2243.457	12	186.955	252.205	< 0.001
Within Groups	19.273	26	.0741		
Total	2262.731	38			

IZ: inhibition zone, ME: methanol extract, EE: ethanol extract, AE: acetone extract, M: methanol, E: ethanol, A: acetone. Values are expressed as mean ± SD, analysis was performed with one-way ANOVA. Mean values represented by different letters are significantly different $p < 0.05$.

Table 5. Results of MIC and MFC tests.

Plant	Extract	MIC / MFC (mg/ml)		
		F. oxysporum	***F. solani***	***R. solani***
Artemisia herb alba	ME	0.75 ± 0.00 / 2.50 ± 0.00	2.50 ± 0.00 / 10.00± 0.00	2.50 ± 0.00 / 5.00 ± 0.00
	EE	0.75 ± 0.00 / 2.50 ± 0.00	2.50 ± 0.00 / 10.00 ± 0.00	2.50 ± 0.00 / 10.00 ± 0.00
	AE	1.08 ± 0.23 / 5.00 ± 0.00	10.00 ± 0.00 / 10.00 ± 0.00	5.00 ± 0.00 / 10.00 ± 0.00
Pulicaria undulata	ME	2.50 ± 0.00 / 10.00 ± 0.00	1.25 ± 0.00 / 5.00 ± 0.00	2.50 ± 0.00 / 10.00 ± 0.00
	EE	1.25 ± 0.00 / 10.00 ± 0.00	0.91 ± 0.23 / 5.00 ± 0.00	5.00 ± 0.00 / 20.00 ± 0.00
	AE	5.00 ± 0.00 / 10.00 ± 0.00	2.50 ± 0.00 / 10.00 ± 0.00	5.00 ± 0.00 / 20.00 ± 0.00
Gymnocarpos decandrus	ME	5.00 ± 0.00 / 10.00 ± 0.00	5.00 ± 0.00 / 10.00 ± 0.00	5.00 ± 0.00 / 10.00 ± 0.00
	EE	1.25 ± 0.00 / 5.00 ± 0.00	0.75 ± 0.00 / 5.00 ± 0.00	10.00 ± 0.00 / (-)
	AE	5.00 ± 0.00 / 10± 0.00	5.00 ± 0.00 / 10± 0.00	16.66 ± 4.71 / (-)

MIC: minimum inhibition concentration, MFC: minimum fungicidal concentration, (-) not detected, ME: methanol extract, EE; ethanol extract, AE; acetone extract.

3.7. Quantification of Phenolic Acids and Flavonoids in Plant Extracts by HPLC

Figures 3–5 illustrate the HPLC chromatograms of phenolic acids and flavonoids in study plants. *A. herb-alba* contains the highest amount of phenolic and flavonoid as indicated in Figure 3 and Table 8. Main peaks belonged to phenolic acids were identified as vanillic acid, caffeic acid, syringic acid, p-coumaric acid, benzoic acid, ferulic acid, o-coumaric acid, salicylic acid, and cinnamic acid; while peaks belonged to flavonoids were catechol, quercitin, neringein, myricetin, and kaempherol). *P. undulata* extract come second in amount of separated phenolic acids and flavonoids compounds with 6452.50 mg/kg. Main peaks belonged to phenolic acids and flavonoids are listed in Figure 4 and Table 9. The least amount of phenolic acids and flavonoids was recorded in extract of *G. decandrus* (826.22 mg/kg). Separated peaks and identified compounds are listed in Figure 5 and Table 10.

3.8. Discussion

3.8.1. Extraction and Antifungal Activity

Screening wild plants for active compounds had acquired much attention recently as a method to find new botanical pesticides and developing agribusiness industry. Plant extract is a rich source of compounds that could be effective against plant pathogenic fungi. The current study tested the antifungal activity of *A. herb alba, P. undulata* and *G. decandrus* plants collected from Sadat city, Menoufiya governorate, Egypt against fungal isolates *F. oxysporum, F. solani* and *R. solani.* Using methanol, ethanol and acetone in extraction of study plants played an important role in extraction yield. Methanol and ethanol alcohols showed the highest yield of plant material content followed by acetone. Results indicated that chemical content of study plants are highly dissolved in methanol and ethanol rather than acetone. Therefore, they are recommended as the optimal solvents to obtain high content of phytochemicals from study plants.

Different solvent extracts showed varying antifungal activity against different fungal species. Statistical analysis of data indicated that all solvent extracts were highly effective against tested fungi compared with control and commercial fungicide

Table 6. Effect of plant extracts on secretion of extracellular enzymes by *Fusarium oxysporum*.

Plant extract	Amylase	Catalase	Protease
A. herb alba	++	-	-
P. undulata	++	+	+
G. decandrus	++	++	++
Control	++	++	++

(-) = absence of enzyme,(+) = presence and weak activity of enzyme, (++) = presence and strong activity of enzyme, T = treatment, C = control.

(mancozeb). Treatment of *F. oxysporum* and *F. solani* with different solvent extracts of *A. herb alba*, *P. undulata* and *G. decandrus* indicated significant differences in inhibition zone diameter means compared with control. *F. oxysporum* and *F. solani* were more sensitive to plant extracts than *R. solani* that showed resistance against *G. decandrus* acetone extract. Resistance of *R. solani* to some plant extracts could be due to their lower content of bioactive antimicrobial agents or its ability to overcome their effect [18]. Resistance of *R. solani* could relate also to their cell wall structure. The fungal cell wall is composed of a complex network of proteins and polycarbohydrates that varies in composition depending on the fungal species [19]. These proteins could be involved in plant extract resistance. Variability in inhibition effect of different solvent extracts of the same plant is related to differences in polarity between solvents that in turn effect on their phytochemicals solubility. The antifungal effect of study plant extracts is related mainly to their chemical composition. Phytochemical screening of study plants showed their high content with phenolic and flavonoid compounds in addition to their content with alkaloids and saponins as indicated in Table 5. Several studies have shown that alkaloid, saponin, flavonoid, and phenolic compounds possess antimicrobial activities [20]. From chemical analysis, the antifungal activity of plant extracts related mainly to the presence of polyphenol compounds that are usually the major antifungal compounds of most plant extracts.

Further experiments were conducted to determine MIC and MFC of study plants against tested fungi. The MIC and MFCs values of plant extracts are summarized in Table 5. The MIC and MFC values differed according to the type of extract and fungal species. *A. herb alba* and *P. undulata* extracts succeeded to induce fungicidal effect on all tested fungi at tested concentrations while *G. decandrus* showed fungicidal activity against *Fusarium sp*. only and fungistatic activity against *R. solani*. Results ensured the high resistance of *R. solani* to plant extracts than *F. oxysporum* and *F. solani*. Values of MIC and MFC of tested plants differed from that obtained by early studies of Ghareeb and Issa [21] on other fungal species. This difference could be related to different plant collection time, different preparation methods, and different resistance of tested fungi. Moreover, variations in values of MICs and MFCs among tested fungi are due to their intrinsic properties and permeability of their cell wall to different extracts.

3.8.2. Mode of Action

Understanding the modes of action of botanical fungicides could enable specialists to use phytochemicals with similar mechanism sequentially or in combination to delay fungal resistance to control agents. Table 6 illustrates qualitatively the effect of plant extracts on secretion of extracellular hydrolases amylase, protease and catalase enzymes in *F. oxysporum* cell. Results indicated absence of protease and catalase enzymes in growth media containing *A. herb alba* extract and weak activity of both enzymes in presence of *P. undulata* extract. At the same time activity of amylase enzyme not affected by all plant extracts. Hydrolytic enzymes are secreted during host plant infection, which allow fungal cells to enter host plant cells by breaking down cell walls and causing pathogenesis in the host plant. Moreover, microbial proteases are among the most important hydrolytic enzymes. They catalyze the hydrolysis of proteins into smaller peptides and amino acids for subsequent absorption into cells, constituting a very important step in nitrogen metabolism [22]. Results suggest that antifungal components of *A. herb alba* and *P. undulata* extracts can interact with hydrolytic enzymes of fungal cell disturbing their structures. Moreover, inhibition of protease enzymes by *A. herb alba* could be related to its high content with flavonoids and phenolic acids. As described in early studies, flavonoids can interfere with fungal cell membrane, disrupting ergosterol biosynthesis, interact with eukaryotic enzymes through phenyl ring or chelate heavy metals present in enzymes structural architecture and thereby inactivate them [23][24]. According to Treutter, flavonoids may directly involved in the inhibition of the pathogen's enzymes, especially those digesting the plant cell wall, by chelating metals required for their activity [25]. Thus, flavonoids can interact with various fungal enzymes thereby influencing fungal growth, metabolism and pathogenicity. Some fungi have efflux pump proteins found in cell membranes of the fungus composed of nucleotide binding domains aim to break down ATP to transport substances out of the cell [26]. Flavonoids can activate these efflux pumps and leads to drug resistance [27]. Understanding the details of these interactions including binding sites and mechanisms can lead to development of more effective antifungal agents. Current study demonstrated the interfering of plant extract with fungal cell wall by studying the ultrastructure of *F. oxysporum* cell treated with *A. herb alba* extract under TEM. Results revealed clear differences in structure of fungal cell between control and treated plates. As shown in Figures 1 and 2, treatment of *F. oxysporum* growth media with *A. herb alba* extract affected the fungus morphology and led to irregular cell shape. The antifungal effect of extract leads to a loss in mycelial membrane integrity that responsible for maintaining fungal viability. Results indicate marked disruption in conidial cell wall, the

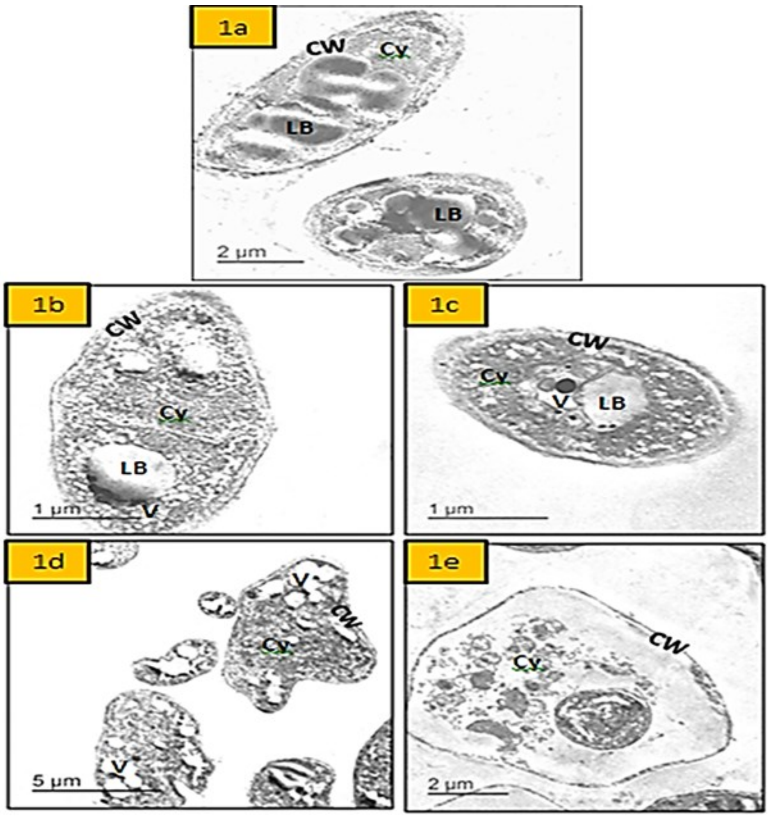

Figure 1. Ultrastructure of *F. oxysporum* conidia treated with *A. herb alba* extract under transmission electron microscope; (1a): control conidia (untreated); (1b, 1c): *A. herb alba* treated conidia with vacuolation in the cytoplasm and decrease in number of lipid bodies; (1d): *A. herb alba* treated conidia with irregular and thin cell wall and (1e): *A. herb alba* treated conidia with very thin cell wall and damaged cytoplasm. CW= cell wall, Cy= cytoplasm, LB= lipid body, and V= vacuoles.

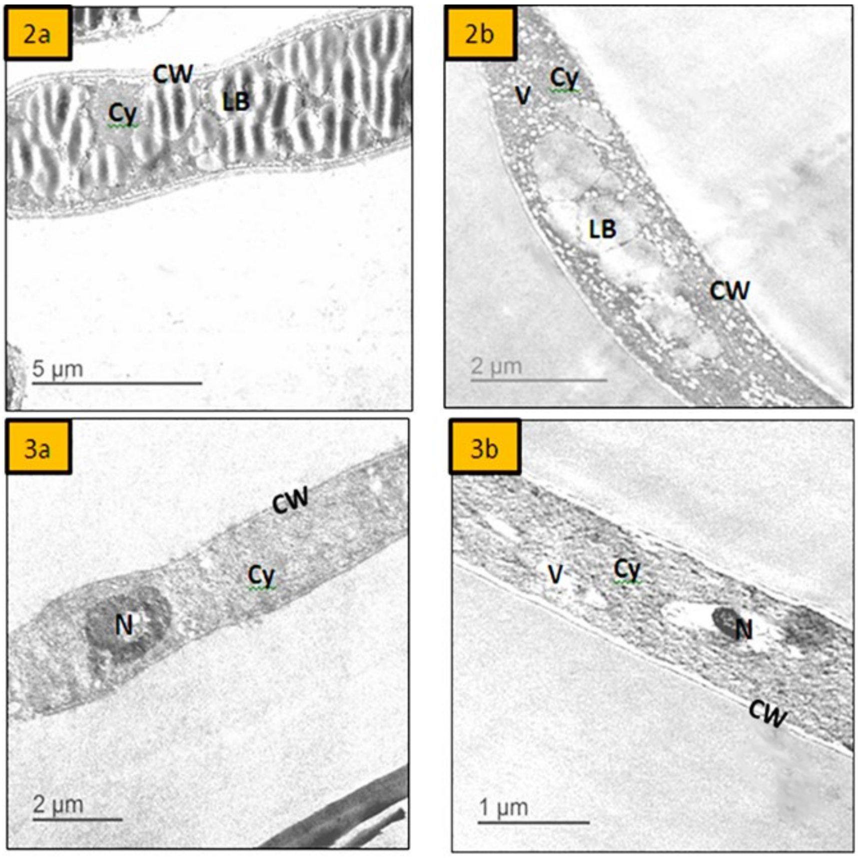

Figure 2. Ultrastructure of *F. oxysporum* hyphae treated with *A. herb alba* extract under transmission electron microscope; (2a): control (untreated), (2b): decrease in number of lipid bodies, thin cell wall and vacuolation in the cytoplasm. (3a): control (untreated), (3b): vacuolation in the cytoplasm and damaged nucleus. CW= cell wall, Cy= cytoplasm, N= nucleus and V= vacuoles.

plasmalemma and the cytoplasm of *F. oxysporum*. Ultrastructure changes obtained was similar to that caused by *Matricaria chamomilla* essential oil and *Berberis vulgaris* extract on *Aspergillus niger* and *Botrytis cinerea* conidia, respectively [28][29].

Antifungal components in *A. herb alba* extract like benzoic acid can interfere with cell wall composition and change its permeability. HPLC analysis of *A. herb alba* indicated their high content with benzoic acid, ferulic acid, coumaric acid and quercetin, as listed in Table 8. Benzoic acid is a plant secondary metabolite, and considers a key component in the phenylpropanoid pathway that regulates the defense system in plants against biotic and abiotic conditions [30]. A previous study reported that benzoic acid was the greatest inhibitor of the growth and production of enzymes by *G. boninense* [31]. Furthermore, ferulic acid is a phenolic acid compound commonly found in plants with antimicrobial activity. It can inhibit the growth of *Bacillus cereus* and *Pseudomonas fluorescens* by changing the properties of the cell membrane surface. The target site of ferulic acid antifungal action was the cell membrane as confirmed by Yan et al. [32]. Similarly Khakpour *et al*. consider quercetin and gallic acid are among the major bio-active constituents of *C. sinensis, J. regia, V. faba,* and *U. urens,* that are responsible for their antimicrobial activity on *D. chrysanthemi, P. carotovorum, P. syringae,*and *R. solancearum* and even other bacteria [33]. Mentioned acids can inhibit or interrupt thegrowth of fungi by affecting on fungal cell functioning. They can alter many parts of the cell espectially cell membrane. As confirmed by results of transmissiom electron microscope, *A. herb alba* extract partially destroyed the organelles and the nucleus of fungus and caused shrunken in hyphae (Figures 1 and 2). These bioactive polyphenol compounds found in plant extracts, singly or in combination could interfere with the life process of fungi by binding their protein molecules acting as chelating agents, altering structural component synthesis, weakening or destroying the permeability barrier of the cell membrane and changing the physiological status of the cells. These alterations may also modify the activity of membrane enzymes involved in the formation of the cell wall leading to cell death. The current study suggeste the mechanism of inhibition of *F. oxysporum* is related to the alteration of the morphological and ultracellular structure of mycelia that attributed to the antifungal components of plant extracts.

3.8.3. Phytochemical Screening

The phytochemical screening of methanol, ethanol and acetone fractions of study plant revealed the presence of saponins, steroids, phenolic acids, flavonoids and alkaloids in all studied plants (Table 7). Methanol and ethanol solvents succeeded in extracting most phytochemical components of study plant which explain the high antifungal activity of methanol and ethanol extracts of study plants compared with acetone one. Results of phytochemical screening illustrated that *A. herb alba* and *P. undulata* extracts were rich with phenolic acids and flavonoids that were in agreement with previous studies [34]-[36]. Moreover, early phytochemical analysis of *Pulicaria* species indicated the occurrence of flavonoids and steroids [37]. *G. decandrus* extracts was rich with steroids and flavonoids which were reported in different species of family Caryophyllaceae [38].

3.8.4. Quantification of Phenolic Acids and Flavonoids in Study Plants by HPLC

Phenolic compounds constitute a very large group of secondary metabolites distributed in plant kingdom. They are synthesized either as soluble or cell wall bound compounds during plant growth and especially in response to environmental conditions. Phenolic acids are occurring in plants as esters or glycosides conjugated with other natural compounds as flavonoids, sterols and glucosides. Plant derived phenolics as phenolic acids and flavonoids can display various antimicrobial effects as destabilization of plasma membrane and inhibition of extracellular enzymes [39]. In this regard, phenolic acid and flavonoid content of study plants were determined using HPLC.The amount of phenolic acids and flavonoid concentrations varied widely between study plants. The highest phenolic acids and flavonoid content were 1.5238×10^4 mg/kg in *A. herb alba* followed by 6452.505 mg/kg in *P. undulata* and 826.222 mg/kg in *G. decandrus.* Although study plants had grown at the same environment and under the same stress conditions, they showed different accumulation of phenolic acids and flavonoids that could be related to genetic factors. Moreover, the level of phenolic compounds including flavonoids could vary in quantity and quality depending on the plant harvest time. Results showed clearly that *A. herb alba* contains the highest phenolic acids and flavonoids content. The peak chromatogram indicated the presence of phenolic acids (benzoic acid, ferulic acid, salicylic acid, *o*-coumaric acid, ellagic acid, chlorogenic acid, *p*-coumaric acid, caffeic acid, vanillic acid, syringic acid, and cinnamic acid) and flavonoids (quercetin, neringein, myricitin, and kaempherol) (see Table 8 and Figure 3). Plants have the capacity to biosynthesize different phenolic acids in response to changing environmental conditions, so that total phenolic and flavonoid content of *A. herb alba* was more or less differ from that identified in other

Table 7. Results of preliminary phytochemical screening test of plant extracts.

Extract	*A. herb alba*			*P. undulata*			*G. decandrus*		
	ME	EE	AE	ME	EE	AE	ME	EE	AE
Saponins	+	+	+	+	+	+	+	+	+
Phenolic acids	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+
Alkaloids	+	+	+	+	+	-	-	-	-
Steroids	-	-	-	+	+	+	+	+	+

(+): presence; (-): absence; ME: methanol extract; EE: ethanol extract; AE: acetone extract.

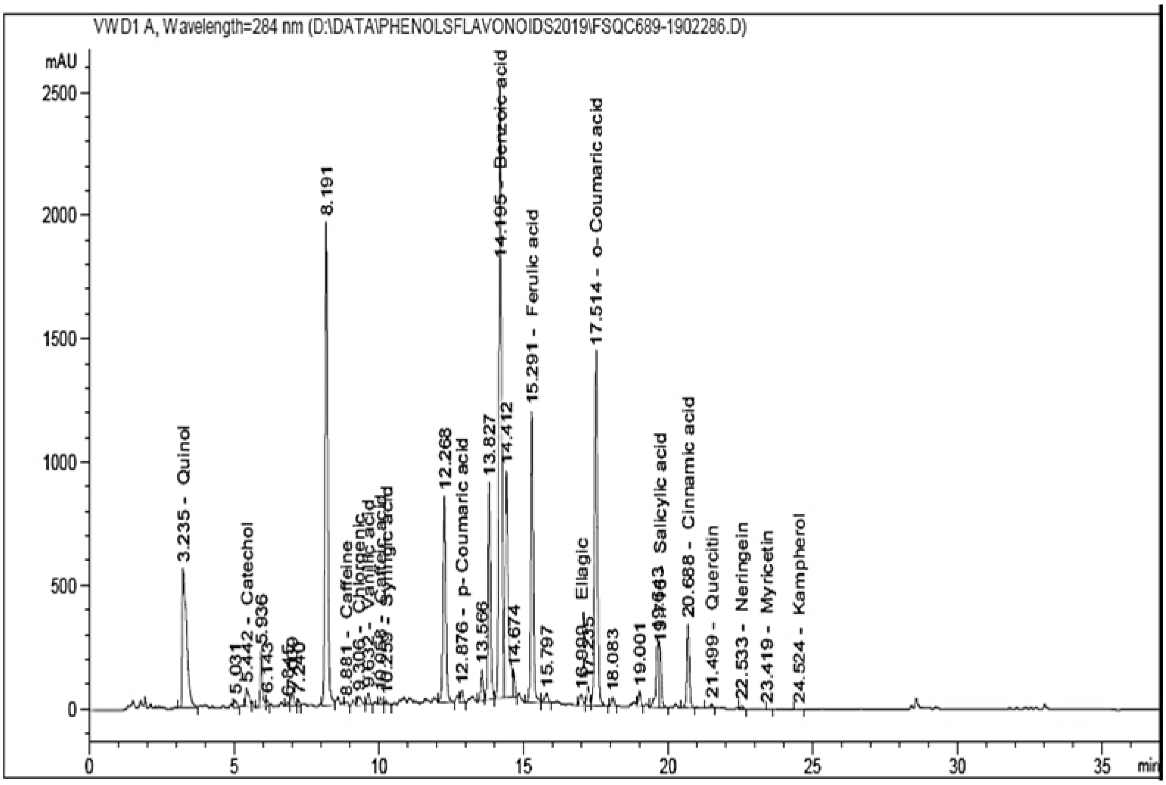

Figure 3. HPLC chromatogram of phenolic acids and flavonoids in the extract of *A. herb alba.*

Table 8. Phenolic acid and flavonoid compounds in *A. herb alba extract*.

Peak no	Compound name	Rt (min)	Area (mAU*s)	Amount (mg/kg)
1	Quinol	3.235	5941.53223	972.91847
2	Catechol	5.442	558.81598	100.48324
3	Caffeine	8.881	121.85777	11.07976
4	Chlorgenic	9.306	353.80408	21.26829
5	Vanillic acid	9.632	306.71252	32.49038
6	Caffeic acid	10.058	187.18616	6.11044
7	Syringic acid	10.259	98.72724	4.70598
8	*p*-Coumaric acid	12.876	306.87030	9.91356
9	Benzoic acid	14.195	1.54635×10^4	1.30736×10^4
10	Ferulic acid	15.291	7064.63965	307.01559
11	Ellagic	16.999	326.00574	22.12285
12	*o*-Coumaric acid	17.514	9638.87695	220.78970
13	Salicylic acid	19.716	1171.21460	287.63294
14	Cinnamic acid	20.688	2111.90918	59.64448
15	Quercitin	21.499	97.14295	50.40681
16	Neringein	22.533	67.39668	50.87980
17	Myricetin	23.419	7.70443×10^{-1}	4.75816
18	Kaempherol	24.524	5.71456	2.93830

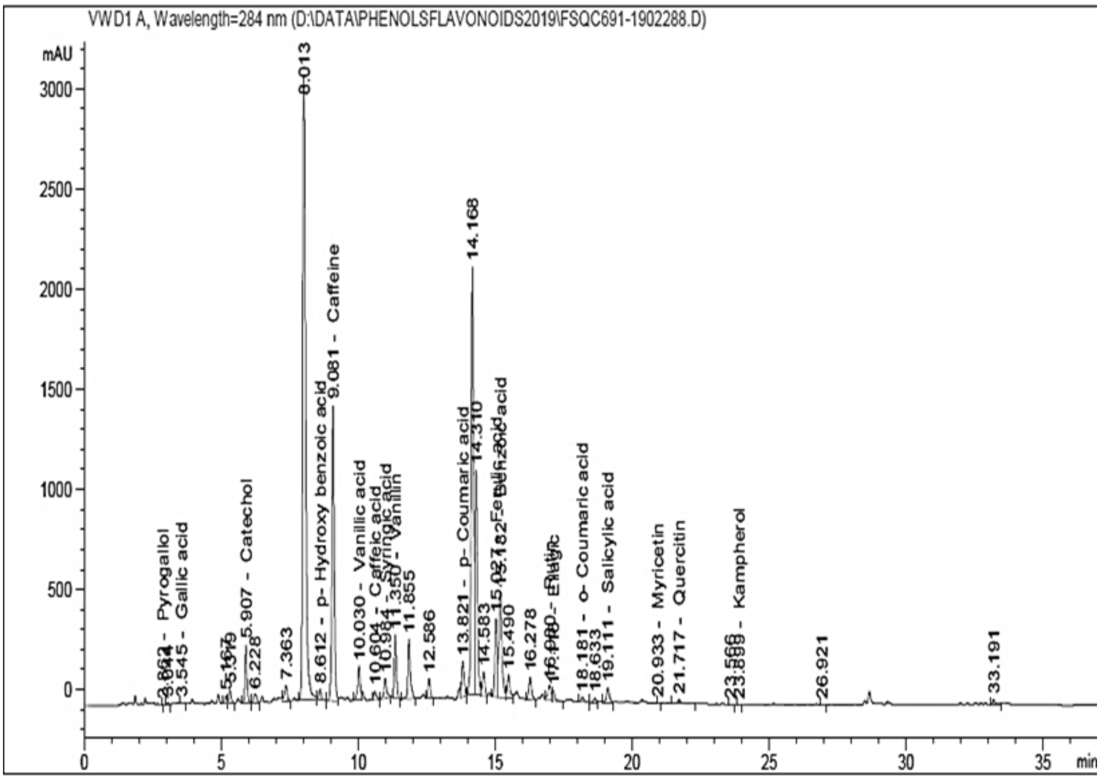

Figure 4. HPLC chromatogram of phenolic acids and flavonoids in the extract of *P. undulata* extract.

Table 9. Phenolic acid and flavonoid compounds in the extract of *P. undulata*.

Peak no	Compound name	Rt (min)	Area (mAU*s)	Amount (mg/kg)
1	Pyrogallol	2.862	1.02065	1.45958×10^{-1}
2	Gallic acid	3.545	35.86327	2.34899
3	Catechol	5.907	1483.91443	325.12729
4	*p*-Hydroxybenzoic acid	8.612	384.29080	81.64754
5	Caffeine	9.081	8752.3759	1103.27540
6	Vanillic acid	10.030	1080.69617	142.36919
7	Caffeic acid	10.604	310.55038	12.31559
8	Syringic acid	10.984	721.55731	41.53314
9	Vanillin	11.350	1707.85596	86.04171
10	*p*-Coumaric acid	13.821 1	1171.64612	47.79259
11	Ferulic acid	15.027	2281.31519	120.91204
12	Benzoic acid	15.182	3843.66211	3962.09350
13	Rutin	16.980	581.17615	222.80454
14	Ellagic	17.116	324.10291	26.82045
15	*o*-Coumaric acid	18.181	179.65379	5.54613
16	Salicylic acid	19.111	537.07898	159.40201
17	Myricetin	20.933	3.50421	7.52546
18	Quercitin	21.717	163.55061	102.22888
19	Kaempherol	23.899	3.68396	2.57555

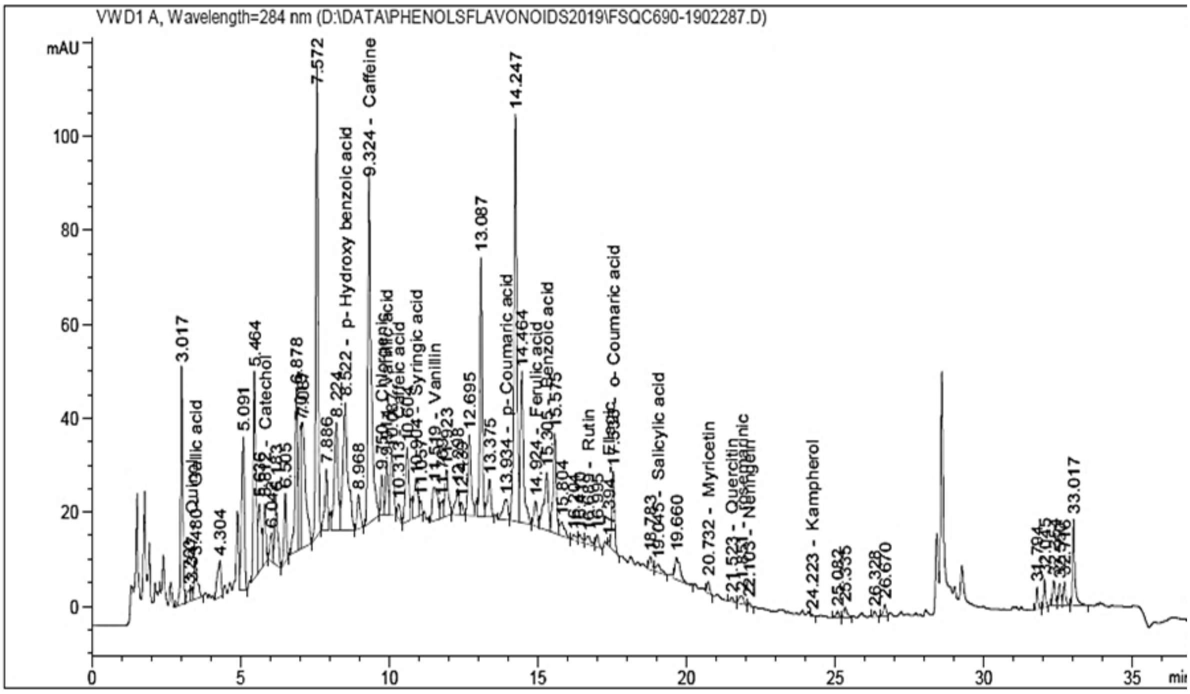

Figure 5. HPLC chromatogram of phenolic acids and flavonoids in the extract of *G. decandrus* extract.

Table 10. Phenolic acid and flavonoid compounds in the extract of *G. decandrus*.

Peak no	Compound name	Rt (min)	Area (mAU*s)	Amount (mg/kg)
1	Quinol	3.347	13.71519	2.26910
2	Gallic acid	3.480	60.98121	3.19326
3	Catechol	5.812	73.14532	513.43256
4	*p*-Hydroxybenzoic acid	8.522	317.93192	56.03347
5	Caffeine	9.324	583.41595	59.58754
6	Chlorgenic	9.750	46.53905	2.82093
7	Vanillic acid	9.914	68.98265	6.69282
8	Caffeic acid	10.313	20.70338	7.36947×10^{-1}
9	Syringic acid	10.904	70.82682	3.43252
10	Vanillin	11.519	89.76189	3.76383
11	*p*-Coumaric acid	13.934	50.09307	1.23544
12	Ferulic acid	14.924	39.48585	1.74630
13	Benzoic acid	15.305	129.78949	110.11964
14	Rutin	16.689	21.90650	3.03437
15	Ellagic	17.394	4.74864	1.63606×10^{-1}
16	*o*-Coumaric acid	17.535	105.49876	2.88740
17	Salicylic acid	19.045	22.31716	3.36718
18	Myricetin	20.732	15.23818	12.38376
19	Quercitin	21.523	6.06788	4.62736
20	rosemarinic	21.851	19.52471	18.68105
21	Neringein	22.103	4.56698	14.85728
22	Kampherol	24.223	1.30077	1.15605

Artemisia species [40]. Similar results were obtained by Seddik *et al.* who identified and quantified caffiec acid, gallic acid, and ferulic acid from the ethyle acetate extract of *A. herb alba* leave [41]. While, it differed from that obtained from different phytogeographical region as described by Souhila *et al.* [42]. Ferulic acid was previously identified and described in *A. herb alba* and other *Artemisia* species [43].

HPLC analysis of *P. undulata* revealed the presence of phenolic acids (benzoic acid, vanillic acid, caffeic acid, syringic acid, coumaric acid, ferulic acid, and gallic acid) and flavonoids (myricetin, quercetin, rutin, and kaempherol) (Table 9 and Figure 4). Similar components was detected in other species of *Pulicaria* as quercetin in previous reports of Elmann *et al.* [44] and ferulic acid in study of Triana *et al.* [45]. Furthermore, HPLC analysis of *G. decandrus* indicated the presence of phenolic acids (benzoic acid, ferulic acid, caffeic acid, ellagic acid, coumaric acid, salicylic acid, rosmarinic acid, syringic acid, chologenic acid, and gallic acid) and flavonoids (neringein, kaempherol, quercetine, myricetin, rutin, and vanillin) (see Table 10 and Figure 5). In accordance with our results, El-hawary *et al.* isolated vitexin, protocatechuic acid and quercetin from ethanol extract of *G. decandrus* [46]. They reported that *G. decandrus* contained nineteen compounds (twelve flavonoid, five saponins, and two phenolic compounds) as identified by HPLC/MS/MS. Benzoic acid was the most prominent component in study plants. Plant benzoic acids are aromatic carboxylic acids that serve as precursors for a wide variety of natural products including plant hormones, cofactors, defense compounds, and attractants for pollinators and seed disperser [47]. Various studies demonstrated the role of benzoic and salicylic acids in improving plant growth and productivity [48]-[50].

4. CONCLUSIONS

The current study provide a scientific base for using desert plants grow wild as natural alternatives to chemical fungicides. The total phytochemical screening and antifungal activity varied significantly among different plants. The current study suggests that *A. herb alba* and *P. undulata* extracts can interfere with fungal cellwall and enzymes causing their inhibition. Furthermore, the antifungal potential of *A. herb alba* and *P. undulata* extracts is related to their flavonoid and phenolic acid content. Thus, the antifungal activity of studied plants could be enhanced if active components are purified and used. In the future, further studies will be performed to purify and characterize the polyphenolic compounds of tested plant species and test their stability, and field efficiency under different environmental conditions.

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Conflicts of Interest

The authors declare no conflict of interest.

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