



# Antifungal activity and GC-MS detection of leaves and roots parts of *Chenopodium album* extract against some phytopathogenic fungi

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## ABSTRACT

Antifungal potential of leaves and roots parts of *Chenopodium album* was evaluated against five phytopathogenic fungi including *Alternaria alternata*, *Fusarium solani*, *Rhizoctonia solani*, *Pythium aphanidermatum* and *Sclerotinia sclerotium* using four concentrations viz. 0, 5, 10 and 15% of leaves and roots water extract led to significantly reduced of fungi mycelial growth. GC-MS analysis of leaves and roots water extract showed the presence of 7 compounds. 2(3H)-furanone, dihydro-4,4-dimethyl; 9-octadecenoic acid (Z), methyl ester; 9,12-octadecenoic acid (Z), methyl ester; 6-methylene bicyclo (3.2.0) hept-3-en-2-one., 1,2-benzene dicarboxylic acid, mono (2-ethylhexyl) ester and hexadecanoic acid, methyl ester. Five phytopathogenic fungi could be managed and controlled by the water leaves and roots extract of *C. album*. The antifungal activity of this extract was possible could be used to control a wide range of fungi.

**Key words:** *Alternaria alternata*, *Chenopodium album*, *Fusarium solani*, GC-MS, *Pythium aphanidermatum*, *Rhizoctonia solani*, *Sclerotinia sclerotium*.

## INTRODUCTION

*Chenopodium album* Linn. is an annual shrub, polymorphous, erect herb, mealy white, up to 3.5 m in height and widely. It has been grown in Asia, Africa, North America and Europe (Agrawal *et al.*, 2014). The tender shoots are cooked as a vegetable and eaten raw with curd or in a salad; as well as it is also used as fodder (Pande and Pathak, 2010). *C. album* is very nutritious and rich in vitamin C, vitamin A, calcium, protein, iron, potassium and phosphorus, content. It is used as the laxative, anthelmintic against hookworms and round, as spleen enlargement, blood purifier in hepatic disorders, burns and intestinal ulcers. Also, it has been used as folk medicine and bioactivities such as antifungal, antinociceptive, antipruritic and hypotensive properties of crude (Agrawal *et al.*, 2014). Recently, *C. album* has spread heavily during winter and summer in gardens, the field of barley and wheat of Wasit province in Iraq. The major factor for the spread and persistence of *C. album* is their ability to resist diseases and pests compared with the field crops in their environment. Thus, it can be a potential source of anti-phytopathogens compounds and their identification is needful to develop fungicides. It could be cheap and produced from plants available in the environment.

Chemical fungicides are mostly using to control plant disease of fungi because of their high effectiveness. However, many of their disadvantages cause environmental pollution, diseases for humans and animals. The killing of beneficial insects, also, most pathogenic fungal strains began to resist many types of these fungicides (Kamurthy *et al.*, 2016).

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Plant extracts played an important role in inhibiting the growth of various pathogenic pathogens in the soil and improving the quality of plant seeds. Thembo *et al.* (2010) pointed that the possibility of using chemical extracts from some of the weeds as fungicides that environmentally friendly and they have been tested four weed plants such as *Lippia javanica*, *Tagetes minuta*, *Vigna unguiculata* and *Amaranthus spinosus* against four isolates of phytopathogen fungi, *Aspergillus parasiticus*, *A. flavus*, *Fusarium proliferatum* and *F. verticillioides*.

The water and alcoholic extracts of *Oxalis corniculata* and *Mimosa pudica* weeds were tested as antifungal against fungus *Candida Albicans*; they showed high efficiency in inhibition to the pathogen (Kamurthy *et al.*, 2016). Based on that, we selected *C. album* weed to the absence of fungal infections on their stems and leaves. In Iraq, the antifungal activity of leaves and roots of *C. album* extracts of this

species has not been previously reported, the aim of our objective was to the identification of compounds extracted using GS-MS and test the efficacy of this compounds to inhibit mycelial growth of some phytopathogens invitro. Therefore, the main aim of this study to understand the secret of *C. album* resistance to plant pathogens and the diagnosis of compounds extracted from its roots and leaves. Also, to identify the possibility of using them to control various plant diseases as an alternative to synthetic fungicides.

## MATERIALS AND METHODS

### Plant materials

Leaves and roots of *C. album* were collected from Wasit University during March 2018. It is identified by at Field Crop Department, College of Agriculture, Wasit University. Herbarium of *C. album* was prepared and preserved in the Crop field laboratory, Wasit University. The roots have been separated from the stem by a sharp blade and washed by rinsing in tap water. They allowed to dry and then dried by electric oven at 75°C. The plant materials were ground to a fine powder using a mechanical grinder.

### Fungal pathogens

Five isolates of phytopathogens fungi *A. alternata*, *F. solani*, *R. solani*, *P. aphanidermatum* and *S. sclerotium* were obtained from the Field Crops Department, Agriculture College, Wasit University, Wasit, Iraq. These fungi have been grown in Potato dextrose agar (PDA) media with autoclaved at 120°C for 20 min.

### Preparations of water extracts

Two hundred gram of *C. album* were soaked in 1 L of distilled water separately and incubated overnight. Then, filtered through a Whatmann No. 41 filter paper. The filtered extract has been evaporated by a rotary evaporator and oven dried at 45°C. Different concentrations 0, 5, 10 and 15 % of dried powder extraction were prepared from leaves and roots dry powder to 100 ml sterilized distilled water separately. After that, they are ready to use them in inhibition growth of pathogens fungi.

### Antifungal activity

To determine the effects of the leaves and roots of *C. album* water extract on the mycelia length growth of pathogens in dual-culture techniques. Four concentration 0, 5, 10 and 15 % concentrations have been added as 3 ml for each concentration into 60 ml sterilized P.D.A media (40°C ± 2°C) separately. The media was mixed properly and poured into Petri dishes and then inoculated with 5-mm mycelia plugs of the pathogens placed in the centers of the plates separately. Control treatments consisted of PDA and disks of pathogens, without filtered extract. There were three repeated plates for each concentration per pathogen. The inoculated plates have been incubated at 27°C ± 1°C until the fungus grows completely in the control dish. Percent mycelia growth inhibition was calculated according to the following formula:

$$\text{Percent inhibition} = C - T / C * 100$$

C = Control of radial growth of the pathogen,  
T = Treatment of radial growth of the pathogen with leaves or roots of *C. album* water extract.

### GC-MS analysis

GC-MS 7820A gas chromatography equipped with detector 5977E mass spectrometer (Agilent Technologies, USA). Helium was used as a carrier gas with 99.999% supplied by (BOC, gas, Sydney, AU). Inlet temperature of the instrument was 250°C. Oven was preset at 50°C for 5 min, raised up to 250°C. The total run time for equipment was 45 min. the flow rate was 0.7 ml/min, the split less was 20 ml/min at 1.5 min. The total run time for equipment was 45 min. The compounds were identified by comparison of the mass spectrum with the US National Institute of Standards and Technology (NIST, 2014) database.

The samples have been analyzed in biosecurity lab., Murdoch University, Australia. Limit of detection (LOD) has been identified in this study as well. Alkane standard C7-C30 (Supelco, Bellefonte, USA) has been used to identify limit of detection. One microliter of standard was used in injection into GC-MS with 250°C injection port.

### Statistical analysis

*In vitro* experiments, three replicates were performed for each treatment according to the randomized design of the experimental experiments. The results data were analyzed for the variance using by Principal component analysis (PCA) and One-way Analysis of Variance (ANOVA) methods using online MetaboAnalyst 3.0 a comprehensive tool for metabolomics analysis and interpretation.

## RESULTS AND DISCUSSION

### Antifungal activity

The results of *In vitro* screening showed that the ability of *C. album* water extraction to inhibition of radial growth of some fungi pathogens. The percent mycelial inhibition of *Alternaria alternata*, *Fusarium solani*, *Rhizoctonia solani*, *Pythium aphanidermatum* and *Sclerotinia sclerotium* reached at 5 % concentration of leaves water extract at 27.90, 8.55, 20.12, 10.73 and 3.52 % respectively, while reached 48.18, 37.62, 62.40, 74.26 and 40.52 % respectively at 10 % concentration and reached 100, 83.60, 100, 9.33 and 91.42% respectively at 15 % concentration. For roots water extract at 5 % concentration, it reached 32.18, 17.76, 21.60, 16.30 and 19.00% respectively, while reached 63.80%, 71.95, 78.10, 80.46 and 85.15.52 % respectively at 10 % concentration and reached 100 % for all fungi at 15 % concentration. Where reached at 15 % concentration of leaves water extract at 100%, 83.60%, 100%, 9.33% and 91.42% respectively, while reached 48.18, 37.62, 62.40, 74.26 and 40.52 % respectively at 10 % concentration. For roots water extract at 15 % concentration, it reached 100 % for all fungi growth. While reached 63.80%, 71.95, 78.10, 80.46 and 85.15.52 % respectively at 10 % concentration. However, mycelia

inhibition of all fungi reached 0% at 5 and 0% concentration of both leaves and roots water extract (Table 1).

There are evidence from earlier studies that various weed species possess antifungal properties, Iqbal *et al.* (2001) found that the growth of mycelial growth of *Phomopsis theae*, *Aspergillus niger* and *Rhizoctonia solani* was reduce after treated with water extracts of *Phyllanthus debilis*, *Desmodium trifolium*, *Ageratum conyzoides*, *Vernonia cinerea* and *Oxalis corniculata* weed species. Some extracts of *C. album* such as roots, leaves, stem and inflorescence were examined against *F. oxysporum* f. sp. *cepae* at different concentrations (0.5, 1.0,3.0%) extract suppressed pathogen growth by 2.5–100% (Rauf and Javaid, 2013). *C. album* has been found to have cinnamic acid amide, saponin, phenolic amide, apocortinoid, alkaloid chinoalbicin, lignans, phenols and xyloside as active phytoconstituents (Agrawal *et al.*, 2014). Kamurthy *et al.* (2016) reported that some compounds extracted from weeds including, phenolic compounds, organic acids, malic acid, steroids, tannins, glycosides, triterpenoids, tartaric acid, alkaloids, flavonoids and citric acid may have the important role on inhibition of *C. albicans* growth.

#### GC-MS detection

GC-MS has been detected seven main compounds belonging to the aromatic dicarboxylic ester, fatty acid methyl esters and ketone groups which water leaves and roots extract from *C. album* (Table 2). The peak areas for these compounds were 6-methylenebicyclohept- 3-en-2-one (159.984); 1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester (377.746); 23h-furanone, dihydro-4,4-

dimethyl (288.391); hexadecanoic acid, methyl ester (211.644); 9-octadecenoic acid (Z)-, methyl ester (153.649); 9,12-octadecadienoic acid (Z,Z)-, methyl ester (58.281) and farnesene (86.406) (Table 2). The names of compounds are shown alongside comparison with molecular formulas, molecular weights and time retention of the NIST database and peak areas in Table 2. Some of these compounds belong to the methyl ester, methyl ethylethyl, methyl ethyl group, linoleic, Esther and fatty acids (Agoramoorthy *et al.* 2007., Chandrasekharan *et al.* 2008., Lima *et al.* 2011., Hussein *et al.* 2016).

Agoramoorthy *et al.* (2007) have been described that the methyl esters and fatty acid were known to possess antifungal properties. Khurshid *et al.* (2018) have been evaluated the antimicrobial activity of methanolic leaf, stem and inflorescence extract of *Cenchrus pennisetiformis* against *Fusarium oxysporum* f. sp. *lycopersici* causing wilt disease in tomato, they found that the grass significantly reduced fungal biomass and GC-MS analysis showed the presence of 10 compounds 1-eicosene, 1-propanol2-2-hydroxypropoxy, benzofuran 2,3-dihydro, hexadecanoic acid, ethyl-ester, Phenol, 2,4-bis, 1,1-dimethylethyl, Heptanal, 2-[phenylmethylene]-, E-15-heptadecenal, Ethanone, 1-[2,4,5-tri ethoxyphenyl]- and Benzene, 1-[1,1-dimethylethyl]-3,5-dimethyl-2,4,6-trinitro. Fatty acid of seeds of *Annona cornifolia*, have been examined by Lima *et al.* (2011), palmitic acid methyl ester (16.9%), linoleic acid methyl ester (19.1%) and mainly oleic acid methyl ester (51.5%). Heat map and ANOVA analyses in these experiments have been described the differences of the compounds with their peak areas. The PCA visualizes both the covariance and correlation between

**Table 1:** *In vitro* screening the leaves and roots of *C. album* water extraction to inhibition of radial growth of some fungi pathogens.

<i>C. album</i> water extract		Mcelial growth inhibition %				
		<i>A. alternata</i>	<i>F. solani</i>	<i>R. solani</i>	<i>P. aphanidermatum</i>	<i>S. sclerotium</i>
Leaves	0 %	0	0	0	0	0
	5 %	27.90	8.55	20.12	10.73	3.52
	10 %	48.18	37.62	62.40	74.26	40.52
	15 %	100	83.60	100	93.33	91.42
L.S.D				3.277		
Root	0 %	0	0	0	0	0
	5 %	32.18	17.76	21.60	16.30	19.00
	10 %	63.80	71.95	78.10	80.46	85.15
	15 %	100	100	100	100	100
L.S.D				3.215		

**Table 2:** Compounds were identified (one unit corresponds to a 10<sup>4</sup> area) from leaves and roots extract of *C. album* using GC-MS analysis.

Compounds	RT	Peak area	p-value	FDR
23H-Furanone, dihydro-4,4-dimethyl	9.008	288.391	0.002	0.093
Hexadecanoic acid, methyl ester	10.743	211.644	0.033	0.117
9,12-Octadecadienoic acid Z,Z-, methyl ester	17.886	58.381	0.306	0.672
6-Methylenebicyclo3.2.0hept-3-en-2-one	9.523	159.984	0.439	0.672
Farnesene	18.322	86.406	0.539	0.571
1,2-Benzenedicarboxylic acid, mono2-ethylhexyl ester	24.654	377.746	0.646	0.621
9-Octadecenoic acid Z-, methyl ester	20.110	153.649	0.672	0.653

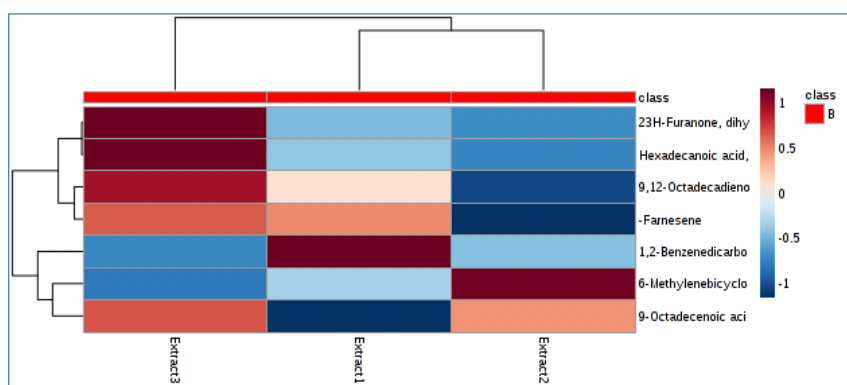


Fig 1: Heat map showing the changes of abundance values normalized to the compounds that are significantly influenced.

the metabolites and the modeled class designation. Thereby the PCA-plot helps to identify statistically significant and potentially biochemically significant metabolites based both on contributions to the model and their reliability. An extension of the PCA, the heat map, was applied to compare the outcome of multiple classification models compared the peak area. The used example is a GC coupled MS-based metabolomics study in extracted samples were extracted from the weed seeds (Fig1). In the current study, the method explain inhibited the growth of five phytopathogenic fungi *Alternaria alternata*, *Fusarium solani*, *Rhizoctonia solani*, *Pythium aphanidermatum* and *Sclerotinia sclerotium*. Based on Agoramoorthy *et al.* (2007) showed the ability of fatty acid methyl ester extract of *E. agallocha* to suppressed growth of four species of *Candida* including *C. parapsilosis*, *C. tropicalis*, *C. albicans* and *C. krusei* because it is rich in palmitic acid methyl ester (56.02%). The 6-methylenebicyclo (3.2.0) hept-3-en-2-one has been used to show normalization of the compounds that are significantly influenced 1,2-benzene dicarboxylic 5acid and for mono (2-ethylhexyl) ester has been used successfully from marine metabolites of a cyanobacterium *Oscillatoria terebriformis*, *Alternaria* sp. and *Streptomyces* sp.

This compound has been used for a large number of biological studies, including cytotoxicity, anti-inflammatory, antioxidant, antimicrobial and antiviral activity (Mukund *et al*, 2014., Krishnan *et al*, 2014; Govindappa *et al*, 2014). It was a major compound, which has been found in the current study (377.746). Therefore, there was a possibility that it may have had a role in the antifungal activity of water leave and roots extract of *C. album* in this study.

## CONCLUSION

As a result of the spread of this weed in a wide area of Iraq, it can be used as an environment-friendly fungicide. It can also be managed many important diseases that affect the fields of wheat and barley, which are grown primarily in Iraq instead of using the pesticides. This study concluded that the five phytopathogenic fungi *A. alternata*, *F. solani*, *R. solani*, *P. aphanidermatum* and *S. sclerotium* could be managed and control by the water leaves and roots extract

of *C. album*. Antifungal activity of these extracts can be used to control a wide range of phytopathogens.

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