Mycopath (2017) 15(1): 29-32 **Research Article**

Antifungal constituents of *n*-butanol soluble fraction of leaf extract of nettleleaf goosefoot weed

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Abstract

The present study was undertaken to investigate antifungal activity and GC-MS analysis of *n*-butanol fraction of methanolic leaf extract of *Chenopodium mural* L. Dried leaves of *C. murale* were extracted with methanol for 2 weeks. After evaporating the solvent on a rotary evaporator, the remaining extract was fractioned using various organic solvents. *n*-Butanol fraction was assayed for antifungal activity against *Fusarium oxysporum* f. sp. *lycopersici,* isolated from diseased tomato roots. Different concentrations of the extract ranging from 1.562 to 200 mg mL^{-1}, reduced fungal biomass significantly over control. A 100 mg mL^{-1} concentration almost completely controlled fungal growth and reduced its biomass by 98% over control. The extract was subjected to GC-MS analysis that revealed presence of 34 compounds. The predominant compounds in the extract were 1-heptanol (10.01%), 3-hydroxyhexanoic acid (9.70%), *n*-methyldodecylamine (6.63%), decane (6.54%), 1,2-decanediol (5.50%) and 1-octanol (4.71%).

Keywords: Antifungal activity, *Chenopodium murale,* GC-MS analysis, *n*-butanol extract.

Introduction

Nettleleaf goosefoot (*Chenopodium murale* L.) is an annual weed growing in different regions of Pakistan, especially in lower areas. This weed is native to Europe and commonly found in gardens, fields, roadsides and in moist areas. It is distributed in temperate and tropical regions and found abundantly in Pakistan. It grows in winter season and is usually marked as a pest in agro-ecological systems (Alam and Shaikh, 2007). This weed is also used as a traditional medicine in some regions of the world. It is a rich source of nutrients, antioxidants and dietary elements (Batish *et al.*, 2007a). It belongs to family *Chenopodiaceae*. Members of this family are known for their antifungal potential (Amin and Javaid, 2007; Javaid and Rauf, 2015)

Fausarium oxysporum f. sp. *lycopersici* is an important soil-borne fungus causing vascular wilts in tomato. Effected tomato plants show symptoms like leaf yellowing and wilting. Dried, wilted leaves drop prematurely and eventually whole plant dies producing very few or no fruit at all. Chemical control of fusarium wilt is mostly in practice all around the world. Chemical fungicides such as benomyl, thiram, thiabendazole and carbendazim, are widely used for its control (Akram and Anjum, 2011). Researchers are now searching for alternative disease management strategies as these chemicals are hazardous to environmental and human health (Anitha and Rabeeth, 2009). In order to identify natural antifungal compounds as alternate to synthetic fungicides, scientists screened a large number of plants for their antifungal activity against fungal pathogens and very encouraging results have been obtained (Ali *et al.*, 2017; Javaid *et al.*, 2017a, Sana *et al.*, 2017). The present study aimed to evaluate antifungal activity of *n*-butanol extract obtained from nettleleaf goosefoot leaves on growth of *Fausarium oxysporum* f. sp. *lycopersici.*

Materials and Methods

Fresh leaves of nettleleaf goosefoot weed were collected from Punjab University Lahore, rinsed thoroughly under tap water and sun dried. Dried leaves were ground to a fine powder. Two kilograms of dried leaves were soaked in 5L of methanol for 14 days in a bucket. Afterwards extract was separated from leaves residue by filtering through cheese cloth and filter paper, respectively. Methanolic extract was then evaporated under vacuum on a rotary evaporator and finally a thick paste was achieved by dry heating in electric oven at 40 ˚C. Distilled water (200 mL) was mixed thoroughly with methanolic leaf extract and successively partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol in order of increasing polarity. The required *n*-butanol fraction was evaporated using rotary evaporator acquiring 4 g of thick extract. Afterwards, different concentrations of this fraction (1.562 to 200 mg mL^{-1}) were prepared and evaluated for their antifungal activity against *F. oxysporum* f. sp. *lycopersici* following procedure given by Javaid *et al.* (2017b). GC-MS analysis of *n*-butanol fraction was performed using GC-MS QP-2010 model.

Data regarding the effect of different concentrations of *n*-butanol fraction on biomass of *F. oxysporum* f. sp. *lycopersici* was subjected to

ANOVA followed by LSD test at $P = 0.05$ using software Statistix 8.1.

Results and Discussion

Data regarding antifungal activity of different concentrations of *n*-butanol fraction of methanolic leaf extract of *C. murale* against *F. oxysporum* f. sp. *lycopersici* is presented in Fig. 1. In general, all the concentrations significantly suppressed fungal biomass, however, the effect of higher concentrations was far more pronounced than lower ones. The lower concentration *viz.* 1.562–6.25 mg mL^{-1} reduced fungal biomass just by 10–27% over control. Similarly, 12.5–50 mg mL^{-1} concentrations markedly reduced the fungal biomass by 49–67% over control. Higher concentrations (100 and 200 mg mL^{-1}) almost completely arrested the fungal growth and reduced its biomass up to 98% over control (Fig. 2). Earlier, Amin and Javaid (2007) estimated the antifungal potential of *C. murale* against *Macrophomina phaselina*, the cause of charcoal rot of sunflower. Different concentrations of leaf extracts of *C. murale* significantly reduced the biomass of target fungal pathogen by 57–68%. Likewise, Qasem and Abu-Blan (1995) reported that extracts of *C. murale* also possess antifungal activity against *Alternaria solani* and *Penicillium digitatum*.

GC-MS analysis of *n*-butanol fractions showed the presence of 34 organic compounds in the fraction (Fig. 3). Names and formulas of these compounds are presented in Table 1.

Table 1: Compounds identified from *n*-butanol fraction of methanolic leaf extract of *Chenopodium murale* through GC-MS analysis.

S. No.	Names of compounds	Molecular Formula	Molecular Weight	Retention time (min)	Peak area (%)
1	*n*-Methyldodecylamine	$C_{13}H_{29}N$	199	5.003	6.63
2	1-Heptanol	$C_7H_{16}O$	116	5.142	10.01
3	2-Hexanol	$C_6H_{14}O$	102	5.250	2.77
4	Diethoxymethane	$C_5H_{12}O_2$	104	5.575	2.80
5	3-Hexanol	$C_6H_{14}O$	102	5.900	3.04
6	3-Hydroxyhexanoic acid	$C_6H_{12}O_3$	132	6.067	9.70
7	1,1-Dipropoxyethane	$C_8H_{18}O_2$	146	6.267	2.13
8	1-Iodoheptane	$C_7H_{15}I$	226	6.400	1.49
9	1-Butoxy-1-ethoxyethane	$C_8H_{18}O_2$	146	6.467	3.79
10	1-Butoxypentane	$C_9H_{20}O$	144	6.742	3.17
11	3-Nitropropionic acid	$C_3H_5NO_4$	119	7.042	1.15
12	2,5-Dimethyl-2,5-hexanediol	$C_8H_{18}O_2$	146	7.124	1.17
13	1,1-Diethoxy-butane	$C_8H_{18}O_2$	146	7.258	1.87
14	17-Cyclohexyltritriacontane	$C_{39}H_{78}$	546	7.750	0.77
15	1-Dodecanol	$C_{12}H_{26}O$	186	7.800	1.24
16	3-Methylnonane	$C_{10}H_{22}$	142	7.900	1.29
17	1-Butoxypentane	$C_9H_{20}O$	144	8.150	3.37
18	Octanoic acid	$C_8H_{16}O_2$	144	8.192	3.05
19	1-Nonyne	C_9H_{16}	124	8.342	4.31
20	Butoxypropanol	$C_7H_{16}O_2$	132	8.450	1.17
21	Decane	$C_{10}H_{22}$	142	8.575	6.54
22	Methyl octanoate	$C_9H_{18}O_2$	158	8.817	1.03
23	Decyne	$C_{10}H_{18}$	138	9.442	1.22
24	Triazine	$C_{10}H_{22}$	142	9.908	1.30
25	1,2-Decanediol	$C_{10}H_{22}O_2$	174	10.442	5.50
26	Dibutoxymethane	$C_7H_{14}O_3$	146	11.750	4.31
27	2-Hydroxyheptanoic acid	$C_9H_{20}O_2$	146	12.033	1.02
28	1,12-Dodecanediol	$C_{12}H_{26}O_2$	202	13.017	2.80
29	Pimelic acid	$C_7H_{12}O_4$	160	13.167	0.70
30	1-Octanol	$C_8H_{18}O$	130	14.067	4.71
31	Roughanic acid	$C_{16}H_{26}O_2$	250	15.975	1.48
32	Octylphenol	$C_{14}H_{22}O$	206	18.129	0.49
33	Palmitic acid	$C_{16}H_{32}O_2$	256	21.200	2.40
34	Dioctyl phthalate	$C_{24}H_{38}O_4$	390	26.017	1.26

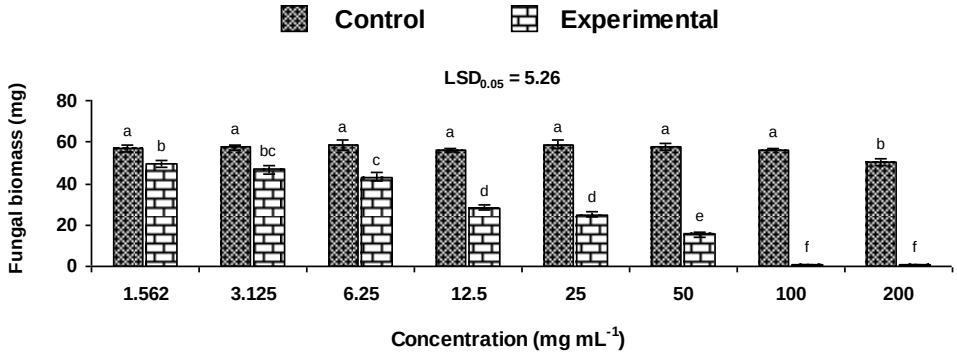

Fig. 1: Effect of different concentrations of *n*-butanol sub-fraction of methanolic leaf extract of *Chenopodium murale* on biomass of *Fusarium oxysporum* f. sp. *lycopersici.* Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

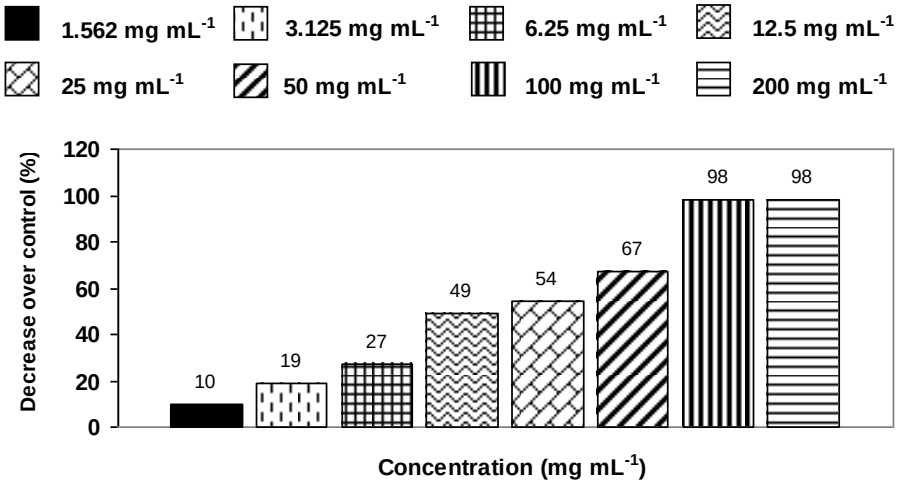

Fig. 2: Percentage decrease in biomass of *Fusarium oxysporum* f. sp. *lycopersici* due to different concentrations of *n*-butanol sub-fraction of methanolic leaf extract of *Chenopodium murale.*

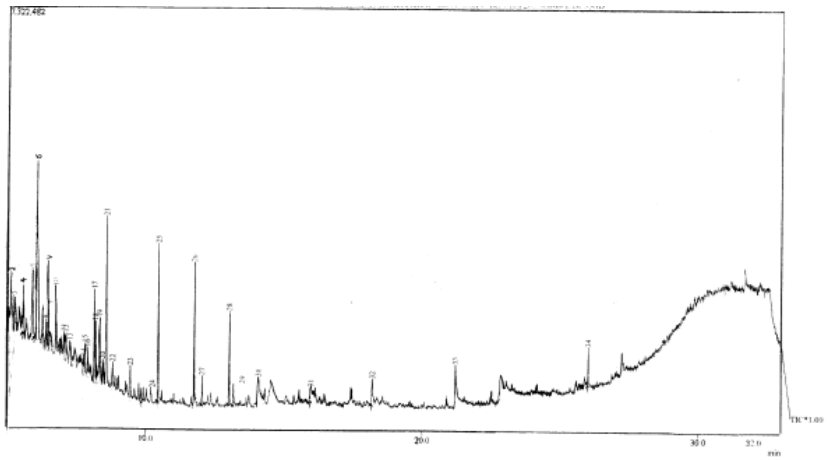

Fig. 3: GC-MS chromatogram of *n*-butanol sub-fraction of methanolic leaf extract of *Chenopodium murale.*

Most abundantly compounds were 1-heptanol (10.01%), 3-hydroxyhexanoic acid (9.70%), *n*-methyldodecylamine (6.63%), decane (6.54%) and 1,2-decanediol (5.50%). Moderately abundant compounds were 1-octanol (4.71%), dibutoxymethane (4.31%), 1-nonyne (4.31%), 1-butoxy-1-ethoxyethane (3.79%), 1-butoxypentane (3.37%), 1-butoxypentane (3.17%), Octanoic acid (3.05%), 3-hexanol (3.04%), diethoxymethane (2.80%), 1,12-dodecanediol (2.80%), 2-hexanol (2.77%) and palmitic acid (2.40%). Rest of the compounds namely 1-iodoheptane (1.49%), 3-nitropropionic acid (1.15%), 2,5-dimethyl-2,5-hexanediol (1.17%), 1,1-diethoxy-butane (1.87%), 17-cyclohexyltritriacontane (0.77%), 1-dodecanol (1.24%), 3-methylnonane (1.29%), butoxypropanol (1.17%), methyl octanoate (1.03%), decyne (1.22%), triazine (1.30%), 2- hydroxyheptanoic acid (1.02%),

pimelic acid (0.70%), roughanic acid (1.48%), octylphenol (0.49%) and dioctyl phthalate (1.26%) were present in low concentrations. Bayan *et al.* (2016) identified 1-octanol and octanoic acid from the essential oil of *Heracleum platytaenium*, evaluated against *Candida albicans* and *Microsporum gypseum.* The isomers of most abundant compound 1-heptanol in this study have also been reported from other allelopathic plants (Swamy *et al.,* 2015). Sana *et al*. (2017) identified heptanol from *Melia azadrach* against *Sclerotium rolfsii*. In the present study dioctyl phthalate is found with 1.26% peak area. Phthalates have also been reported from other plant species such as *Euphorbia hylonoma* as well as from *Streptomyces bangladeshiensis* and are known to exhibit antimicrobial activity against some pathogenic fungi (Al-Bari *et al*., 2006). Similarly, phthalic acid esters and fatty acids such as palmitic acid present in essential oil of *Leea indica* leaves as major constituents, also known to possess antifungal activity (Chandrasekaran *et al.,* 2007). Palmitic acid, octanoic acid, roughanic acid and pimelic acid are known to have antifungal activities against various fungal species (McGaw *et al*., 2002; Seidel and Taylor, 2004).

From the present study, it was concluded that *n*-butanol extract of *C. murale* possess various antifungal compounds for the control of *F. oxysporum* f. sp. *lycopersici.*

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