Mycopath (2017) 15(1): 41-45 **Research Article**

GC-MS analysis of ethyl acetate fraction of leaf extract of London rocket weed for identification of possible antifungal constituents

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Abstract

The present study was undertaken to find out the antifungal constituents of ethyl acetate soluble fraction of methanolic leaf extract of London rocket weed (*Sisymbrium irio* L.). To achieve this objective, methanolic leaf extract was partitioned using various organic solvents including ethyl acetate. Different concentrations of ethyl acetate fraction were prepared which ranged from 1.562 to 200 mg mL^{-1}, and evaluated against *Fusarium oxysporum* f. sp. *cepae* isolated from diseased onion. A significant reduction in fungal biomass of 77–93% was recorded due to different concentrations of this fraction. GC-MS analysis revealed the presence of 7 constituents in the fraction. Among these 1,3-Cyclopentadiene, 5-(1-methylethyllidene) (26.31%) and Di-n-octyl phthalate (26.28%) were the most abundant followed by Acetic Acid, butyl ester (11.67%) and γ-Sitosterol (11.64%). Presence of these compounds may be responsible for antifungal behavior of this fraction against the pathogen of basal rot disease of onion.

Keywords: Antifungal activity, ethyl acetate fraction, GC-MS analysis, *Sisymbrium irio.*

Introduction

Brassicacae is a very large family distributed all over the world having 338 genera and more than 3709 species including both cultivated and crop plants (Al-Shehbaz *et al.,* 2006). Members of this family are known for their antifungal properties (Ye *et al.,* 2011; Aguilar-Gonzalez *et al.,* 2015). *Sisymbrium irio*, a member of Brassicacae family, is an annual winter weed found in roadside and fence areas of Punjab, Pakistan. Besides, having economical and agricultural importance, *S. irio* also possesses many applications in folk medicines. It has been used in treatment of inflammation and rheumatoid (Bolus, 1983). It was found to have flavanoids (Khan *et al.,* 1991), alkaloids, oils, steroids and anthraquinones which play an important role in antifungal activities (Arayno and Zafor, 1983; Krets *et al*., 1987; Soulier, 1994; Shah *et al*., 2013). *S. irio* also has antipyretic and analgesic properties (Vohora *et al.,* 1980). Literature showed its antifungal potential against *Macrophomina phaseolina* (Javaid *et al.,* 2017). Plant extract of *S. irio* also showed toxic effects against *Alternaria solani* (Qasem and Hifzi, 2008). According to Shabnam *et al*. (2015), ethyl acetate fraction of *S. irio* leaves inhibited the growth of *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeroginosa* whereas, all extracts of leaves exhibited antifungal activity against *Aspergillus flavus* and *Fusarium oxysporum*.

F. oxysporum f. sp. *cepae* causes significant yield and economical losses in onion (Ozer and Koycu, 2004). Different strategies have been employed for its management including chemicals application also (Rial-Otero *et al*., 2005). However, there have been many environmental issues as well as health problems regarding the use of synthetic chemical fungicides. To compensate these harmful effects, biological approaches have been used to minimize losses and maximize yield growth and economy (Parka *et al*., 2002). In the present study, ethyl acetate fraction of *S. irio* leaf extract was tested against *F. oxysporum* f. sp. *cepae* (FOC) and investigation of possible chemical constituents responsible for antifungal activity.

Materials and Methods

To prepare methanolic extract of leaf 1 kg dried crushed material was soaked in methanol (2 × 5 L) for 2 weeks. The crude extract was prepared by evaporating the filtrate on rotary evaporator. The extract was suspended in water (200 mL) and then subjected to fractionation using different organic solvents namely *n*-hexane, chloroform, ethyl acetate and *n*-butanol following Javaid and Rauf (2015). *In vitro* antifungal bioassays of ethyl acetate, *n*-butanol and aqueous fractions were conducted in 10-mL volume test tubes. The stock solutions of 200 mg mL^{-1} were prepared by adding 1 mL DMSO in 1.2 g extract and 5 mL malt extract broth. Lower concentrations 1.56 to 100 mg mL^{-1}were prepared by adding appropriate quantities of malt broth to stock solution. Parallel control treatments were made by diluting control solution of 1 mL DMSO in 5 mL

malt broth. Inoculum of FOC was prepared in autoclaved distilled water and 50 µL was added to each tube. All treatments were replicated thrice. The experiment was incubated at 27 °C. After a week, the fungal biomass was filtered, dried and weighed. All the data were analyzed by ANOVA followed by LSD test (P = 0.05) using computer software Statistix 8.1.

Results and Discussion

The effect of ethyl acetate fraction on fungal biomass is described in Fig. 1 and 2. All the concentrations of ethyl acetate fraction showed significant fungal growth reduction with reference to control treatments. As the concentration of extract increased the inhibitory effect became more pronounced. The reduction in fungal biomass was 77–93%. The *n*-butanol fraction of the extract also exhibited inhibitory effect against *F. oxysporum* f. sp. *cepae* but the effect was less pronounced than the effect of ethyl acetate fraction. The higher concentrations (50–200 mg mL^{-1}) exhibited more pronounced inhibitory effect on fungal growth causing 82–90% reduction. Similarly, these higher concentrations of aqueous fraction exhibited reduction in fungal biomass by 79–90%. Previously, the aqueous extract of *S. irio* showed significant reduction in growth of *Alternaria alternata* (Shafique *et al*., 2006). Javaid *et al.* (2017) reported 77% and 87% reduction in biomass of *M. phaseolina* due to 200 mg mL^{-1} concentration of *n*-butanol and ethyl acetate fractions of leaf extract of *S. irio*, respectively. *S. irio* aqueous extract significantly inhibited colony growth of *Verticillium dahliae* (Qasem and Abu-Blan, 1995). Members of Brassicacae family including *S. irio* produce various metabolites including glucosinolates, which are responsible for their antifungal action (Sun *et al*., 2011). *S. irio* also have flavanoids (Khan *et al.*, 1991), which are also known for antifungal potential against the pathogenic fungi (Kanwal *et al.*, 2010).

GC-MS chromatogram showed the presence of 7 constituents in ethyl acetate fraction of *S. irio* leaf extract (Fig. 3). The identified compounds along with their retention times, molecular formulae and molecular masses are listed in Table 1. 1,3-Cyclopentadiene, 5-(1-methylethyllidene) and Di-n-octyl phthalate were the most abundantly present compounds having peak areas 26.31% and 26.28%, respectively. The compounds acetic acid, butyl ester (11.67%) and γ-sitosterol (11.64%) were moderately abundant whereas 1,3-benzenedicarboxylic acid, bis(2-ethylhexyl) ester; 1,2-benzenedicarboxylic acid, butyl 2-methylpropyl ester and cyclohexanone were present in less quantities having peak areas of 9.79%, 7.25% and 6.54%, respectively. Structures of these compounds are presented in Fig. 4. By using the technique column chromatography previously 15 compounds were isolated by Al-Qudah and Abu-Zarga (2010), including two new constituents namely (sitosteryl-60-Oundecanoate -D-glucoside and (Z)-8,11,12 trihydroxyoctadec-9-enoic acid) from aerial parts of *S. irio*. Similarly, Al- Jaber (2011), also isolated different flavanoids from shoot of *S. irio.*

From the present study, it can be concluded that ethyl acetate fraction of *S. irio* leaf extract possesses potent antifungal compounds to control growth of *F. oxysporum* f. sp. *cepae.*

Table 1: Compounds identified in ethyl acetate fraction of methanolic leaf extract of *Sisymbrium irio* through GC-MS.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	Acetic Acid, butyl ester	$C_6H_{12}O_2$	116	3.568	11.67
2	1,3-Cyclopentadiene, 5-(1-methylethyllidene)	C_8H_{10}	106	4.591	26.31
3	Cyclohexanone	$C_6H_{10}O$	98	5.147	6.54
4	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	$C_{16}H_{22}O_4$	278	19.227	7.25
5	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390	24.011	26.28
6	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	390	25.515	9.79
7	γ-Sitosterol	$C_{29}H_{50}O$	414	29.656	11.64

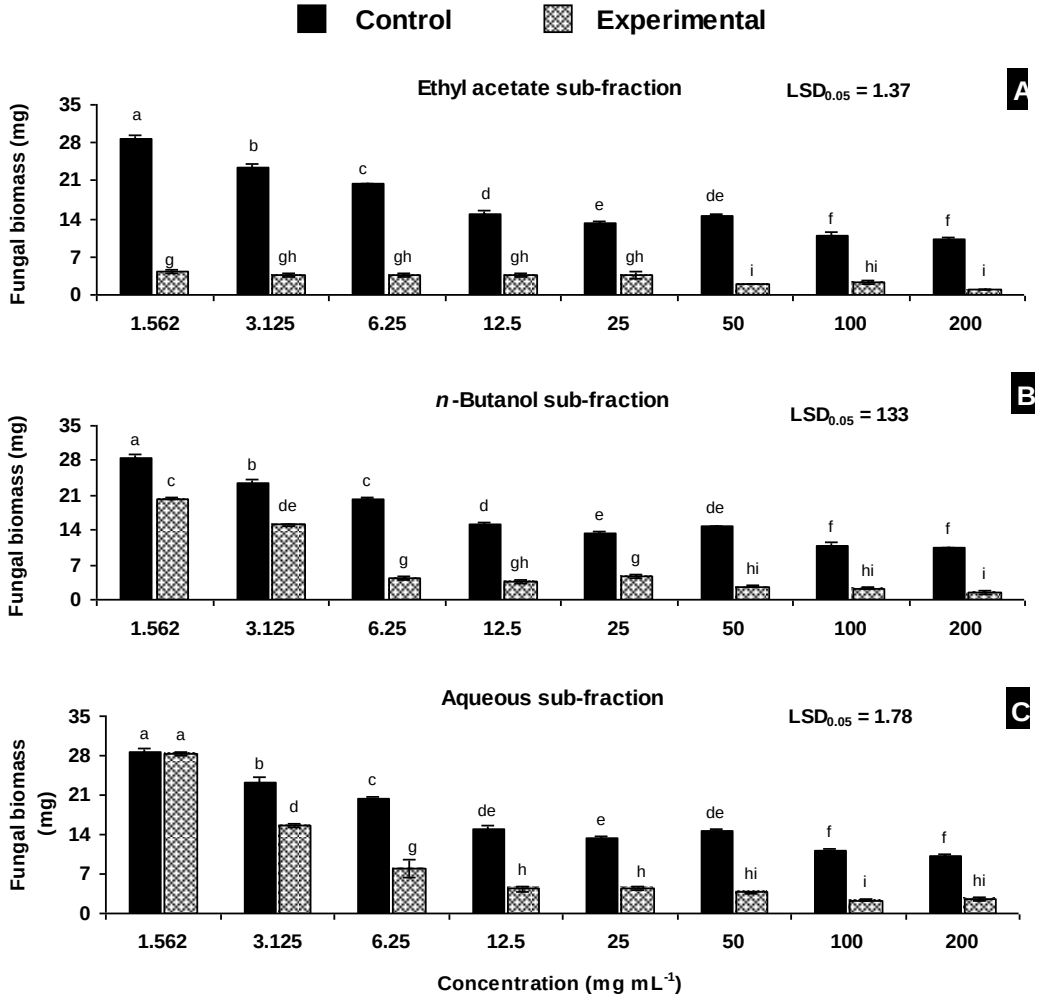


Fig. 1 (A-C): Effect of ethyl acetate, *n*-butanol and aqueous fractions of methanol leaf extract of *Sisymbrium irio* on biomass of *Fusarium oxysporum* f. sp. *cepae.* Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

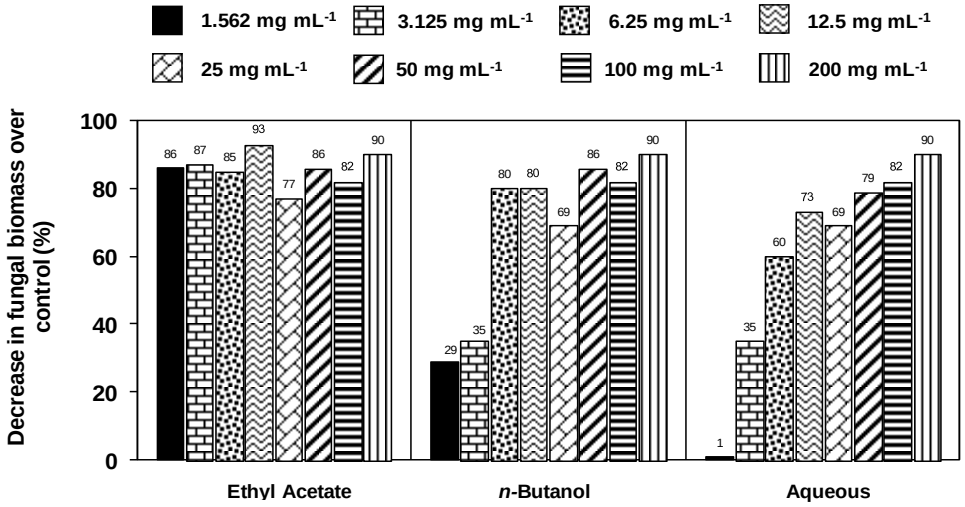


Fig. 2: Percentage decrease in biomass of *Fusarium oxysporum* f. sp. *cepae* due to different concentrations of ethyl acetate, *n*-butanol and aqueous fractions of methanolic leaf extract of *Sisymbrium irio.*

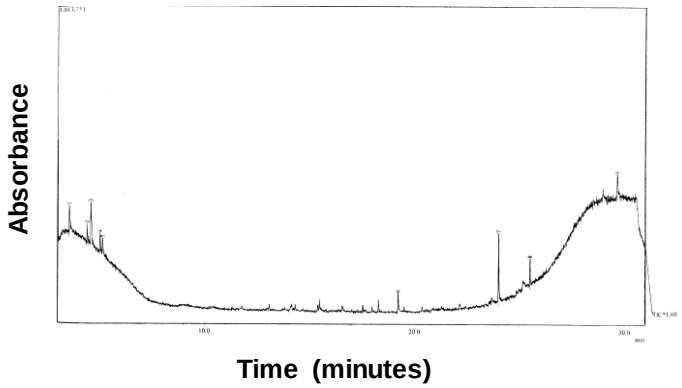
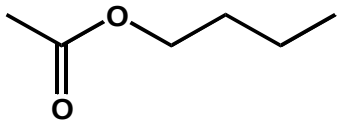
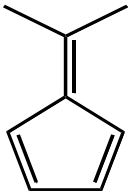


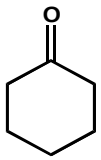
Fig. 3: GC-MS chromatogram of ethyl acetate fraction of methanolic leaf extract of *Sisymbrium irio.*



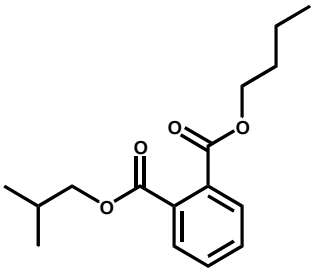
1. Acetic acid, butyl ester



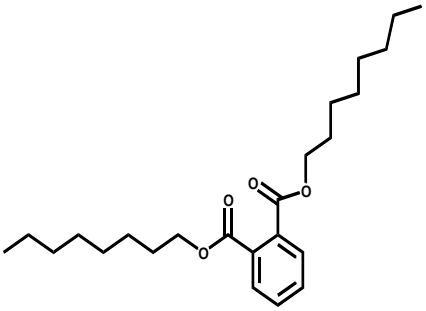
2. 1,3-Cyclopentadiene, 5-(1 methylethyllidene)



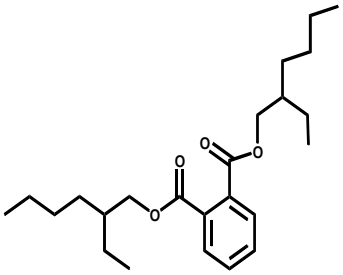
3. Cyclohexanone



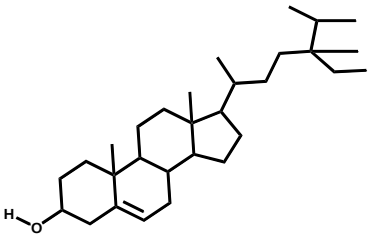
4. 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester



5. Di-*n*-octyl phthalate



6.1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester



7. γ-Sitosterol

Fig. 4: Structures of compounds identified in ethyl acetate fraction of methanolic leaf extract of *Sisymbrium irio* through GC-MS.

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