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ANTIFUNGAL ACTIVITY OF *N*-BUTANOL STEM EXTRACT OF QUINOA AGAINST *MACROPHOMINA PHASEOLINA*

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a recently introduced pseudo-cereal in Pakistan. The present study aimed to assess the antifungal potential of a polar solvent *n*-butanol soluble fraction of methanolic stem extract of quinoa against *Macrophomina phaseolina.* The study was further extended to identify the possible antifungal constituents through GC-MS analysis. *In vitro* bioassays revealed that a 25 mg mL^{-1} concentration of the *n*-butanol fraction in malt extract broth can completely control the fungal growth. GC-MS analysis showed the presence of 9 volatile compounds namely stigmasta-7, 16-dien-3-ol, (3.beta.,5.alpha.)- (23.95%), gamma-sitosterol (16.92%), stigmasterol (13.46%), decane (7.88%), 2-propylcyclohexanol (6.90%), 1,2-benzedicarboxylic acid, diisooctyl ester (5.07%), cyclohexene,3-nonyl- (4.36%), 4-butyl (dimethyl) silyloxpentadecane (3.09%), and 2-pyrrolidinecarboxylic acid-5-oxo-, ethyl ester (1.60%) in this fraction. Literature survey showed that stigmasterol and 1,2-benzedicarboxylic acid, diisooctyl ester had antifungal activities against a variety of fungal species and might be responsible for control of *M. phaseolina* in the present study.

Key words: Antifungal, *Macrophomina phaseolina*, *n*-butanol fraction, Quinoa, Stem extract.

Introduction

Macrophomina phaseolina is a cosmopolitan fungal pathogen that belongs to family Botryosphaeriaceae, and is responsible for charcoal rot, stem rot, root rot and wilting in many economically important host crops (Saima & Wu, 2019; Banaras *et al*., 2021). Being polyphagous in nature, it presents a huge variability in pathogenicity by inhabiting the soil or seeds under low moisture and high temperature conditions (Burkhardt *et al*., 2019). The pathogen survives in the form of sclerotia for long time so it is very difficult to control it completely. In this context, various chemicals are in practice to avoid losses caused by this devastating pathogen but with some limitations as they possess hazardous effects on the environment and humans so these are not recommended. To combat this issue, plant-based antifungal materials have been investigated as an alternate to synthetic fungicides for the control of *M. phaseolina* (Javaid *et al*., 2018; Banaras *et al*., 2020; Javed *et al*., 2021). This management strategy does not create imbalance in the environment and has less residue effects in the soil with the production of non-toxic metabolites so their application is considered to be safe for use (Siddiq *et al*., 2019; Akbar *et al.*, 2020).

Chenopodium quinoa Willd. is an emerging pseudo-cereal native to costal and Andean climatic zones of South America, now becoming popular in non-native areas too. It is recently introduced in Pakistan because of its agronomic desirable traits. It can grow well under harsh climatic conditions such as in saline soils, drought, cold, and frost, with less input of fertilizers (Maughan *et al*., 2019). It has gained importance due to its high nutritious values, good quality amino acid profile and rich in protein contents. All plant parts are consumed vigorously but seeds are excellent source of minerals (0.5-0.8%), proteins (12-15%), saponins (4.8-5.5%), fibers (18-21%) and lipids (8-12%) (Pereira *et al*., 2019). In addition, quinoa is a rich source of many secondary metabolites including phytic acid, flavonoids, steroids, benzoic acid, syringic acid, ferulic acid, isoflavones, genistein, aglycones, kaempferol, quercetin, terpenoids and glycosides, and exhibit a variety of biological activities such as anti-inflammatory, anti-diabetic, antioxidant, cytotoxic, anticancer, antimicrobial and insecticidal (Khan *et al*., 2018, 2020; Khan & Javaid, 2020a). However, the studies regarding its antifungal efficacy against the pathogenic microorganisms are scarce and especially lacking against *M. phaseolina* (Hernandez-Ledesma, 2019). Therefore, the objective of the present study was to focus on antifungal efficacy of *n*-butanol fraction of methanolic stem extract of quinoa against *M. phaseolina* and identification of various volatile constituents in this fraction.

Materials and Methods

Stems of quinoa were collected and shade dried. The plant material (2 kg) was coarsely powdered and dipped in methanol (5 L) for two weeks followed by filtration by an ordinary filter paper. The solvent was evaporated under vacuum at 45°C in order to obtain a concentrated crude extract. The obtained extract was mixed in distilled water and the mixture was fractionated successively by using different solvents *viz. n*-hexane (400 × 5 mL), chloroform (400 mL), ethyl acetate (400 mL) and *n*-butanol (400 mL). Finally, the *n*-butanol fraction was selected and the solvent was evaporated on a rotary evaporator to get a gummy mass that was tested against *M. phaseolina* (Khan *et al.*, 2021).

For antifungal bioassay, 1.2 g of the extract was stirred in 1 mL of dimethyl sulphoxide (DMSO) and raised its volume up to 6 mL with the addition of malt extract broth (MEB) in order to prepare the highest concentration of 200 mg mL^{-1}. A portion of this medium (3 mL) was serially diluted by adding autoclaved MEB to obtain the lower concentrations *viz.* 100, 50, 25, 1.562 mg mL^{-1}. A series of control treatments was similarly prepared by adding 0.5 mL DMSO but without addition of the extract. The tested fungus *M. phaseolina* conidial suspension was prepared by scratching the 10-day-old culture plates of the fungus in distilled water and adding 50 µL in each test tube containing 1 mL of growth medium and incubated for 7 days at 28°C with three replicates of each treatment. Later, the fungal mat was filtered on pre-weighted filter papers and dried for 2 hours at 70ºC (Akhtar and Javaid, 2018). All the data were analyzed by one-way ANOVA followed by LSD Test ($p \leq 0.05$) using software Statistix 8.1. The *n*-butanol fraction was subjected to GC-MS analysis for the identification of antifungal compounds present in it (Khan & Javaid, 2020b).

Results and Discussion

The results showed the antifungal effect of *n*-butanol fraction on growth of *M. phaseolina* (Fig. 1). Lower concentrations of the extract (1.562 to 12.5 mg mL^{-1}) significantly reduced biomass of *M. phaseolina* by 52–65%. A concentration of 25 mg mL^{-1} completely arrested the fungal growth resulting in 100% reduction in its biomass (Fig. 1). Previously, Stuardo & Martin (2008) tested the alkaline treated quinoa against the *Botrytis cinerea* and concluded that the extract completely arrested the mycelial growth of the fungal pathogen. Recently, Khan & Javaid (2019) reported the presence of strong antibacterial and antifungal compounds in ethyl acetate fraction of *C. quinoa* stem. Moreover, the *n*-hexane extract of *C. quinoa* inflorescence and leaves revealed the presence of enormous antifungal, antioxidant and anti-inflammatory compounds (Khan *et al.*, 2018, 2020).

GC-MS chromatogram of *n*-butanol fraction of methanolic stem extract is shown in Fig. 2 that indicates the presence of 9 phytoconstituents belonging to diverse groups of volatile natural organic compounds. The identified compounds detail is given in Table 1, whereas their structures are shown in Fig. 3. The major identified compounds were stigmasta-7, 16-dien-3-ol, (3.beta., 5.alpha.)- (**7**), gamma-sitosterol (**8**) and stigmasterol (**6**) with 23.95%, 16.92% and 13.46% peak areas, respectively. Decane (**2**) was found as moderately abundant compound followed by 2-propylcyclohexanol (**3**), 1, 2-benzedicarboxylic acid, diisooctyl ester (**9**), cyclohexene, 3-nonyl- (**4**) and 4-butyl (dimethyl) silyloxpentadecane (**1**) showing 7.88%, 6.90%, 5.07%, 4.36% and 3.09% of peak areas, respectively. The compound 2-pyrrolidinecarboxylic acid-5-oxo-, ethyl ester (**5**) with 1.60% peak area was found to be the least abundant one.

Literature showed that some of the isolated compounds in the present study possess strong antifungal activities against a variety of fungal species. Bhardwaj (2018) identified compound **6** from a medicinal plant *Tecomella undulata* and evaluated its antifungal efficacy against *Trichoderma reesei*, *Fusarium oxysporum*, *Aspergillus niger*, *Candida albicans*, *Penicillium funiculosum* and *Trichoderma viride*. The *T. undulata* leaf extract was found highly effective against *T. viride* and *F. oxysporum* even at lower concentrations. Earlier, Bawankar *et al*., (2013) also reported that this compound was effective against *A. niger*, *C. albicans*, *Aspergillus flavus*, *Aspergillus terreus* and *Penicillium* sp. Compound **9** was reported from the methanolic extract of *Cynodon dactylon* and was found effective against *A. niger*, *A. flavus*, *Alternaria alternata* and *C. albicans* (Balasundari & Boominathan, 2018). Therefore, it is concluded that antifungal activity of *n*-butanol fraction of methanolic stem extract of quinoa against *M. phaseolina* is possibly because of presence of compounds **6** and **9**.

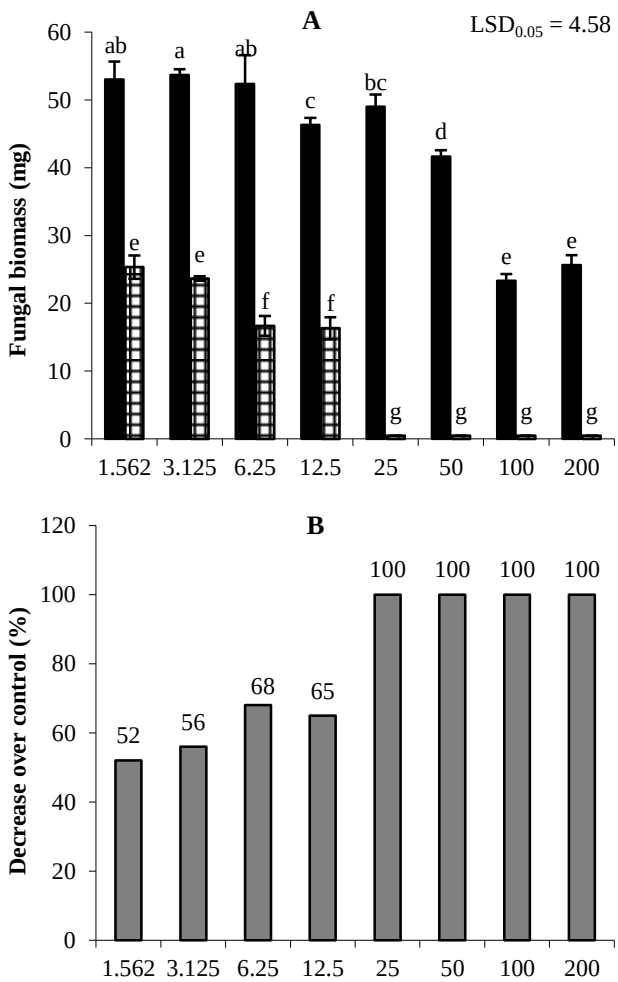


Fig. 1. A: Effect of different concentrations of *n*-butanol fraction of methanolic stem extract of *Chenopodium quinoa* on biomass of *Macrophomina phaseolina.* B: Percentage decrease in fungal biomass due to different concentrations of the extract over corresponding control treatments. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($p \leq 0.05$) as determined by LSD Test.

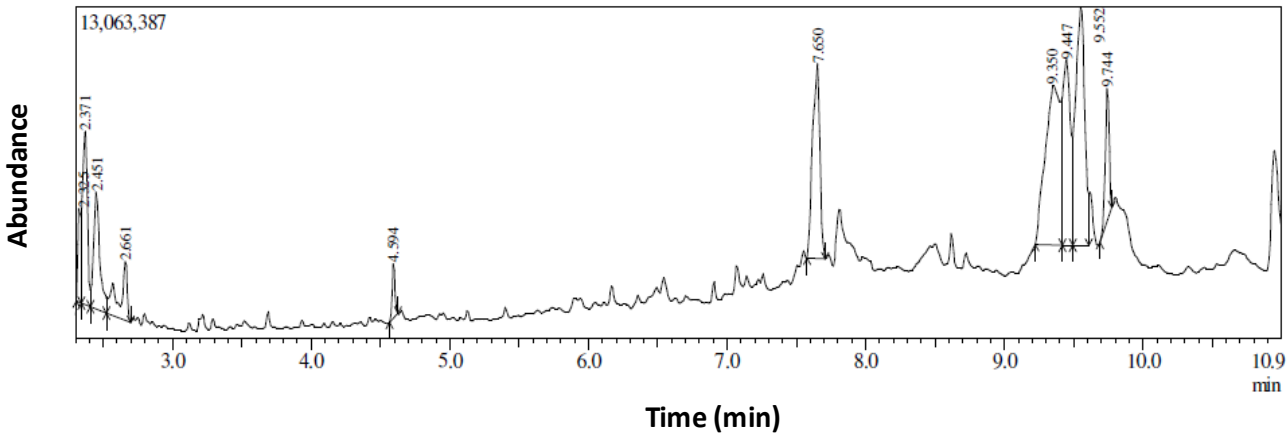
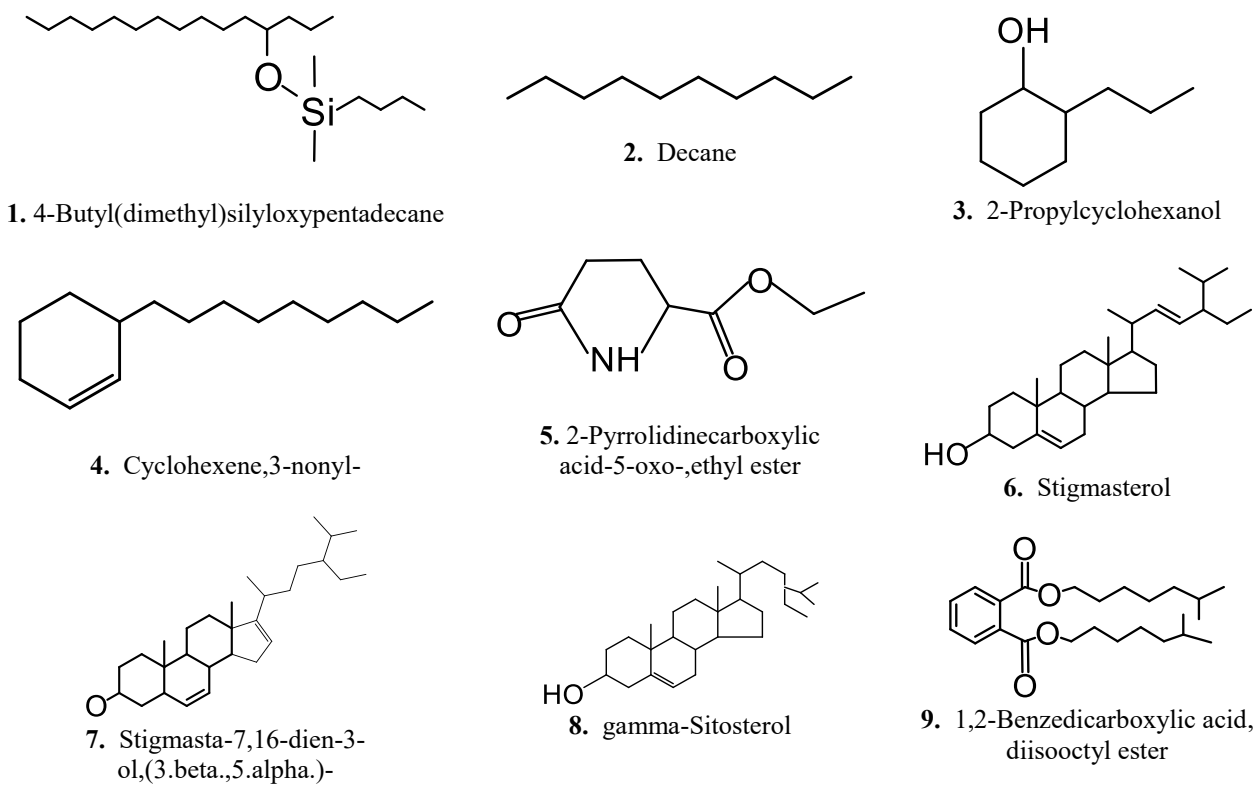


Fig. 2. GC-MS chromatogram of *n*-butanol fraction of methanolic stem extract of *Chenopodium quinoa.*



1. 4-Butyl(dimethyl)silyloxypentadecane

2. Decane

3. 2-Propylcyclohexanol

4. Cyclohexene,3-nonyl-

5. 2-Pyrrolidinecarboxylic acid-5-oxo-,ethyl ester

6. Stigmasterol

7. Stigmasta-7,16-dien-3-ol,(3.beta.,5.alpha.)-

8. gamma-Sitosterol

9. 1,2-Benzedicarboxylic acid, diisooctyl ester

Fig. 3. Structures of compounds identified in *n*-butanol fraction of stem extract of *Chenopodium quinoa* through GC-MS.

Table 1. Compounds identified through GC-MS analysis in *n*-butanol fraction of methanolic stem extract of *Chenopodium quinoa*.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peakarea (%)
1.	4-Butyl (dimethyl) silyloxypentadecane	$C_{21}H_{46}OSi$	342	2.325	3.09
2.	Decane	$C_{10}H_{22}$	142	2.371	7.88
3.	2-Propylcyclohexanol	$C_9H_{18}O$	142	2.451	6.90
4.	Cyclohexene, 3-nonyl-	$C_{15}H_{28}$	208	2.661	4.36
5.	2-Pyrrolidinecarboxylic acid-5-oxo-,ethyl ester	$C_7H_{11}NO_3$	157	4.594	1.60
6.	Stigmasterol	$C_{29}H_{48}O$	412	7.650	13.46
7.	Stigmasta-7, 16-dien-3-ol, (3.beta.,5.alpha.)-	$C_{29}H_{48}O$	412	9.350	23.95
8.	gamma-Sitosterol	$C_{29}H_{50}O$	414	9.552	16.92
9.	1,2-Benzedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	9.744	5.07

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