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ANTIFUNGAL, ANTIBACTERIAL AND ANTIOXIDANT COMPONENTS OF ETHYL ACETATE EXTRACT OF QUINOA STEM

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ABSTRACT

Quinoa (*Chenopodium quinoa* L.) is a recently introduced pseudocereal crop in Pakistan. In the present study, methanolic extract was partitioned with *n*-hexane, chloroform and finally with ethyl acetate. The ethyl acetate fraction was subjected to GC-MS analysis for the identification of various antimicrobial and antioxidant constituents. Thirteen compounds were identified through GC-MS analysis of this fraction. Among these, 11 compounds possessed antibacterial, antifungal and/or antioxidant properties as reported in literature. These include naphthalene; tetradecane; hexadecane; pentadecane; cyclohexadecane; 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester; dibutyl phthalate; 1-nonadecene; 1-octadecanol; 9-hexacosene; and 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester.

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INTRODUCTION

The use of plant products is an old-age practice for the treatment of infectious diseases and pathogens. Interest in plant products has been revived again due to the hazardous effects of chemical products in contrast with natural plant derived products which are safe in use, environment friendly and have low cost. Currently, in Asian and African countries, 80% of the population depends upon folk medicines prepared from traditional phytochemicals isolated from plants being used in healthcare products, medicines, pesticides and fungicides (Pereira et al., 2017). In general, plants are a rich source of secondary metabolites with enormous chemical diversity possessing antifungal, antibacterial, antimicrobial, antioxidant and nematicidal properties (Waltenberger et al., 2018). Some of them are constitutive and exist only in their bioactive forms whereas many others are responded as inactive precursors in response to pathogenic attacks. Well known examples of constitutive phytochemicals are spermidine, coumarins, alkaloids, carotenoids, flavonoids, terpenes, tocopherol, campesterol, kaempferol, sesquiterpenes, quercetin, triterpenoids, polyphenols, glycosides, rutin, phenols, lactones, saponins and glucosinolates (Meela et al., 2019).

Chenopodium quinoa Willd. is a multipurpose agro-industrial pseudo grain native to Andean regions and cultivated thousands of years ago. Because of nutritional characteristics and wide ability to stress tolerance against drought and saline soils, it has justified the cultivation interest not only in Pakistan but worldwide. Many other countries like Europe, China, India, Canada and Columbia are increasing their demand towards quinoa (Gámez et al., 2019). It is a short duration high yielding crop that can also be used as a break crop because of resistance to pathogens. It is rich in minerals,

lysine, amino acid and gluten free making it a more valuable crop than others (Vergara et al., 2019). Quinoa seed coat contains saponins, glycosides and triterpenoids making it bitter in taste. It also possesses diverse bioactive constituents which are antifungal, antibacterial, antioxidant and antimicrobial in nature (Maliro and Njala, 2019). Therefore, the current study was aimed to elucidate the phytoconstituents present in ethyl acetate stem extract of quinoa.

MATERIALS AND METHODS

Quinoa accession Ames 13737, New Mexico USA, was sown in November 2017 for the collection of plant material and uprooted at plant maturity. Stem (2 kg) was washed under tap water in order to remove unwanted soil particles and debris. The fresh cleaned material was then dried under shade and chopped down in order to prepare a homogenized mixture using a mechanical grinder. Methanolic extract of the powder was obtained by maceration in methanol (5 L) for two weeks followed by filtration with Whatman No.1 filter paper. Next, the solvent was evaporated under vacuum at 45°C to obtain concentrated material and stirred in distilled water. After that, the obtained biomass was mixed with 200 mL water and successively partitioned with *n*-hexane, chloroform and finally with ethyl acetate in a separating funnel (Akhtar and Javaid, 2018). The ethyl acetate fraction was subjected to GC-MS analysis for the evaluation of bioactive constituents. GC-MS analysis was conducted using a chromatographic system comprised of a Shimadzu GC-2010plus installed with auto injector AOC-20i, auto sampler AOC-20s and gas chromatograph equipped with a QP2010ultra mass-selective detector (Shimadzu).

RESULTS AND DISCUSSION

GC-MS analysis of ethyl acetate fraction of methanolic stem extract is given in Figure 1 which indicates the presence of 13 constituents belonging to a diverse group of natural volatile organic compounds. Details of identified compounds are presented in Table 1 and structures of these identified compounds are shown in Figure 2. The major prevailing phytochemical constituents were benzene, nitro (2) and 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (13) with peak areas of 28.28% and 12.01%, respectively. The compounds namely naphthalene (3), 1-nonadecene (10), dibutyl phthalate (9), 9-hexacosene (12) and cyclohexadecane (6) showing abundance at peak areas of 9.31, 8.27, 5.16, 4.35 and 4.11%, respectively were recorded as moderate ones. On the other hand, the compounds present in less concentrations were phenol, 3-methyl (1), 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester (8), hexadecane (7), pentadecane (5), tetradecane (4) and 1-octadecanol (11) with peak areas ranging from 1.70 to 2.79%.

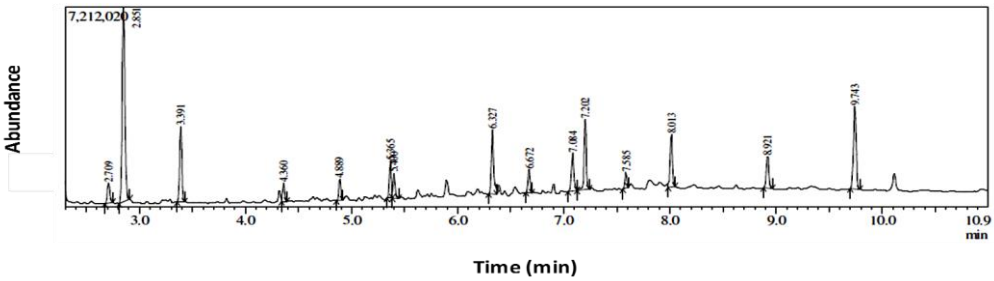

Figure 1. GC-MS chromatograms of ethyl acetate fraction of methanolic stem extract of *Chenopodium quinoa.*

The most abundant compound 13 was previously identified by Deepak et al. (2017) from *n*-hexane extract of *Turbinaria ornata* and is known to possess antimicrobial properties against *Bacillus subtilis*, *Escherichia coli*, *Aspergillus niger*, *Pseudomonas aeruginosa* and *Candida albicans*. This compound is also known for its antiviral, antioxidant, anticancer and anti-inflammatory properties. Likewise, compound 3 was previously isolated from *Zingiber officinale* with a strong antioxidant and inhibitory efficacy against *Klebsiella pneumonia*, *Staphylococcus aureus*, *Proteus mirabilis*, *P. aeruginosa*, *E. coli* and *Proteus mirabilis* (Shareef et al., 2016). Similarly, compounds 10, 11 and 12 were also reported to possess strong antibacterial and antifungal efficacy against human pathogenic bacterial species including *E. coli*, *P. aeruginosa*, *Streptococcus mutans*, *B. circulans* and *S. aureus* as well as fungal pathogens namely *Sclerotinia sclerotiorum*, *Fusarium oxysporum*, *Botrytis cinerea* and *Rhizoctonia solani* (Balachandar et al., 2018; Servi et al., 2019).

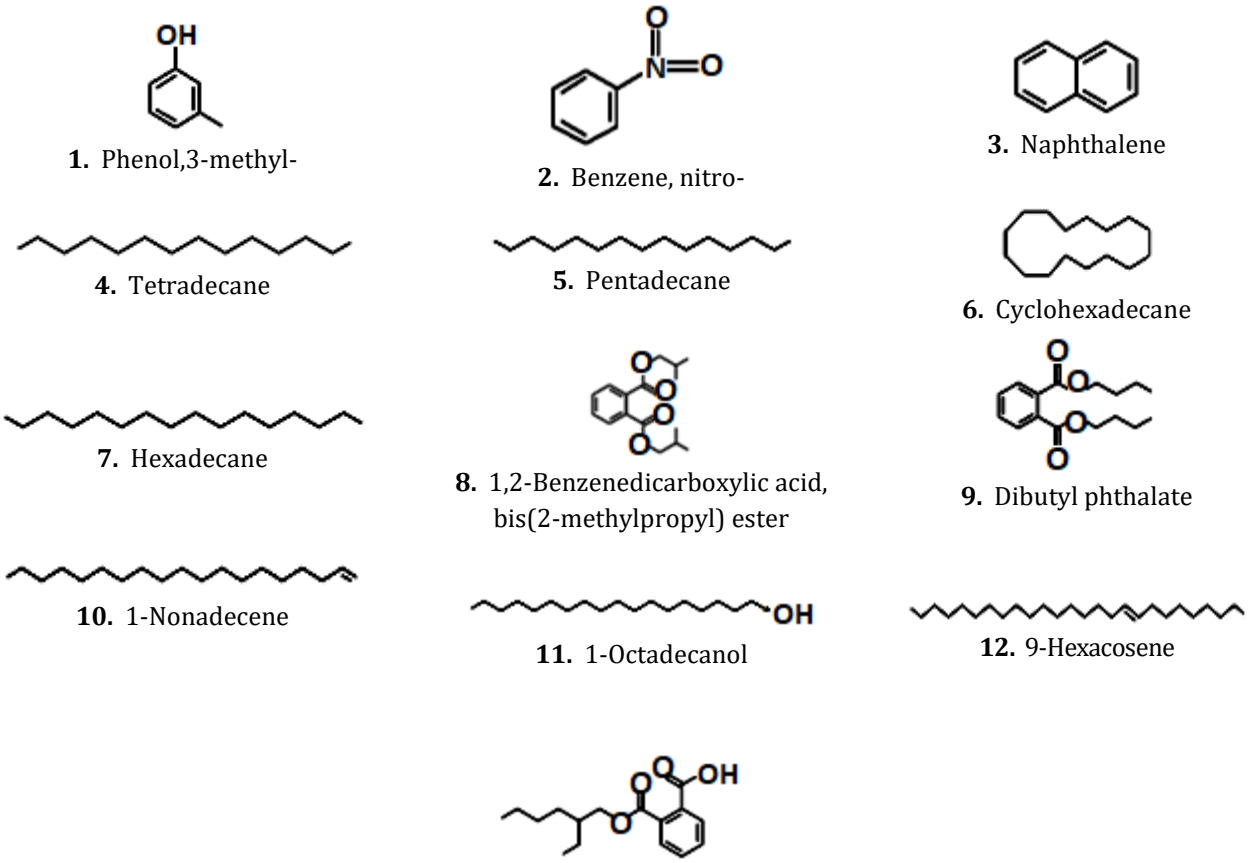

Figure 2. Structures of compounds identified in ethyl acetate fraction of stem extract of *Chenopodium quinoa* through GC-MS.

Table 1. List of compounds in ethyl acetate fraction of methanolic stem extract of *Chenopodium quinoa* identified by GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	Phenol,3-methyl-	C_7H_8O	108	2.709	2.79
2	Benzene, nitro-	$C_6H_5NO_2$	123	2.851	28.28
3	Naphthalene	$C_{10}H_8$	128	3.391	9.31
4	Tetradecane	$C_{14}H_{30}$	198	4.360	1.95
5	Pentadecane	$C_{15}H_{32}$	212	4.889	2.30
6	Cyclohexadecane	$C_{16}H_{32}$	224	5.365	4.11
7	Hexadecane	$C_{16}H_{34}$	226	5.400	2.52
8	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	278	6.672	2.63
9	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	7.084	5.16
10	1-Nonadecene	$C_{19}H_{38}$	266	7.202	8.27
11	1-Octadecanol	$C_{18}H_{38}O$	270	7.585	1.70
12	9-Hexacosene	$C_{26}H_{52}$	364	8.921	4.35
13	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	$C_{16}H_{22}O_4$	278	9.743	12.01

Table 2. Bioactivity of compounds in ethyl acetate fraction of methanolic stem extract of *Chenopodium quinoa*.

Sr. No.	Names of compounds	Known bioactivity	Reference
1	Phenol,3-methyl-	No activity reported	-
2	Benzene, nitro-	No activity reported	-
3	Naphthalene	Antioxidant, antibacterial,	Shareef et al. (2016), Matsushita et al. (2011)
4	Tetradecane	Antioxidant, antifungal	Karpagasundari and Kulothungan (2014), Mends et al. (2012)
5	Pentadecane	Antioxidant, antimicrobial	Gnanasundaram and Balakrishnan (2017), Girija et al. (2014)
6	Cyclohexadecane	Insecticidal, antioxidant, anticancer	Habib and Karim (2016), Kumar et al. (2015)
7	Hexadecane	Antimicrobial, antioxidant, anti-inflammatory	Molehin et al. (2017), Das et al. (2016) Konovalova et al. (2013)
8	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	Antioxidant, antibacterial, antifungal	Hifnawy et al. (2016), Al-Youssef and Hassan (2015)
9	Dibutyl phthalate	Antimicrobial	Alagammal et al. (2012), Maruthupandian and Mohan (2011)
10	1-Nonadecene	Antibacterial, antifungal	Balachandar et al. (2018), Chowdhary and Kaushik (2018), Wagay et al. (2017)
11	1-Octadecanol	Antimicrobial,	Servi et al. (2019), Chatterjee et al. (2018)
12	9-Hexacosene	Antibacterial	Matloub et al. (2018), Gherraf et al. (2017)
13	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester	Antifungal, antiviral, antibacterial, antioxidant, anticancer, anti-inflammatory	Deepak et al. (2017), Jinfeng et al. (2017), Ahmed et al. (2015)

Previously, compounds 9 and 6 have been reported from the ethanolic extracts of *Polygala javana* and *Calotropis gigantea* plants with strong antimicrobial and insecticidal activities against *Tribolium castaneum* pest of stored wheat grains (Alagammal et al., 2012; Habib and Karim, 2016). Likewise, antimicrobial efficacy of compounds 7 and 8 was reported against *Aspergillus niger*, *A. fumigatus*, *Candida albicans*, *E. coli*, *P. aeruginosa* and *S. aureus* (Al-Youssef and Hassan, 2015; Das et al., 2016). Gnanasundaram and Balakrishnan (2017) isolated the compound 5 from the ethanolic leaf extract of *Cissus vitiginea* and reported the antioxidant efficacy along with strong antibacterial potential against *S. aureus*, *K. pneumoniae* and *E. coli* (Girija et al., 2014). Likewise, the antioxidant properties of compound 4 was identified previously and was also found to be effective against pathogenic fungi namely *A. flavus* and *R. solani* with promising results (Karpagasundari and Kulothungan, 2014; Mends et al., 2012). This study concludes that ethyl acetate fraction of quinoa stem extract is very rich in bioactive compounds especially possessing antimicrobial and antioxidant properties.

Authors' contribution: IHK and AJ conceived and designed the study; IHK conducted the research work and wrote the initial draft; AJ supervised the work, reviewed and edited the manuscript.

Conflict of Interest: The authors declare no conflict of interest.

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