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Anticancer, antimicrobial and antioxidant compounds of quinoa inflorescence

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Abstract

Background: *Chenopodium quinoa* is a newly introduced drought resistant crop in Pakistan. Studies regarding the efficacy of bioactive compounds present in this plant are scarce. Therefore, the current investigation was carried out to identify the antimicrobial, antioxidant and anticancer compounds present in ethyl acetate fraction of methanolic extract of inflorescence of *C. quinoa*.

Methods: Dry powdered inflorescence of the test plant was macerated with methanol and partitioned through different organic solvents on the basis of increase in polarities beginning with *n*-hexane followed by chloroform and ethyl acetate. GC-MS analysis was performed for the identification of bioactive constituents present in ethyl acetate fraction.

Results: The GC-MS analysis revealed the presence of 15 different phytochemicals. Among these, 1,2-benzedicarboxylic acid, diisooctyl ester (15); 9,12-octadecadienoic_acid-(Z,Z) (13); 8,11-octadecadienoic_acid, methyl ester (12); hexacosanoic acid, methyl-ester (11); hexadecenoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester (14); *n*-hexadecanoic acid (10); hexadecenoic-acid, methyl ester (8); 2-propenoic acid,3-[4-(acetyloxy)-3-methoxyphenyl]-, methyl ester (7); 1,6,10,14,18,22-tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl-,(all-E)-(9) and undecane (1) were present in moderate to abundant concentrations. Biological activities of the identified compounds were searched in the previous literature.

Conclusion: The present study concluded that ethyl acetate fraction of methanolic extract of the inflorescence of *C. quinoa* contains a diverse range of potent bioactive constituents with antimicrobial, antifungal, antibacterial, cancer preventive, anti-inflammatory and cytotoxic properties.

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Introduction

Plants are considered as a natural source of certain bioactive molecules that are used as raw material for pharmaceutical, medicine and agrochemical industry [1]. The use of herbal medicines to cure infectious diseases is an old age practice [2]. Numerous studies have shown antimicrobial and antioxidant potential [3, 4]. Synthetic antimicrobial drugs have toxic side effects on the host cells, which make grounds for the search and development of novel antibiotics isolated from plant origin [5]. Plant products are considered as a potential source for the discovery of organic compounds with beneficial medicinal effects [6]. These compounds are mostly secondary metabolites such as flavonoids, ketones, alcohols, steroids, tannins, alkaloids, terpenes, diterpenes, sesquiterpene lactones, triterpenes, phytoestrogens, carotenoids, curcumin and curcuminoids with the ability to produce definite physiological effect on hosts [7]. World Health Organization (WHO) is also working to screen antimicrobial agents from medicinal plants for the exploitation of traditional medicine system [8].

Chenopodium quinoa Willd., family Amaranthaceae (previously Chenopodiaceae), is native to the South America and is being used as a food crop for more than 5000 years [9]. It is known as a pseudo-cereal recently introduced in Asia, Southeast Asia, Australia and European countries. It is an annual herbaceous drought, cold resistant plant easily grown on acidic soils [10]. It is a rich source of amino acids, carbohydrates, fibers, proteins, vitamins, iron, calcium, minerals and magnesium [11]. It is also low in fat contents and naturally gluten free plant, making it a wonderful choice for the people [12]. In addition, quinoa seeds contain essential phenolics, saponins, flavonol glycosides, betaines, tannins, triterpene, ecdysteroids, and terpenoids that are rich in anti-inflammatory and antimicrobial properties [13]. Knowledge of the biological activities of phytochemical compounds present in quinoa inflorescence is limited. Therefore, the present study was carried out to analyze ethyl acetate fraction of methanolic inflorescence extract of *C. quinoa* through GC-MS to search compounds with antimicrobial, antioxidant and anticancer properties.

Methods

Seeds of quinoa var. 2WANT (origin was New Mexico, USA) were obtained from Prof. Dr. Shahzad Ahmed Basra, Department of Agronomy, University of Agriculture, Faisalabad. Seeds were sown in the field at Experimental Station, Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan during quinoa growing season of 2017-18. Inflorescence was collected from mature quinoa plants before drying. Quinoa inflorescence (10 g) was washed carefully under running tap water for the removal of associated debris. The material was shade dried first and then completely dried at 40 °C in an electric oven. The obtained dried biomass was passed through a mechanical grinder to pulverize it into a fine powder. Next, the powder was dipped in methanol (200 mL) for two weeks at room temperature followed by a filtration process using double layer of Whatman No. 1 filter paper and the filtrates were evaporated on a rotary evaporator at 45 °C to obtain a concentrated crude methanolic extract. The traces of methanol were evaporated by putting the crude extract in an electric oven at 45 ºC. The obtained extract was then mixed well in autoclaved distilled water (50 mL) and partitioned with *n*-hexane (5 × 100 mL) using a separating funnel. The remaining aqueous material was then extracted with chloroform (100 mL) followed by ethyl acetate (100 mL) [14].

Thereafter, ethyl acetate fraction was subjected to analysis of different volatile organic compounds using GC-MS. Analysis was performed by using a Shimadzu GC-2010 plus system coupled with an auto injector AOC-20i, an auto sampler AOC-20s and a gas chromatograph. Helium was used as a carrier gas, sample volume 1.0 µL was injected through setting injector at a temperature of 250 °C and interface temperature was calibrated at 320 °C. After injection of sample, the initial column temperature was 100 °C for 60 s that was enhanced from 100 to 200 °C at 20 °C min^{-1} and hold for 2.0 min, finally from 200 °C to 300 °C at 40 °C min^{-1}. The total run time was 10.9 min [15].

A thorough literature survey was conducted for the evaluation of possible bioactive constituents.

Results

GC-MS analysis was performed to identify possible bioactive phytoconstituents present in the ethyl acetate fraction of methanolic extract of inflorescence of quinoa plant. GC-MS chromatogram indicates the presence of 15 major peaks (Figure 1).

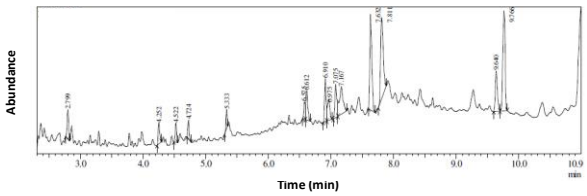

Figure 1: GC-MS chromatogram of ethyl acetate fraction of methanolic extract of quinoa inflorescence.

Details of the retention times, peak area percentages and molecular weights of the isolated compounds are provided in Table 1 whereas their structures are illustrated in Figure 2. The most abundant compounds were 1,2-benzedicarboxylic acid, diisooctyl ester (15); 9,12- octadecadienoic acid (Z,Z) (13) and 8,11-octadecadienoic acid, methyl ester (12) with peak areas of 18.62%, 17.93% and 15.68%, respectively. The compounds namely hexacosanoic acid, methyl ester (11); hexadecenoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester (14); *n*-hexadecanoic acid (10); hexadecanoic acid, methyl ester (8); 2-propenoic acid, 3-[4-(acetyloxy)-3-methoxyphenyl]-, methyl ester (7); 1,6,10,14,18,22-tetracosahexaen-3-ol, 2,6,10,15,19,23-hexamethyl-,(all-E)- (9) and undecane (1) were present in moderate concentrations with 7.92%, 7.18%, 5.41%, 5.21%, 4.26%, 3.94% and 3.28% peak areas, respectively. On the other hand, the least

Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
Undecane	$C_{11}H_{24}$	156	2.799	3.28
Benzaldehyde,4-hydroxy-	$C_7H_6O_2$	122	4.252	2.76
Homovanillyl alcohol	$C_9H_{12}O_3$	168	4.522	1.99
Methylparaben	$C_8H_8O_3$	152	4.724	1.84
Nonanedioic acid, monomethyl ester	$C_{10}H_{18}O_4$	202	5.333	1.80
1H-Indole-3-carboxaldehyde	C_9H_7NO	145	6.575	2.19
2-Propenoic acid,3-[4-(acetyloxy)-3-methoxyphenyl]-,methyl ester	$C_{13}H_{14}O_5$	250	6.612	4.26
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	6.910	5.21
1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl-,(all-E)-	$C_{30}H_{50}O$	426	6.975	3.94
n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	7.075	5.41
Hexacosanoic acid, methyl ester	$C_{27}H_{54}O_2$	410	7.167	7.92
8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	7.632	15.68
9,12-Octadecadienoic acid (Z,Z)	$C_{18}H_{32}O_2$	280	7.811	17.93
Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O4$	330	9.640	7.18
1,2-Benzedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	9.766	18.62

Table 1: Compounds identified in ethyl acetate fraction of methanolic extract of quinoa inflorescence through GC-MS analysis.

Compound No.	Names of compounds	Bioactivity	Reference
1	Undecane	Antifungal, antibacterial and antimicrobial	[22]
2	Benzaldehyde, 4-hydroxy-	Antifungal, antibacterial, anti-tyrosinase, pharmaceutical, anti-cancer, medicinal and anti-acetylcholisterenase	[24,26]
3	Homovanillyl alcohol	Antioxidant and anti-inflammatory	[31]
4	Methylparaben	Antioxidant	[32]
5	Nonanedioic acid, monomethyl ester	Antibacterial	[33]
6	1H-Indole-3-carboxaldehyde	Antimicrobial, antifungal, antibacterial, anti-inflammatory, anticancer and anti-plasmodial	[23,25]
7	2-Propenoic acid,3-[4-(acetyloxy)-3-methoxyphenyl]-,methyl ester	No activity reported	
8	Hexadecanoic acid, methyl ester	Antibacterial	[27]
9	1,6,10,14,18,22-Tetracosahexaen-3-ol,2,6,10,15,19,23-hexamethyl-,(all-E)-	Antimicrobial, antiarthritic, anti-inflammatory, cytotoxic, insecticidal and chemo-preventive	[30]
10	*n*-Hexadecanoic acid	Pesticide, bactericide, nematicide, antioxidant, antimicrobial, anti-inflammatory, antiandrogenic and cancer preventive	[29]
11	Hexacosanoic acid, methyl ester	Antibacterial and antioxidant	[34]
12	8,11-Octadecadienoic acid, methyl ester	Antibacterial, antifungal, antioxidant, cancer-preventive	[19-21]
13	9,12-Octadecadienoic acid (Z,Z)	Antibacterial, antifungal, nematicide, anti-inflammatory, anti-coronary, hepatoprotective, antieczemic, insecticide, antiandrogenic antiarthritic, antioxidant, anti-depressant, cancer preventive, anti-histaminic and anti-arthritic	[18]
14	Hexadecanoic acid,2-hydroxy-1-(hydroxymethyl) ethyl ester	Antibacterial, antifungal, anthelmintic, anti-inflammatory and antioxidant	[28]
15	1,2-Benzedicarboxylic acid, diisooctyl ester	Antifungal, antibacterial, antimicrobial, antioxidant and antifouling	[16-17]

Table 2: Bioactivity of components of ethyl acetate fraction of methanolic extract of quinoa inflorescence.

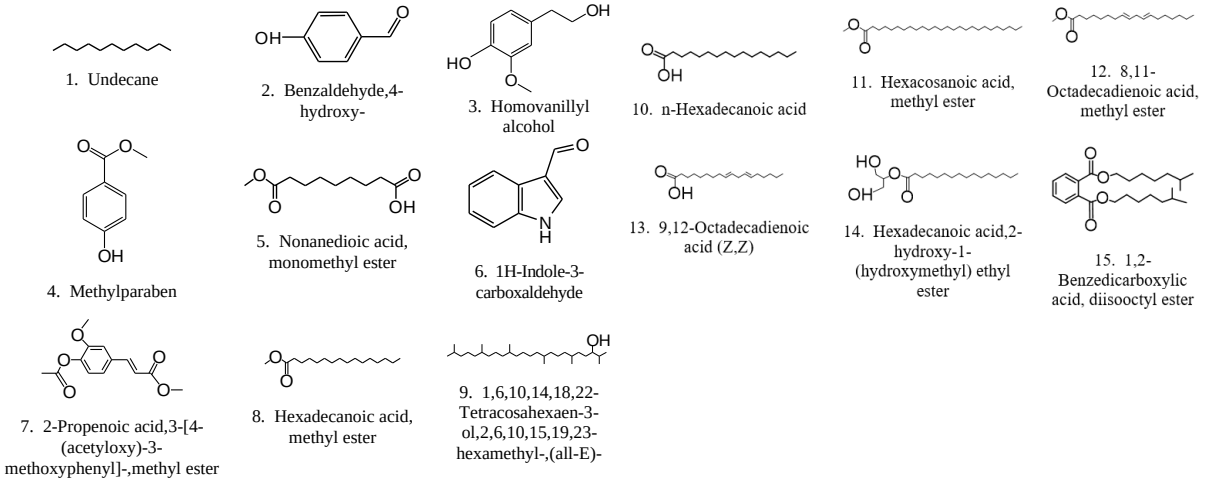

Figure 2: Structures of components of ethyl acetate fraction of methanolic extract of quinoa inflorescence.

abundant compounds were benzaldehyde, 4-hydroxy- (2); 1h-indole-3-carboxaldehyde (6); homovanillyl alcohol (3); methylparaben (4) and nonanedioic acid, monomethyl ester (5) with peak areas ranging from 1.80 to 2.76%.

Discussion

Among identified phytoconstituents, compound 15 was previously isolated from a medicinal plant *Artemisia princeps* leaves with an excellent antimicrobial efficacy against the pathogenic microbes including *Candida albicans*, *Bacillus subtilis*, *Staphylococcus epidermis*, *S. aureus* and *Aspergillus niger* [16]. It has also been reported from a medicinal plant *Saccharum spontaneum* with potent antimicrobial properties [17]. Similarly, compound 13 also known as linoleic acid has been previously identified from the leaf, root and stem extract of *Cenchrus biflorus* with potent antibacterial, antifungal, nematicide, anti-inflammatory, anti-coronary, hepatoprotective, antieczemic, insecticide, antiandrogenic, antiarthritic, antioxidant, anti-depressant, cancer preventive, anti-histaminic and anti-arthritic properties [18]. In previous studies, compound 12 was isolated from methanolic extracts of *Melastoma beccarianum* and *M. malabathricum*. The compound was investigated to evaluate its antibacterial activity against *Bacillus anthracis,* the pathogenic bacterial strain responsible for infections in humans [19,20]. Moreover, it was also tested against phytopathogenic fungal strains such as *Penicillium digitatum* and *Aspergillus niger* and showed profound antifungal activity against these fungi [21]. Likewise, compound 1 was previously isolated from *Zingiber officinale* and tested against a number of fungal and bacterial species namely *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Penicillium* spp., *A. niger*, *Candida albicans* and *Saccharomyces cerevisiae* by disc diffusion method. It was noted that the compound had marked inhibitory activity towards *C. albicans*, *Penicillium* spp. and *P. aeruginosa* [22]. Similarly, compound 2 and 6 were tested against *B. subtilis*, *S. aureus*, *A. niger*, *A. fumigatus*, *A. clavatus*, *S. pyogenes* and *C. albicans*. The compound showed an excellent potential against all the tested microbes but the results were more promising in reducing the population of *C. albicans*, *B. anthracis* and *A. fumigatus* in comparison to the reference drugs namely amphotericin B, gentamicin and ampicillin [23,24]. Both the compounds also possessed strong medicinal, pharmaceutical, anti-plasmodial, antimicrobial, antifungal, antibacterial, anti-tryosinase, anti-cancer and anti-acetylcholisterenase properties [25,26]. Compounds 8, 9, 10 and 14 are well known due to their remarkable anti-inflammatory, cytotoxic, antibacterial, insecticidal, antifungal, anthelmintic, cancer preventive, antimicrobial and antioxidant activities [27-30]. Similarly, compound 3, 4, 5 and 11 were reported to possess strong antioxidant, anti-inflammatory and antibacterial properties [31-34]. This study concludes that ethyl acetate fraction is a potent source of antimicrobial, antioxidant and medicinally important compounds.

Conflict of Interest Statement

The author declares that there is no conflict of interest regarding the publication of this paper.

Author Contributions

Iqra Haider Khan did experimental work and wrote a part of paper. Arshad Javaid supervised the research and contributed in paper writing.

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