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IDENTIFICATION OF VOLATILE CONSTITUENTS OF ETHYL ACETATE FRACTION OF *CHENOPODIUM QUINOA* ROOTS EXTRACT BY GC-MS

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ABSTRACT

The aim of the present study was to identify bioactive constituents of the ethyl acetate fraction of methanolic root extract of quinoa (*Chenopodium quinoa* Willd.) through GC-MS analysis. For this purpose, crushed roots of the plant were extracted with methanol for two weeks. The extract was concentrated by evaporating the solvent on a rotary evaporator followed by addition of distilled water and subsequent fractionation using *n*-hexane, chloroform and ethyl acetate. The ethyl acetate fraction was subjected to GC-MS analysis and nine compounds were identified, including undecane (**1**), benzene, nitro- (**2**), -ascorbic acid 2,6-dihexadecanoate (**3**), octadec-9-enoic acid (**4**), benzene,1,1'-(oxydi-2,1-ethanediyl) bis 3-ethyl- (**5**), cis-9-hexadecenal (**6**), 1,2-benzedicarboxylic acid, diisooctyl ester (**7**), 10-nonadecanone (**8**) and 1-triacontanol (**9**). Literature survey revealed that most of the identified compounds possess diverse biological activities. Compounds **1**, **6** and **7** are reported to possess antimicrobial activity. Compounds **3** and **6** are antioxidants. Compounds **3**, **4**, **7**, **8** and **9** possess diverse medicinal properties including anti-inflammatory, anti-infertility, cancer and cardio protective, anti-mutagenic, and/or wound healing, antifouling. The present study concludes that ethyl acetate fraction of root extract is a good store house of bioactive constituents.

Keywords: Bioactive components, *Chenopodium quinoa*, ethyl acetate fraction, GC-MS analysis.

INTRODUCTION

In recent decades, plants are being used as source of folk medicine for the treatment and prevention of various diseases because of infrequent side effects and significant results. Plant derived medicines are a cheap source of novel compounds and are in practice for the prevention and procurement of human, animal and plant diseases. Worldwide, many countries have dependency on plant-based products as a source of potent drugs for the remedies of traditional ailment (Malik *et al*., 2018). Chemicals directly extracted from plants are referred to as phytochemicals formed during plant normal metabolic process, are organic in nature gaining much importance because of their incredible benefits. So far, phytochemicals have been implemented in agriculture to combat the problems caused by synthetic herbicides, fungicides, virucides, nematicides and pesticides. Alternative strategies of prime means are now emerging from bioactive compounds isolated from plants to limit the specific pathogens with no toxic effects on beneficial microbes, less environment retention and to rapidly degrade (Mitrani *et al*., 2018).

Chenopodium quinoa Willd., a nutrition rich pseudo cereal crop belongs to family Chenopodiaceae, is widely cultivated in Andean highlands of South and North America. This crop is recently introduced in Pakistan because of its high commercial value and increasing demand all around the world. It is grown as a staple food with extreme tolerance to abiotic stresses, less water and fertilizers demand. All parts of the plant are utilized but the seeds are outstanding because of their protein contents (10-16%), lipids (6-10%), free sugars (9-16%), and minerals (0.6-1%) (Maughan *et al*., 2019). It is a rich source of flavonol glycosides and saponins which are found to be pharmaceutically and biochemically highly interesting compounds, potentially beneficial for human health. It has many biological activities including its toxic effects against many fungal and viral diseases, increase of mucosal drug absorption and lowering cholesterol effects (Jarvis *et al.*, 2017). Therefore, the present study was carried out to investigate the bioactive potential of ethyl acetate fraction of methanolic root extract of *Chenopodium quinoa* using GC-MS analysis.

MATERIALS AND METHODS

Chenopodium quinoa was grown at University of the Punjab Lahore and its roots were obtained at maturity. The roots (2 kg) were washed thoroughly with water to remove adhered soil particles. The cleaned material was shade dried and coarsely powdered using a mechanical grinder and extracted with methanol (6 L) for 14 days at room temperature with occasional shaking. The extract was filtered through Whatman No. 41 filter paper and concentrated in a rotary evaporator at 45 °C to get dry residue (98 g) which was suspended in 200 mL distilled water and successively partitioned with *n*-hexane (4 × 500), chloroform (400 mL) and ethyl acetate (400 mL) in a separating funnel. Later on, ethyl acetate fraction was evaporated and as a result 3.9 g gummy biomass was obtained. It was subjected to GC-MS analysis to identify volatile compounds (Iftikhar *et al.*, 2019). A thorough literature survey was conducted to explore various bioactivities of the identified compounds.

RESULTS AND DISCUSSION

Gas chromatography- mass spectroscopy (GC-MS) is one of the best techniques to identify the constituents of branched chain hydrocarbons, volatile matter, esters and alcoholic acids (Iftikhar *et al*., 2019). The analysis of ethyl acetate fraction of methanolic root extract of quinoa revealed the existence of 9 major and minor constituents belonging to diverse groups of natural compounds (Table 1). The GC-MS chromatogram of the compounds is shown in Fig. 1 and the structures of corresponding components to the peaks are given in Fig. 2. The major prevailing compounds were detected as 1,2-benzedicarboxylic acid, diisooctyl ester (**7**), octadec-9-enoic acid (**4**) and 10-nonadecanone (**8**) with peak areas of 20.48, 19.79 and 18.88%, respectively. The compounds namely benzene,1,1'-(oxydi-2,1-ethanediyl) bis 3-ethyl- (**5**) and 1-triacontanol (**9**) representing peak areas of 11.38% and 7.86% were recorded as moderately abundant ones. Compounds present in less concentrations were ascorbic acid 2,6-dihexadecanoate (**3**), nitro-benzene, (**2**), *cis*-9-hexadecenal (**6**) and undecane (**1**) with peak areas ranging from 1.52 to 4.26%.

Table 1. Compounds identified in ethyl acetate fraction of methanolic extract of the roots of *Chenopodium quinoa* through GC-MS.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	Undecane	$C_{11}H_{24}$	156	2.795	1.52
2	Nitro-benzene	$C_6H_5NO_2$	123	2.857	4.17
3	Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	7.067	4.26
4	Octadec-9-enoic acid	$C_{18}H_{34}O_2$	282	7.815	19.79
5	Benzene,1,1'-(oxydi-2,1-ethanediyl) bis 3-ethyl-	$C_{20}H_{26}O$	282	8.252	11.38
6	Cis-9-hexadecenal	$C_{16}H_{30}O$	238	8.620	1.89
7	1,2-Benzedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	9.753	20.48
8	10-Nonadecanone	$C_{19}H_{38}O$	282	9.992	18.88
9	1-Triacontanol	$C_{30}H_{62}O$	438	10.357	7.86

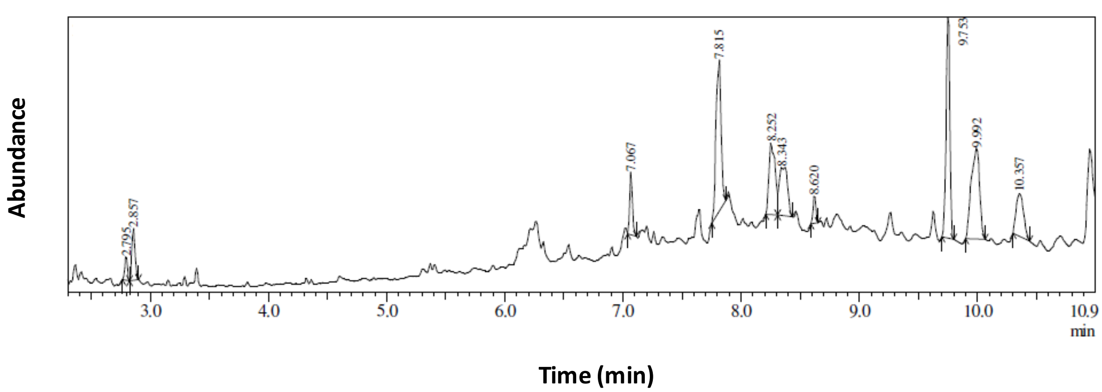


Fig. 1. GC-MS chromatogram of ethyl acetate fraction of methanolic extract of quinoa roots.

Table 2. Bioactivity of components of ethyl acetate fraction of methanolic extract of *C. quinoa* roots.

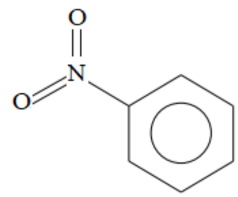
Compound No.	**Names of compounds**	**Bioactivity**	**Reference**
1	Undecane	Antifungal, antibacterial	Jeong-Ho *et al.,* (2008) Sasidharan and Menon (2010) Moya *et al.,* (2018) Okla *et al.,* (2019)
2	Benzene, nitro-	No activity reported	
3	Ascorbic acid 2,6-dihexadecanoate	Antioxidant, cancer and cardio protective, anti-infertility, vitamin C, immunomodulator, anti-scorbutic, anti-inflammatory, anti-nociceptive, anti-mutagenic, wound healing property	Sosa *et al.,* (2016) Kadam and Lele (2017) Anburaj *et al.,* (2016) Rajashyamala and Elango (2015)
4	Octadec-9-enoic acid	Cancer preventive, cardio protective, reduces blood level of cholesterol, hypotensive, atherosclerosis	Dubal *et al*., (2013)
5	Benzene,1,1’-(oxydi-2,1-ethanediyl) bis 3-ethyl-	No activity reported	
6	*Cis*-9-hexadecenal	Antimicrobial, antioxidant	Arora and Meena (2017) Qadir *et al.* (2018) Kumar and Kumar (2016)
7	1,2-Benzedicarboxylic acid, diisooctyl ester	Antimicrobial, antifouling	Deepa and Boominathan (2018) Kalaivani *et al.* (2012) Lakshmi and Rajalakshmi (2011) Balasundari and Boominathan (2018) Sermakkani and Thangapandian (2012) Devi and Muthu (2014)
8	10-Nonadecanone	Antimicrobial, medicinal properties	Kumar *et al.* (2015) Agarwal *et al.* (2018)
9	1-Triacontanol	Plant growth stimulator, anti-inflammatory, antiviral, medicinal properties	Mendez *et al.* (2003) Riedo *et al.* (2011) Verma and Batra (2013)

The most abundant compound **7** is a plasticizer in nature known to possess antimicrobial and antifouling properties and has been previously isolated from leaves of *Andrographis paniculata* (Kalaivani *et al.,* 2012); *Aloe vera* (Lakshmi and Rajalakshmi, 2011); *Cynodon dactylon* (Balasundari and Boominathan, 2018); *Cassia italica* (Sermakkani and Thangapandian, 2012) and whole plant ethanolic extract of *Saccharum spontaneum* (Devi and Muthu, 2014).

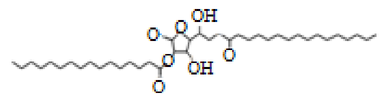
Compound **4** previously isolated from *Tectaria coadunata* (Dubal *et al*., 2013); compound **8** identified in endophytic bacteria *Bacillus cereus* (Kumar *et al.,* 2015); and compound **1** earlier obtained from the cones of *Pinus koraiensis* are known to have significant antibacterial and antifungal activities against pathogenic gram positive and gram negative bacteria, fungal strains such as *Aspergillus niger, Cryptococcus neoformans* and *Candida glabrata* (Jeong-Ho *et al*., 2008). Compounds **9** and **6** are fatty acids in nature and were previously isolated from the leaves of *Stevia rebaudiana* (Verma and Batra, 2013) and *Avicennia marina* (Kumar and Kumar, 2016), respectively. Both have potent antimicrobial, antioxidant and a number of medicinal properties, and have been used widely in pharmaceuticals in ancient times (Qadir *et al.,* 2018). Likewise, compound **3** belongs to a class of vitamins previously identified in methanolic seed extracts of *Euphorbia lathyrus* (Sosa *et al*., 2016), *Nigella sativa* (Kadam and Lele, 2017), *Tecoma stans* (Anburaj *et al.,* 2016) and *Evolvulus alsinoides* and found to be antioxidant in nature

(Rajashyamala and Elango, 2015). The present study concludes that ethyl acetate fraction of methanolic root extract is a rich source of potent bioactive substances.

1. Undecane

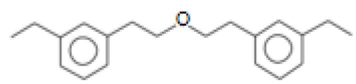


2. Nitro-benzene

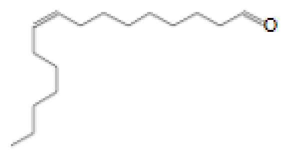


3. 1-(+)-Ascorbic acid 2,6-dihexadecanoate

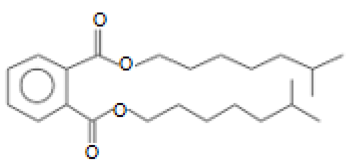
4. Octadec-9-enoic acid



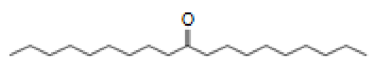
5. Benzene,1,1'-(oxydi-2,1-ethanediyl) bis 3-ethyl-



6. *Cis*-9-hexadecenal



7. 1,2-Benzedicarboxylic acid, diisooctyl ester



8. 10-Nonadecanone

9. 1-Triacontanol

Fig. 2. Structures of components of ethyl acetate fraction of methanolic extract of *Chenopodium quinoa* roots.

REFERENCES

Agarwal, D., S.N. Saxena, L.K. Sharma and G. Lal (2018). Prevalence of essential and fatty oil constituents in fennel (*Foeniculum vulgare* Mill) genotypes grown in semi-arid regions of India. *Journal of Essential Oil-bearing Plants,* 21: 40-51.

Anburaj, G., M. Marimuthu, V. Rajasudha and R. Manikandan (2016). Phytochemical screening and GC-MS analysis of ethanolic extract of *Tecoma stans* (family: Bignoniaceae) yellow bell flowers. *Journal of Pharmacognosy and Phytochemistry,* 5: 172-175.

Arora, S. and S. Meena (2017). GC-MS profiling of *Ceropegia bulbosa* Roxb. var. *bulbosa*, an endangered plant from thar desert Rajasthan. *The Pharma Innovation Journal*, 6: 568-573.

Balasundari, T. and M. Boominathan (2018). Screening of bioactive compounds by GC-MS, antimicrobial activity and *In silico* studies in *Cynodon dactylon* L. Pers leaves. *World Journal of Science and Research,* 3: 7-15.

Deepa, S. and M. Boominathan (2018). *In silico* determination and biological evaluation of methanol extract of *Zingiber officinale*. *Asian Journal of Innovative Research,* 3: 27-33.

Devi, J.A.I. and A.K. Muthu (2014). Gass chromatography-mass spectrometry analysis of bioactive constituents in the ethanolic extract of *Saccharum spontaneum* Linn. *International Journal of Pharmacy and Pharmaceutical Sciences*, 6: 755-759.

Dubal, K.N., P.N. Ghorpade and M.V. Kale (2013). Studies on bioactive compounds of *Tectaria coadunata* Wall. Ex Hook. & Grev. *Asian Journal of Pharmaceutical and Clinical Research*, 6: 186-187.

Iftikhar, H., D. Ahmed and M.T. Qamar (2019). Study of phytochemicals of *Melilotus indicus* and alpha-amylase and lipase inhibitory activities of its methanolic extract and fractions in different solvents. *Chemistry Select*, 4: 7679-7685.

Jarvis, D.E., Y.S. Ho, D.J. Lightfoot, S.M. Schmockel, B. Li, T.J. Borm, H. Ohyanagi, K. Mineta, C.T. Michell, N. Saber and N.M. Kharbatia (2017). The genome of *Chenopodium quinoa*. *Nature*, 542: 307.

Jeong-Ho, L., H. Yang, H. Lee and S. Hong (2008). Chemical composition and antimicrobial activity of essential oil from cones of *Pinus koraiensis*. *Journal of Microbiology and Biotechnology,* 18: 497-502.

Kadam, D. and S.S. Lele (2017). Extraction, characterization and bioactive properties of *Nigella sativa* seed cake. *Journal of Food Science and Technology,* 54: 3936-3947.

Kalaivani, C.S., S.S. Sathish, N. Janakiraman and M. Johnson (2012). GC-MS studies on *Andrographis paniculata* (Burm.f.) Wall. ex Nees-A medicinally important plant. *International Journal of Medicinal and Aromatic Plants,* 2: 69-74.

Kumar, A.G., R.A. Antony and V.R. Kannan (2015). Exploration of endophytic microorganisms from selected medicinal plants and their control potential to multi drug resistant pathogens. *Journal of Medicinal Plants Studies*, 3: 49-57.

Kumar, D.G. and R.R. Kumar (2016). Gas chromatography mass spectrometry analysis of bioactive components from the ethanol extract of *Avicennia marina* leaves. *Innovare Journal of Sciences*, 4: 9-12.

Lakshmi, P.T.V. and P. Rajalakshmi (2011). Identification of phyto-components and its biological activities of *Aloe vera* through the gass chromatography-mass spectrometry. *International Research Journal of Pharmacy,* 2: 247-249.

Malik, K., M. Ahmad, G. Zhang, N. Rashid, M. Zafar, S. Sultana and S.N. Shah (2018). Traditional plant based medicines used to treat musculoskeletal disorders in Northern Pakistan. *European Journal of Integrative Medicine*, 19: 17-64.

Maughan, P.J., L. Chaney, D.J. Lightfoot, B.J. Cox, M. Tester, E.N. Jellen and D.E. Jarvis (2019). Mitochondrial and chloroplast genomes provide insights into the evolutionary origins of quinoa (*Chenopodium quinoa* Willd.). *Scientific Reports*, 9: 185.

Mendez, E., M. Blanco, A. Laguna and E. Garcia (2003). Isolation and characterization of a mixture of higher primary aliphatic alcohols of high molecular weight from henequen (*Agave furcroydes* L.) wax. *Ciencias Quimicas,* 34: 35-38.

Mitrani, E., E. Perdum, O.G. Iordache and I. Dumitrescu (2018). Advantages and disadvantages of pesticide analysis methods used in agricultural samples. *Scientific Papers-Series B-Horticulture*, 62: 709-714.

Moya, P., J.R. Girotti, A.V. Toledo and M.N. Sisterna (2018). Antifungal activity of *Trichoderma* VOCs against *Pyrenophora teres*, the causal agaent of barley net blotch. *Journal of Plant Protection Research,* 58: 45-53.

Okla, M.K., S.A. Alamri, M.Z.M. Salem, H.M. Ali, S.I. Behiry, R.A. Nasser, I.A. Alaraidh, S.M. Al-Ghtani and W. Soufan (2019). Yeild, phytochemical constituents, and antibacterial activity of essential oils from the leaves/twigs, branches, branch wood, and branch bark of sour orange (*Citrus aurantium* L.). *Processes,* 7: 362-376.

Qadir, A., A. Ali, M. Arif, A.H. Al-Rohaimi, S.P. Singh, U. Ahmad, M. Khalid and A. Kumar (2018). Solvent extraction and GC-MS analysis of sesame seeds for determination of bioactive antioxidant fatty acid/fatty oil components. *Drug Resistance,* 68: 344-348.

Rajashyamala, G. and V. Elango (2015). Identification of bioactive components and its biological activities of *Evolvulus alsinoides* linn. A GC-MS study. *International Journal of Chemical Studies*, 3: 41-44.

Riedo, C., D. Scalarone and O. Chiantore (2011). Pyrolysis-GC/MS for the identification of macromolecular components in historical recepies. *Analytical and Bioanalytical Chemistry*, 401: 1761-1769.

Sasidharan, I. and A.N. Menon (2010). Comparative chemical composition and antimicrobial activity fresh and dry ginger oils (*Zingiber officinale* Roscoe). *International Journal of Current Pharmaceutical Research*, 2: 39-43.

Sermakkani, M. and V. Thangapandian (2012). GC-MS analysis of *Cassia italica* leaf methanol extract. *Asian Journal of Pharmaceutical and Clinical Research,* 5: 90-94.

Sosa, A.A., S.H. Bagi and I.H. Hameed (2016). Analysis of bioactive chemical compounds of *Euphorbia lathyrus* using gas charomatography-mass spectrometry and fourier-transform infrared spectroscopy. *Journal of Pharmacognosy and Phytotherapy,* 8: 109-126.

Verma, R.N. and A. Batra (2013). Isolation and analytic characteriaztion of rebaudioside A and GC-MS analysis of methanolic leaves extract of *Stevia rebaudiana* Bert. *Annals of Phytomedicine,* 2: 108-114.

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