Journal of Animal & Plant Sciences, 32(2): 2022, Page: 609-614
ISSN (print): 1018-7081; ISSN (online): 2309-8694
https://doi.org/10.36899/JAPS.2022.2.0461
Short Communication

HEXANE SOLUBLE BIOACTIVE COMPONENTS OF LEAF EXTRACT OF QUINOA

I. H. Khan and *A. Javaid

Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Lahore, Pakistan
*Corresponding author's email: arshad.iags@pu.edu.pk; arshadjpk@yahoo.com

ABSTRACT

Aim of the present study was to identify various bioactive constituents present in non-polar *n*-hexane fraction of methanolic leaf extract of quinoa (*Chenopodium quinoa* Willd.), a crop of family Chenopodiaceae. Quinoa leaves were extracted in methanol. After evaporation of the solvent, the residues were mixed with distilled water, partitioned with *n*-hexane in a separating funnel and were analyzed by GC-MS, which showed presence of 15 constituents including 10 bioactive components namely 9,12-octadecadien-1-ol, (Z,Z)- (31.31%), 1-(+)-ascorbic acid 2,6-dihexadecanoate (17.39%), 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- (12.99%), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (4.84%), phytol (5.01%), tetradecanoic acid (5.34%), hexadecanoic acid, methyl ester (8.04%), octadecanoic acid (2.98%), 2-pentadecanone, 6,10,14-trimethyl- (1.20%), and octadecanoic acid, methyl ester (0.99%). Literature survey revealed that these compounds exhibit a wide variety of biological properties including antifungal, antibacterial, antioxidant, anticancer, anti-inflammatory, nematicidal and others. This study concludes that *n*-hexane fraction of methanolic leaf extract of quinoa is a rich storehouse of bioactive molecules and can be used for treatment of a number of ailments.

Keywords: Antimicrobial, bioactive compounds, *Chenopodium quinoa,* leaf extract.

Published first online August 13, 2021 Published final March 15, 2022.

INTRODUCTION

In previous decades, plant derived products got much attention in pharmaceutical industry due to their wide applications in traditional, folk and modern medicines for the treatment of a variety of diseases with significant results (Javaid *et al.,* 2018; Khan and Javaid, 2020a). Plants produce terpenoids, phenols, terpenes, tannins, polyphenols, flavonoids, alkaloids, iso-flavonoids, coumarins, glycosides and phenyl propanes, which constitute a rich source of potent bioactive substances (Banaras *et al.,* 2020, 2021). Phytochemicals extracted from plants are most likely to be used in antimicrobial products as an alternative strategy with incredible benefits to combat the health hazards caused by synthetic chemical products (Mitrani *et al.,* 2018).

Chenopodium quinoa, belongs to family Chenopodiaceae, is a nutritionally rich traditional food crop cultivated as a pseudo-cereal. It is native to Andean regions of North and South America, recently introduced in Pakistan because of its nutritious value and increasing demand all over the world. Many researchers from several countries are working on different aspects of this crop to enhance its economic importance (Manaa *et al.,* 2019). It is considered as an extremely tolerant crop against abiotic stresses like frost, drought, cold and heat with less nutrient inputs. The quinoa seeds contain essential amino acids, vitamins, minerals (0.7-1.2%), free sugars (8-15%), lipids (7-11%) and fibers (Hernandez-Ledesma, 2019). Quinoa also contains several naturally bioactive products such as glycosides, saponins and flavanol exhibiting biochemical and pharmaceutical properties with potential benefits to human health. It is a new crop in Pakistan. Recent studies regarding phytochemical analysis of stem and root of quinoa growing in Pakistan demonstrated that these parts contain many bioactive compounds (Khan and Javaid, 2019, 2020b; Khan *et al.,* 2020). However, studies regarding phytochemical profile of leaves of quinoa from Pakistan are lacking. Therefore, the present study was carried out to identify the bioactive components of *n*-hexane fraction of methanolic leaf extract of quinoa using GC-MS analysis.

MATERIALS AND METHODS

Quinoa seeds were sown in the soil and leaves were collected at the flowering stage. The leaves were shade dried followed by drying at 45 °C in an electric oven. The dried leaves were crushed thoroughly and 100 g were soaked in 1000 ml methanol for 14 days. Thereafter, the solvent was separated by passing the soaked material through a muslin cloth. In order to remove all the debris

from this extract, it was passed through a double-layer filter paper. The solvent from this extract was evaporated on a rotary evaporator. One hundred milliliter of water was mixed with the crude methanolic extract obtained after evaporation and mixed vigorously. The mixture was portioned with 200 ml of *n*-hexane in a separating funnel. After the formation of two clear layers, the layers were separated and the upper one was collected and filtered to remove any solid particles (Javaid *et al.,* 2019). This *n*-hexane fraction was then subjected to GC-MS for identification of various phytoconstituents present in it.

GC-MS analysis was done on a chromatographic system comprising of a Shimadzu GC-2010 plus installed with auto injector AOC-20i, auto sampler AOC-20s and gas chromatograph equipped with a QP2010 ultra mass-selective detector. Injection volume was 1.0 µl and helium was used as a carrier gas. Injector temperature was set at 250 °C and interface temperature at 320 °C. Starting temperature of the column was 100 °C for 1.0 min after injection, which was increased with the rate 20 °C min^{-1} from 100 to 200 °C and hold for 2.0 min, finally from 200 °C to 300 °C at 40 °C min^{-1}.

A thorough survey of literature was carried out to search for various bioactivities of the identified compounds.

RESULTS AND DISCUSSION

GC-MS chromatogram of *n*-hexane fraction is shown in Fig. 1 which indicates the presence of 15 compounds in the fraction. Names of these compounds along with their molecular formulae and weights, retention times and peak areas percentages are given in Table 1. Structures of these compounds are given in Fig. 2. Bioactivities of these compounds as available in the literature are tabulated in Table 2. Among the 15 identified compounds, 9,12-octadecadien-1-ol, (Z,Z)- was recognized as the most abundant compound with peak area of 31.31%. It is a fatty alcohol and is also known as linoleyl alcohol. It exhibits antifungal activity (Kalsum *et al.,* 2016). The 2nd most abundant compound was 1-(+)-ascorbic acid 2,6-dihexadecanoate with 17.39% peak area. This compound has been identified in a number of plant species including *Bryonopsis laciniosa* (Ramya *et al.,* 2015). It is a highly bioactive compound and is known for its anticancer, anti-inflammatory, antitumer, antiallergic, antibacterial, antiviral, antidiabetic, antianxiety, anticarcinogenic, antihepatic, antiseptic, neuroprotective and termiticide properties (Ramya *et al.,* 2015). The third most abundant compound was 9,12,15-octadecatrienoic acid, methyl ester, (Z,Z,Z)- with 12.99% peak area. It is an anticancer agent and also possesses antiandrogenic, antihistaminic, antieczemic, anti-inflammatory, antiarthritic, anti-coronary, nematicide, hypocholesterolemia and hepatoprotective properties (Devi and Muthu, 2014; Sheeba and Viswanathan, 2014).

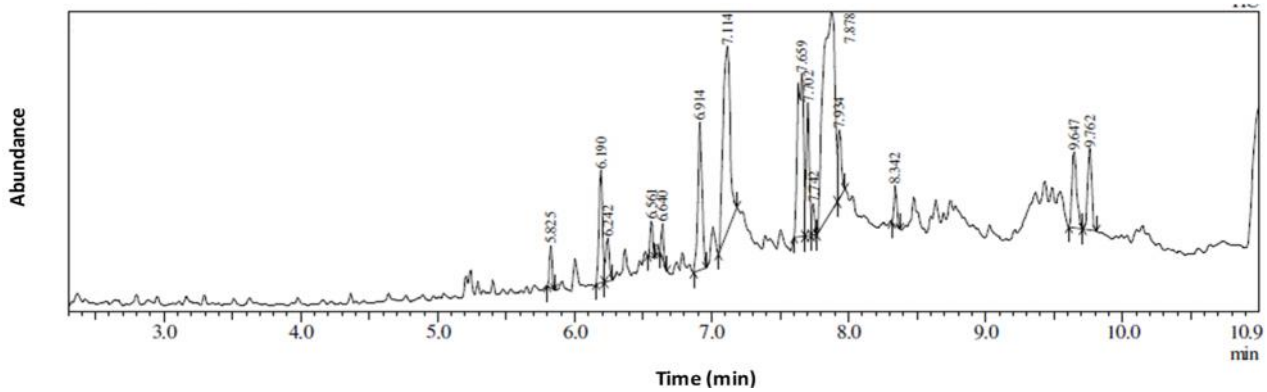

Fig. 1. GC-MS chromatograms of *n*-hexane fraction of methanolic leaf extract of *Chenopodium quinoa.*

Five compounds namely 1,2-benzedicarboxylic acid, diisooctyl ester (4.32%), hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (4.84%), phytol (5.01%), tetradecanoic acid (5.34%) and hexadecanoic acid, methyl ester (8.04%) was identified as moderately abundant. Most of these compounds were found to possess various biological activities against various pathogens and illnesses. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester is an ester of palmitic acid possessing antioxidant, pesticidal, nematicide and hypocholesterolemia properties (Lalitha *et al.,* 2015). Tetradecanoic acid is known for its larvicidal and repellent activity against *Culex quinquefasciatus* and *Aedes aegypti.* A concentration of 5.0 mg cm^{-1} of this compound provided 100% protection against the two tested mosquitoes species (Sivakumar *et al.,* 2011). Hexadecanoic acid, methyl ester possesses both antifungal and antibacterial activities (Chandrasekaran *et al.,* 2011). Phytol is an unsaturated branched chain terpene having antimicrobial activity against a number of bacterial and fungal species including *Escherichia coli*, *Aspergillus niger* and *Candida albicans* with 62.5 µg ml^{-1} MIC_{50} (Ghaneian *et al.,* 2015). Its antimicrobial activity is comparable with traditional disinfectants (Munazir *et al.,* 2012). In addition, its

antidiabetic, antidiuretic, anticancer, immunostimulatory, anti-inflammatory, antioxidant and antinociceptive effects are also known (Venkata *et al.,* 2012; Santos *et al.,* 2013).

Seven compounds *viz.* butanedioic acid, 2,3-bis(benzoyloxy)- (0.86%), octadecanoic acid, methyl ester (0.99%), 2-pentadecanone, 6,10,14-trimethyl- (1.20%), E-10,13,13-trimethyl-11-tetradecen-1-ol acetate (1.39%), benzoic acid, phenyl ester (1.41%), alloaromadendrene oxide-(1) (1.94%) and octadecanoic acid (2.98%) were found as less abundant. Among these, octadecanoic acid or stearic acid occurs in many plant and animal fats. This compound possesses a number of bioactivities including antioxidant, antibacterial, anticonvulsant, anti-analgesic, anti-amoebic, anti-asthmatic,, anti-gastric, antimalarial, anti-obesity, anti-inflammatory, anticancer and hypocholesterolemia (Kumar *et al.,* 2010; Ramya *et al.,* 2015; Manivannan *et al.,* 2017). Octadecanoic acid, methyl ester possesses anti-breast cancer property possibly because of controlling *de novo* diacylglycerol synthesis that induces apoptosis of human breast cancer cells (Manivannan *et al.,* 2017). 2-Pentadecanone, 6,10,14-trimethyl- is an allelopathic and antimicrobial agent (Govindappa *et al.,*2013).

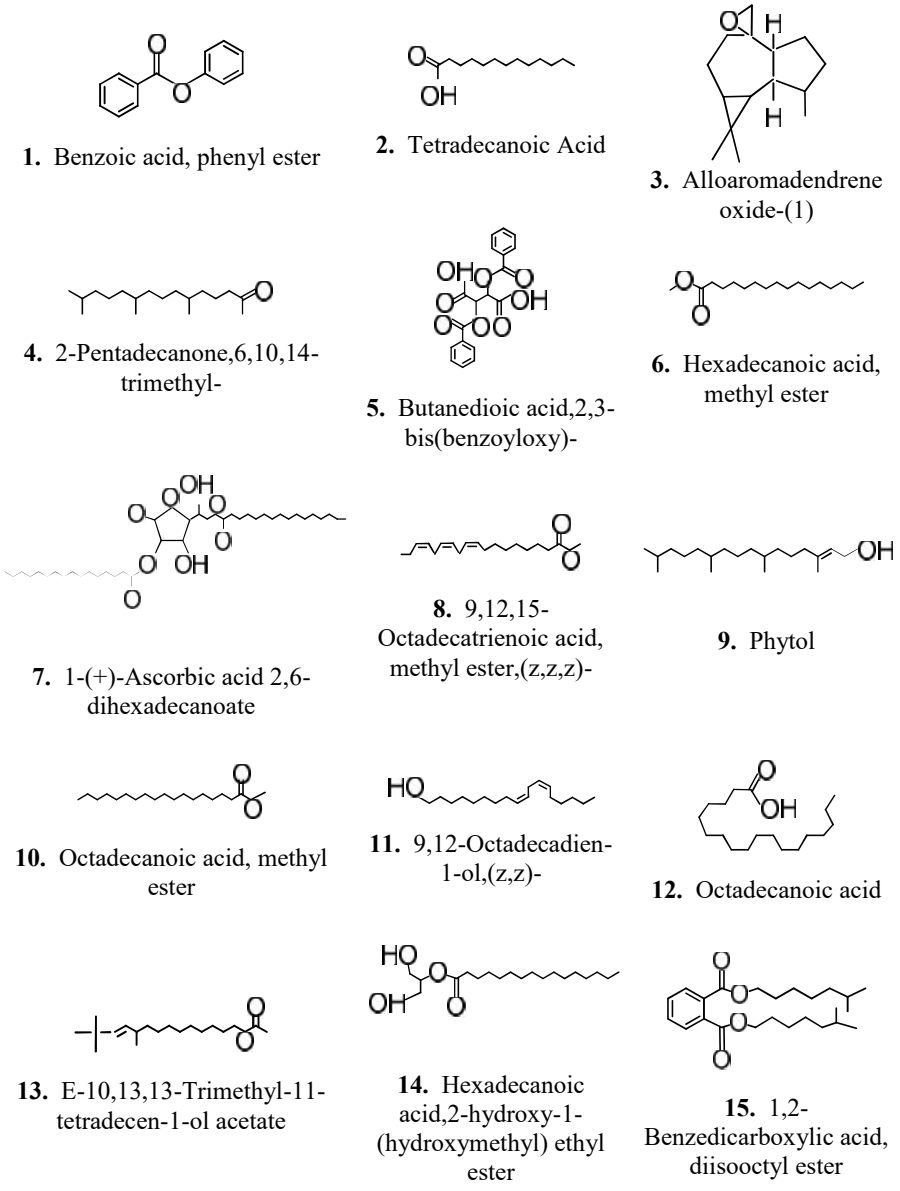

Fig. 2. Structures of compounds identified in *n*-hexane fraction of methanolic leaf extract of *Chenopodium quinoa* through GC-MS analysis

Table 1. List of compounds in *n*-hexane fraction of methanolic leaf extract of *Chenopodium quinoa* identified by GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	Benzoic acid, phenyl ester	$C_{13}H_{10}O_2$	198	5.825	1.41
2	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	6.190	5.34
3	Alloaromadendrene oxide-(1)	$C_{15}H_{24}O$	220	6.242	1.94
4	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	6.561	1.20
5	Butanedioic acid, 2,3-bis(benzoyloxy)-	$C_{18}H_{14}O_8$	358	6.640	0.86
6	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	6.914	8.04
7	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	7.114	17.39
8	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	$C_{19}H_{32}O_2$	292	7.659	12.99
9	Phytol	$C_{20}H_{40}O$	296	7.702	5.01
10	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	7.742	0.99
11	9,12-Octadecadien-1-ol, (Z, Z)-	$C_{18}H_{34}O$	266	7.878	31.31
12	Octadecanoic acid	$C_{18}H_{36}O_2$	284	7.934	2.98
13	E-10,13,13-Trimethyl-11-tetradecen-1-ol acetate	$C_{19}H_{36}O_2$	296	8.342	1.39
14	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O4$	330	9.647	4.84
15	1,2-Benzedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	9.762	4.32

Table 2. Bioactivity of compounds in *n*-hexane fraction of methanolic leaf extract of *Chenopodium quinoa*.

Sr. No.	Names of compounds	Bioactivity	Reference
2	Tetradecanoic acid	Larvicidal, insect repellent	Sivakumar *et al.* (2011)
4	2-Pentadecanone, 6,10,14-trimethyl-	Antimicrobial	Govindappa *et al.* (2014)
6	Hexadecanoic acid, methyl ester	Antifungal and antibacterial	Chandrasekaran *et al.* (2011)
7	1-(+)-Ascorbic acid 2,6-dihexadecanoate	Anticancer, anti-inflammatory, antitumor, antiallergic, antibacterial, antiviral, antidiabetic, antianxiety, anticarcinogenic, antiherpetic, antiseptic, neuroprotective and termiticide	Ramya *et al.* (2015)
8	9,12,15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-	Anticancer, antiandrogenic, antihistaminic, antieczemic, anti-inflammatory, antiarthritic, anti-coronary, nematicide and hypocholesterolemia	Devi and Muthu (2014); Sheeba and Viswanathan (2014)
9	Phytol	Antibacterial, antifungal, anti-schistosome, antioxidant, antinociceptive, antidiuretic, anticancer, immunostimulatory, antidiabetic and anti-inflammatory	Venkata *et al.* (2012); Santos *et al.* (2013); Ghaneian *et al.* (2015)
10	Octadecanoic acid, methyl ester	Anticancer	Manivannan *et al.* (2017)
11	9,12-Octadecadien-1-ol, (Z, Z)-	Antifungal	Kalsum *et al.* (2016)
12	Octadecanoic acid	Antioxidant, antibacterial, anticonvulsant, anti-analgesic, anti-amoebic, anti-asthmatic, anti-gastric, antimalarial, anti-obesity, anti-inflammatory, anticancer and hypocholesterolemia	Kumar*et al.* (2010); Ramya *et al.* (2015); Manivannan *et al.* (2017)
14	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	Antioxidant, pesticidal, nematicidal and hypocholesterolemia	Lalitha *et al.* (2015)

Conclusion: The present study concludes that *n*-hexane fraction of methanolic leaf extract of quinoa is a rich source of bioactive substances, which are not only toxic to a variety of pathogenic organisms but are also useful for treatment of various ailments.

Author's contribution: I. H. Khan carried out experimental work and contributed in paper writing. A. Javaid supervised the whole work, contributed in writing and finalized the manuscript.

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

REFERNCES

Banaras, S., A. Javaid and I.H. Khan (2020). Potential antifungal constituents of *Sonchus oleraceous* against *Macrophomina phaseolina.* Int. J. Agric. Biol. 24(5): 1376-1382.

Banaras, S., A. Javaid and I.H. Khan (2021). Bioassays guided fractionation of *Ageratum conyzoides* for identification of natural antifungal compounds against *Macrophomina phaseolina.* Int. J. Agric. Biol. 25(4): 761-767.

Chandrasekaran, M., A. Senthilkumar and V. Venkatesalu (2011). Antibacterial and antifungal efficacy of fatty acid methyl esters from leaves of *Sesuvium portulacastrum* L. Eur. Rev. Med. Pharmacol. Sci.15: 775-780.

Devi, J.A.I. and A.K. Muthu (2014). Gas chromatography-mass spectrometry analysis of bioactive constituents in the ethanolic extract of *Saccharum spontaneum* Linn. Int. J. Pharm. Pharmaceut. Sci. 6: 755-759.

Ghaneian, M.T., M.H. Ehrampoush, A. Jebali,O. Hekmatim and M. Mahmoudi (2015). Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environ. Health Eng. Manage. J. 2: 13-16.

Govindappa, M., S. Prathap, V. Vinay and R. Channabasava (2014). Chemical composition of methanol extract of endophytic fungi, *Alternaria* sp. of *Tebebuia argentea* and their antimicrobial and antioxidant activity. Int. J. Bio-Pharm. Res.5: 861-869.

Hernandez-Ledesma, B. (2019). Quinoa (*Chenopodium quinoa* Willd.) as source of bioactive compounds: a review. Bioact. Comp. Health Dis. 2: 27-47.

Javaid, A., G.R. Shahzad, N. Akhtar and D. Ahmed (2018). Alternaria leaf spot disease of broccoli in Pakistan and management of the pathogen by leaf extract of *Syzygium cumini.* Pakistan J. Bot. 50: 1607-1614.

Javaid, A., F. Anjum and N.Akhtar (2019). Molecular characterization of *Pyricularia oryzae* and its management by stem extract of *Tribulus terrestris.* Int. J. Agric. Biol. 21: 1256-1262.

Kalsum, N., B. Setiawan and C.U. Wirawati (2016). Phytochemical studies and GC-MS analysis of *Propolis trigona* from two regions in Lampung province of Indonesia. Int. J. Sci. Eng. Res.7: 173-180.

Khan, I.H. and A. Javaid (2019). Antifungal, antibacterial and antioxidant components of ethyl acetate extract of quinoa stem. Plant Prot. 3(3): 125-130.

Khan, I.H. and A. Javaid (2020a). Anticancer, antimicrobial and antioxidant compounds of quinoa inflorescence. Adv. Life Sci. 8(1): 68-72.

Khan, I.H. and A. Javaid (2020b). Comparative antifungal potential of stem extracts of four quinoa varieties against *Macrophomina phaseolina.* Int. J. Agric. Biol. 24(3): 441-446.

Khan, I.H., A. Javaid, D. Ahmed and U. Khan (2020). Identification of volatile constituents of ethyl acetate fraction of *Chenopodium quinoa* roots extract by GC-MS. Int. J. Biol. Biotechnol. 17(1): 17-21.

Kumar, P.P., S. Kumaravel and C. Lalitha (2010). Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. Afr. J. Biochem. Res. 4: 191-195.

Lalitha, S., B. Parthipan and V.R. Mohan (2015). Determination of bioactive components of *Psychotria nilgiriensis* Deb & Gang (Rubiaceae) by GC-MS analysis. Int. J. Pharm. Phytochem. Res. 7: 802-809.

Manaa, A., R. Goussi, W. Derbali, S. Cantamessa, C. Abdelly and R.Barbato (2019). Salinity tolerance of quinoa (*Chenopodium quinoa* Willd) as assessed by chloroplast ultrastructure and photosynthetic performance. Environ. Exp. Bot. 162: 103-114.

Manivannan, P., G. Muralitharan and N.P. Balaji (2017). Prediction aided *in vitro* analysis of octa-decanoic acid from *Cyanobacterium lyngbya* sp. as a proapoptotic factor in eliciting anti-inflammatory properties. Bioinformation.13: 301-306.

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

Munazir, M., R. Qureshi, M. Arshad and M. Gulfraz (2012). Antibacterial activity of root and fruit extracts of *Leptadenia pyrotechnica* (Asclepiadaceae) from Pakistan. Pakistan J. Bot. 44: 1209-1213.

Ramya, B., T. Malarvili and S. Velavan (2015). GC-MS analysis of bioactive compounds in *Bryonopsis laciniosa* fruit extract. Int. J. Pharm. Sci. Res. 6: 3375-3379.

Santos, C.C.M.P., M.S. Salvadori, V.G. Mota, L.M. Costa, A.A.C. Almeida and G.A.L. Oliveira (2013). Antinociceptive and antioxidant activities of phytol *in vivo* and *in vitro* models. Neurosci. J. Article ID 949452.

Sheeba, G.D. and P. Viswanathan (2014). GC-MS analysis of phytocomponents in *Spermacoce articularis* L. leaf. Res. Pharm. 4: 1-7.

Sivakumar, R., A. Jebanesan, M. Govindarajan and P. Rajasekar (2011). Larvicidal and repellent activity of tetradecanoic acid against *Aedes aegypti* and *Culex quinquefasciatus* (Diptera: Culicidae). Asian Pac. J. Trop. Med. 4: 706-710.

Venkata R.B., L.A. Samuel, S.M. Pardha, R.B. Narashimha, V.K.A. Naga, M. Sudhakar and T.M. Radhakrishnan (2012). Antibacterial, antioxidant activity and GC-MS analysis of *Eupatorium odoratum*. Asian J. Pharm. Clin. Res. 5: 99-106.