BIOACTIVE CONSTITUENTS OF WILD *Cannabis sativa* ROOTS FROM PAKISTAN

*Arshad Javaid[1], Iqra Haider Khan[1] and Malik F. H. Ferdosi[2]

ABSTRACT

Hemp (*Cannabis sativa* L.), a medicinal plant of family Cannabaceae, growing wild in most of the rainfed areas of Punjab, Pakistan. In this study, phytochemical profile of methanolic extract of root was assessed through GC-MS analysis and various biologically active compounds were identified through literature survey. There were 14 compounds in the root extract. The most abundant compounds were γ-sitosterol (27.08%) and 9,12-octadecadienoic acid (Z,Z)-, methyl ester (24.09%) and hexadecanoic acid, methyl ester (21.81%). Other identified compounds were methyl stearate (5.51%), stigmasterol (5.12%), campesterol (4.19%), phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-(2.52%), 11-octadecenoic acid, methyl ester (2.10%), squalene (1.89%), β-amyrin (1.88%), eicosanoic acid, methyl ester (0.85%), tetracosanoic acid, methyl ester (0.88%), dronabinol (1.02%) and 2-methoxy-4-vinylphenol (0.96%). Most of the identified compounds possess one or more biological activities *viz.* antitumor, antifungal, antibacterial, antioxidant, anticancer, anti-inflammatory, antidiabetic, and analgesic.

Keywords: Bioactive compounds, Hemp, root extract, Punjab.

Citation: Javaid, A.; I. H. Khan and M. F. H. Ferdosi. 2021. Bioactive Constituents of Wild *Cannabis sativa* Roots from Pakistan. *Pak. J. Weed Sci. Res*., 27 (3):359-368

[1]Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan
[2]Department of Horticulture, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan
*Corresponding author's email: arshad.iags@pu.edu.pk, arshadjpk@yahoo.com

INTRODUCTION

Secondary metabolites produced by plants exhibit a variety of important properties. These can be used as drugs to treat various diseases including cancer and inflammation (Greenwell and Rahman, 2015; Khan and Javaid, 2020a). Likewise, these secondary metabolites also showed antimicrobial activities (Khan and Javaid, 2019; Javed *et al.,* 2021). Weeds have special importance with respect to their use in various folk medicines and as a source of bioactive compounds (Singh and Singh, 2020; Javaid *et al.,* 2021). Extracts and dry biomass of *Coronopus didymus* controlled *Sclerotium rolfsii* and collar rot of bell pepper (Javaid and Iqbal, 2014). Extracts (5% w/v) of different parts of *Sonchus oleraceous* reduced biomass of *Macrophomina phaseolina* by 73–87% (Banaras *et al.,* 2021). Likewise, stem extract of another weed of Asteraceae, *Ageratum conyzoides* caused 83% reduction in growth of *M. phaseolina* (Banaras *et al.,* 2021). Many weeds such as *A. conyzoides*, *Datura metel* and *Chenopodium album* possess herbicidal properties and their extracts can be used to control *Parthenium hysterophorus* (Javaid *et al.,* 2010, 2020a,b).

Cannabis sativa is growing as a weed in Punjab Pakistan, although it is also cultivated in some other countries. It is an important plant that contains a variety of valuable components including phenolic, terpenes and cannabinoids, which are compounds of industrially interest. This valuable, fast-growing herbaceous plant has its origin in Central Asia (Andre *et al.,* 2016). Since ancient times, it has been known due to its medicinal and industrial uses (Skoglund *et al.,* 2013). Its stem provides cellulosic as well as woody fibers. The cortex contains long cellulose-rich bast fibers while the core is lignified and has woody fibers (Guerriero *et al.,* 2013). Hemp seed-oil showed strong antibacterial activity against *Bacillus subtilis* (Ali *et al.,* 2012). Some recent studies have shown antifungal activity of hemp extracts. A 6.25 mg mL^{-1} *n*-butanol leaf extract of hemp completely controlled the growth *Aspergillus versicolor* (Khan *et al.,* 2021). Likewise, leaf extract of hemp showed remarkable antifungal activity to control *Aspergillus flavipes* (Khan and Javaid, 2020b). Most of the previous studies regarding antimicrobial activities of hemp were carried out by using extracts from its aerial part while such studies with the extracts of root are limited. Therefore, the present study was conducted to identify various biologically important compounds present in hemp roots. For this methanolic root extract of hemp was analyzed by GC-MS and activities of the recognized constituents were searched through extensive literature survey.

MATERIALS AND METHODS

Sample collections

C. sativa plants were uprooted from canal bank near Jail Road, Lahore. The plants were placed in paper bags and shifted to the lab for further processing. The plants roots were cut and placed in an oven for two days at the temperature of 35 ºC to evaporate the moisture.

Extract preparation

The fully dried roots of *C. sativa* were cut into small pieces with the help of sharp knife, crushed in a pestle and mortar, and 10 g were soaked in 50 mL of analytical grade methanol. The soaked material was kept for two weeks in the lab for thorough extraction of bioactive compounds. The extract was then filtered by using a double layer of filter paper. Two

milliliters of the extract was taken into a 5 mL vial and the sample was shifted to lab for GC-MS analysis.

GC-MS analysis

GC-MS analysis was performed to identify the possible antimicrobial and other bioactive constituents from methanolic root extract of *C. sativa* as per set conditions (Ferdosi *et al.,* 2020).

GC conditions were as follows:

- Machine model: 7890B, Agilent Technologies (USA)
- Injection volume: 1 µL
- Column; DB-5ms, dimensions (30 m × 0.25 µm × 0.25 µm)
- Oven ramping: initial temperature was 80 ºC and then raised 10 ºC min^{-1} up to 300 ºC.
- Inlet temperature: 280 ºC
- Run time; 50 min
- Carrier gas: Helium
- Solvent Delay: 5 min

MS conditions were as follows:

- Machine Model: 5977A, Agilent Technologies (USA)
- Scan: 50–500 m/z
- Run Time 50 min
- Solvent delay: 5 min
- Source temperature: 230 ºC and
- Quadrupole temperature: 150 ºC
- Library: NIST 2017

Spectra of the compounds were compared with the spectra in library and the compounds were arranged in the ascending order of their retention times and retention indices. The relative abundance was reported by using their peak areas.

Literature survey

An in depth literature review was done to find the evidence of biological activities of chemical constituents of *C. sativa* roots. The ChemDraw software was used to draw structures of major chemical compounds in the extract (Ferdosi *et al.,* 2021a)

RESULTS AND DISCUSSION

GC-MS chromatogram of methanolic root extract is shown in Fig. 1. In the root extract, 14 compounds were identified whose details are given in Table 1. Biological activities of the identified compounds as collected from previous literature are summarized in Table 2 while structures of biologically active compounds are illustrated in Fig. 2. There were three principal compounds in the extract. Among these the most abundant compound was γ-sitosterol (27.08%) followed by 9,12-octadecadienoic acid (Z,Z)-, methyl ester (24.09%) and hexadecanoic acid, methyl ester (21.81%). Three compounds namely methyl stearate (5.51%), stigmasterol (5.12%) and campesterol (4.19%) were identified as moderately abundant ones. On the other hand, phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-(2.52%), 11-octadecenoic acid, methyl ester (2.10%), squalene (1.89%), β-amyrin (1.88%), dronabinol (1.02%), 2-methoxy-4-vinylphenol (0.96%), tetracosanoic acid, methyl ester (0.88%) and eicosanoic acid, methyl ester (0.85%) were ranked as less abundant compounds.

Literature survey revealed that many of the identified compounds possess various biological activities. The predominant compound in the present study was γ-sitosterol. It has been identified in various plant species namely *Lippia nodiflora*, *Acacia nilotica* and *Cirsium arvense*, and is known to have anticancer and antidiabetic activities (Balamurugan *et al.,* 2011; Sundarraj *et al.,* 2012; Ferdosi *et al.,* 2021b).

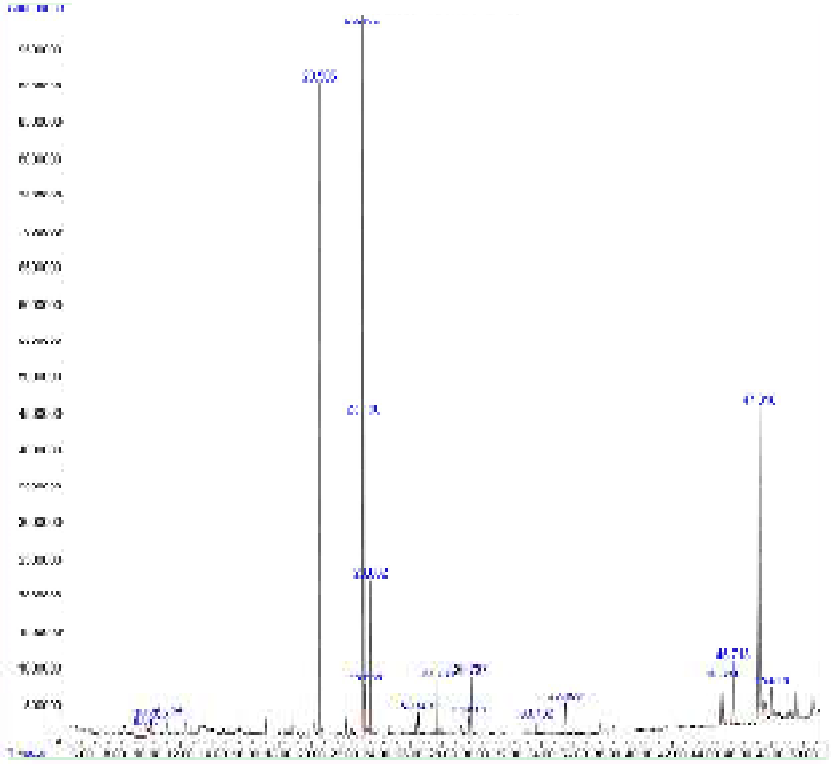

Fig. 1: GC-MS chromatogram of methanolic root extract of *Cannabis sativa*.

Table 1: Compounds identified in methanolic root extract of *Cannabis sativa* through GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150.17	9.877	0.96
2	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270.45	20.505	21.86
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294.47	23.105	24.09
4	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.48	23.286	2.10
5	Methyl stearate	$C_{19}H_{38}O_2$	298.50	23.602	5.51
6	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	326.55	26.501	0.85
7	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	$C_{23}H_{32}O_2$	340.49	27.704	2.52

8	Dronabinol	$C_{21}H_{30}O_2$	314.46	29.619	1.02
9	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	382.66	33.732	0.88
10	Squalene	$C_{30}H_{50}$	410.71	35.460	1.89
11	Campesterol	$C_{28}H_{48}O$	40.68	45.023	4.19
12	Stigmasterol	$C_{29}H_{48}O$	412.69	45.713	5.12
13	γ-Sitosterol	$C_{29}H_{50}O$	414.70	47.318	27.08
14	β-Amyrin	$C_{30}H_{50}O$	426.71	48.046	1.88

Table 2: Bioactivity of components of methanolic root extract of *Cannabis sativa*.

Sr. No.	Names of compounds	Bioactivity	Reference
1	2-Methoxy-4-vinylphenol	Antimicrobial, anti-inflammatory, analgesic, antioxidant	Hameed *et al.* (2015); Rubab *et al.* (2020)
2	Hexadecanoic acid, methyl ester	Urine acidifier, increase zinc bioavailability, antibacterial	Duke (1992); Shaaban *et al.* (2021)
3	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	Urine acidifier, increase zinc bioavailability	Duke (1992)
4	11-Octadecenoic acid, methyl ester	Antibacterial	Shoge and Amusan (2020)
5	Methyl stearate	Antioxidant, antifungal	Pinto *et al.* (2017)
6	Eicosanoic acid, methyl ester	Alpha-glucosidase inhibitors	Elaiyaraja and Chandramohan (2013)
7	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	-	-
8	Dronabinol	Psychoactive, antibacterial	Klingeren and Ham (1976)
9	Tetracosanoic acid, methyl ester	-	-
10	Squalene	Antitumor, antioxidant	Huang *et al.* (2009)
11	Campesterol	Anticancer	Choi *et al.* (2007)

12	Stigmasterol	Antifungal, anti-mutagenic, antidiabetic, anti-tumor, anti-osteoarthritic and anti-inflammatory	Mbambo *et al.* (2012); Kim *et al.* (2014); Wang *et al.* (2017)
13	γ-Sitosterol	Antidiabetic, anticancer	Balamurugan *et al.* (2011); Sundarraj *et al.* (2012)
14	β-Amyrin	Antifungal, antioxidant	Jabeen *et al.* (2011); Cardoso *et al.* (2020); Javed *et al.* (2021)

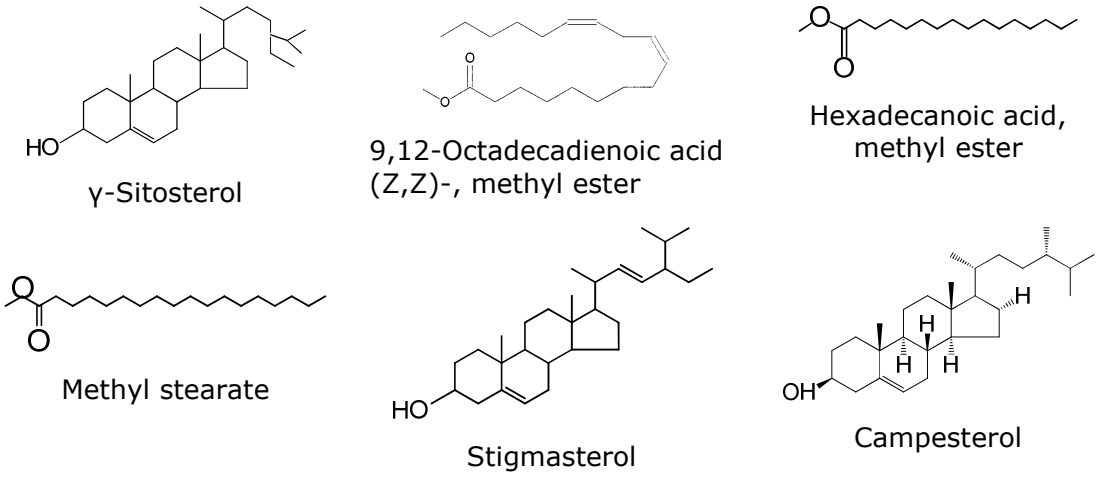

Fig. 2: Structures of major compounds in root extract of *Cannabis sativa*.

The second most abundant compound 9,12-octadecadienoic acid (Z,Z)-, methyl ester is known for a number of bioactivities as it act as urine acidifier, increase zinc bioavailibility and inhibit production of uric acid. Similar properties have also been associated with the third most abundant compound hexadecanoic acid, methyl ester (Duke, 1992). Moreover, hexadecanoic acid, methyl ester also exhibited antibacterial activity (Shaaban *et al.,* 2021). 2-Methoxy-4-vinylphenol was previously identified from red cabbage with antibacterial activity (Rubab *et al.,* 2020). It has also been found in many other studies and is known for its antioxidant, antimicrobial, analgesic, anti-inflammatory and anti-germination (Hameed *et al.,* 2015). β-Amyrin was previously identified in leaves of *Melia azedarach* and *Monotheca buxifolia* with strong antifungal activity against *M. phaseolina* and *Ascochyta rabiei* (Jabeen *et al.,* 2011; Javed *et al.,* 2021). Cardoso *et al.* (2020) isolated this compound from *Myrcianthes pungens* and also reported its antioxidant activity. In addition, it also has antihyperglycemic and

hypolipidemic effects and can be used in the preparation of drugs useful for diabetes and atherosclerosis (Santos *et al.,* 2012). 11-Octadecenoic acid, methyl ester showed antibacterial activity against *Shigella dysentriae*, *E. coli*, *S. aureus* and *Salmonella typhi*, causing diarrhea in humans (Shoge and Amusan, 2020). Root extract contained various fatty acid methyl esters namely methyl stearate; and eicosanoic acid, methyl ester. Earlier studies have shown that fatty acid methyl esters from sunflower, soybean, and corn oil showed strong antifungal activity against *Paracoccidioides* spp. (Pinto *et al.,* 2017). Dronabinol is a primary psychoactive component of cannabis. It is the synthetic form of tetrahydrocannabinol. It also exhibited antibacterial activity (Klingeren and Ham, 1976). Squalene is a triterpene and generally found in sufficient quantity in oils of olive, palm, amaranth and rice bran. It possesses antitumor and antioxidant activities (Huang *et al.,* 2009). Campesterol is a plant sterol having a structure similar to that of cholesterol and causes anticancer effects (Choi *et al.,* 2007). It competes with cholesterol resulting in reduced absorption of cholesterol in the intestine (Choudhary and Tran, 2011). Stigmasterol is generally present in plants and has antifungal, anti-mutagenic, antidiabetic, anti-tumor, anti-osteoarthritic and anti-inflammatory activities (Gabay *et al.,* 2010; Mbambo *et al.,* 2012; Kim *et al.,* 2014; Wang *et al.,* 2017).

Conclusion

Major compounds in the root extract of *C. sativa* were γ-sitosterol, 9,12-octadecadienoic acid (Z,Z)-, methyl ester and hexadecanoic acid, methyl ester. Almost all the compounds possess one or more biological properties.

REFERENCES CITED

Ali, E.M.M., A.Z.I. Almagboul, S.M.E. Khogali and U.M.A Gergeir. 2012. Antimicrobial activity of *Cannabis sativa* L. Chin. Med. 3: 61-64.

Andre, C.M., J.F. Hausman and G. Guerriero. 2016. *Cannabis sativa*: The plant of the thousand and one molecules. Front. Plant Sci. 7: Article 9.

Balamurugan, R., V. Duraipandiyan and S. Ignacimuthu. 2011. Antidiabetic activity of γ-sitosterol isolated from *Lippia nodiflora* L. in streptozotocin induced diabetic rats. Eur. J. Pharmacol. 667: 410-418.

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* extract for the identification of natural antifungal compounds against *Macrophomina phaseolina.* Int. J. Agric. Biol. 25(4): 761-767.

Cardoso, B.K., H.L.M. de Oliveira, U.Z. Melo, C.M.M. Fernandez, C.F.A.A. Campo and J.E. Gonçalves, 2020. Antioxidant activity of α and β-amyrin isolated from *Myrcianthes pungens* leaves. Nat. Prod. Res. 34: 1777-1781.

Choi, J.M., E.O. Lee, H.J. Lee, K.H. Kim, K.S. Ahn, B.S. Shim, S.H. Kim. 2007. Identification of campesterol from *Chrysanthemum coronarium* L. and its antiangiogenic activities. Phytother. Res. 21: 954-959.

Choudhary, S.P. and L.S. Tran. 2011. Phytosterols: Perspectives in

human nutrition and clinical therapy. Curr. Med. Chem. 18: 4557-4567.

Duke, J.A. 1992. Handbook of phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, FL. CRC Press.

Elaiyaraja, A. and G. Chandramohan. 2016. Comparative phyto-chemical profile of *Indoneesiella echioides* (L.) Nees leaves using GC-MS. J. Pharmacogn. Phytochem. 5: 158-171.

Ferdosi, M.F.H., I.H. Khan, A. Javaid, T. Sattar and A. Munir. 2020. Identification of antimicrobial constituents in essential oil from *Paulownia fortunei* flowers. Mycopath 18(2): 53-57

Ferdosi, M.F.H., I.H. Khan, A. Javaid, M.F.A. Fardosi. 2021a. Bioactive components in methanolic flower extract of *Ageratum conyzoides*. Pak. J. Weed Sci. Res. 27 (2): 181-190.

Ferdosi, M.F.H., I.H. Khan, A. Javaid and M.F.A. Fardosi. 2021b. GC-MS examination of methanolic extract of *Cirsium arvense* flower. Pak. J. Weed Sci. Res. 27(2): 173-180.

Gabay, O., C. Sanchez and C. Salvat. 2010. Stigmasterol: a phytosterol with potentialanti-osteoarthritic properties. Osteoarthr. Cartil. 18:106-116.

Greenwell, M. and P.K.S.M. Rahman. 2015. Medicinal plants: Their use in anticancer treatment. Int. J. Pharm. Sci. Res. 6: 4103-4112.

Guerriero, G., K. Sergeant and J.F. Hausman. 2013. Integrated -omics: a powerful approach to understanding the hetero-geneous lignification of fiber crops. Int. J. Mol. Sci. 14: 10958-10978.

Hameed, I.H., H.J. Hussein, M.A. Kareem and N.S. Hamad. 2015. Identification of five newly described bioactive chemical compounds in methanolic extract of *Mentha viridis* by using gas chromatography-mass spectrometry (GC-MS). J. Pharmacog. Phytother. 7: 107-125.

Huang, Z.R., Y.K. Lin and J.Y. Fang. 2009. Biological and pharma-cological activities of squalene and related compounds: potential uses in cosmetic dermatology. Molecules 14: 540-54.

Jabeen, K., A. Javaid, E. Ahmad and M. Athar. 2011. Antifungal compounds from *Melia azedarach* leaves for management of *Ascochyta rabiei* – the cause of chickpea blight. Nat. Prod. Res. 25(3): 264-276.

Javaid, A., S. Shafique and S. Shafique. 2010. Herbicidal effects of extracts and residue incorporation of *Datura metel* against parthenium weed. Nat. Prod. Res. 24 (15): 1426-1437.

Javaid, A. and D. Iqbal. 2021. Management of collar rot of bell pepper (*Capsicum annuum* L.) by extracts and dry biomass of *Coronopus didymus* shoot. Biol. Agric. Hort. 30 (3), 164-172.

Javaid, N., M.H. Shah, I.H. Khan, A. Javaid and S.M. Waleed. 2020. Herbicidal activity of *Ageratum conyzoides* against parthenium. Pak. J. Weed Sci. Res. 26(2):137-146.

Javaid, S., M.H. Shah, I.H. Khan, A. Javaid and S.M. Waleed. 2020. Using extracts of lamb's quarters weed for control of *Alternaria alternata*. Pak. J. Weed Sci. Res. 26(2): 147-155

Javaid, A., S.F. Naqvi and I.H. Khan. 2021. Ethyl acetate extract of *Chenopodium murale* root, a source of bioactive compounds. Pak. J. Weed Sci. Res. 27(1): 93-100.

Javed, S., Z. Mahmood, K.M. Khan, S.D. Sarker, A. Javaid, I.H. Khan and A. Shoaib. 2021. Lupeol acetate as a potent antifungal compound against opportunistic human and phytopathogenic mold *Macrophomina phaseolina*. Sci. Rep. 11: 8417.

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. Antifungal activity of leaf extract of *Cannabis sativa* against *Aspergillus flavipes*. Pak. J. Weed Sci. Res. 26(4): 447-453.

Khan, I.H., A. Javaid and N. Shad. 2021. Comparative efficacy of organic solvent fractions of leaf extract of hemp against *Aspergillus versicolor*. Pak. J. Weed Sci. Res. 27: 101-108.

Kim, Y-S., X-F. Li and K-H. Kang. 2014. Stigmasterol isolated from marine microalgae *Navicula incerta* induces apoptosis in human hepatoma HepG2 cells. BMB Rep. 47: 433-438.

Klingeren, B.V. and M.T. Ham. 1976. Antibacterial activity of delta9-tetrahydrocannabinol and cannabidiol. Anton. Van Leeuw. 42: 9-12.

Mbambo, B., B. Odhav and V. Mohanlall. 2012. Antifungal activity of stigmasterol, sitosterol and ergosterol from *Bulbine natalensis* Baker (Asphodelaceae). J. Med. Plants Res. 6: 5135-5141.

Pinto, M.E.A., S.G. Araújo, M.I. Morais, N.P. Sa, C.M. Lima, C.A. Rosa and L.A.R.S. Lima. 2017. Antifungal and antioxidant activity of fatty acid methyl esters from vegetable oils. An. Acad. Bras. Ciênc. 89: 1671-1681.

Rubab, M., R. Chelliah, K. Saravanakumar, K. Barathi-kannan, S. Wei, J.R. Kim, D. Yoo, M.H. Wang and D.H. Oh. 2020. Bioactive potential of 2-methoxy-4-vinylphenol and benzofuran from *Brassica oleracea* L. var. *capitate f, rubra* (red cabbage) on oxidative and microbiological stability of beef meat. Foods 9: Article 568.

Santos, F.A., J.T. Frota, B.R. Arruda, T.S. de Melo and V.S. Rao. 2012. Antihyperglycemic and hypolipidemic effects of α, β-amyrin, a triterpenoid mixture from *Protium heptaphyllum* in mice. Lipids Health Dis. 11: Article 98.

Shaaban, M.T., M.F. Ghaly, S.M. Fahmi. 2021. Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. J. Basic Microbiol. 61: 557-568.

Shoge, M. and T. Amusan. 2020. Phytochemical, antidiarrhoeal activity, isolation and characterisation of 11-octadecenoic acid, methyl ester isolated from the seeds of *Acacia nilotica* Linn. J. Biotechnol. Immunol. 2: DOI: 10.5281/zenodo.3669434

Singh, R. and A Singh. 2020. Weeds: An important source of natural medicine. J. Drug Metab. Toxicol. 11: Article 253.

Skoglund, G., M. Nockert and B. Holst. 2013. Viking and early Middle Ages northern Scandinavian textiles proven to be made with hemp. Sci. Rep. 3: Article 2686.

Sundarraj, S., R. Thangam, V. Sreevani, K. Kaveri, P. Gunasekaran, S. Achiraman and S. Kannan. 2012. γ-Sitosterol from *Acacia nilotica* L. induces G2/M cell cycle arrest and apoptosis through c-Myc suppression in MCF-7 and A549 cells. J. Ethnopharmacol. 141: 803-809.

Wang, J., M. Huang, J. Yang, X. Ma and P. Zhao. 2017. Anti-diabetic activity of stigmasterol from soybean oil by targeting the GLUT4 glucose transporter. Food Nutr. Res. 61: Article 1364117.