Pak. J. Phytopathol., Vol. 33 (02) 2021. 349-355 DOI: 10.33866/phytopathol.033.02.0707

Official publication of Pakistan Phytopathological Society
Pakistan Journal of Phytopathology
ISSN: 1019-763X (Print), 2305-0284 (Online)
http://www.pakps.com

NATURAL PESTICIDAL COMPOUNDS OF *EUPHORBIA PROSTRATA*

[a]Malik F. H. Ferdosi, [b]Iqra H. Khan, [b]Arshad Javaid*, [c]Muhammad Nadeem, [d]Ayesha Munir

[a] *Department of Horticulture, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan.*
[b] *Department of Plant Pathology, Faculty of Agricultural Sciences, University of the Punjab, Quaid-i-Azam Campus, Lahore 54590, Pakistan.*
[c] *University of Okara, Pakistan.*
[d] *City District Government Girls High School, Karim Park Moman Pura, Ravi Road Lahore, Pakistan.*

A B S T R A C T

This study was carried out to analyze the phytochemical profile of *Euphorbia prostrata* Aiton through GC-MS and identification of possible antifungal, antibacterial and other pesticidal compounds through literature survey. Whole plant material of the weed was collected from Lahore, Pakistan and shade dried followed by two-week extraction in ethyl acetate. The filtrates were analyzed by GC-MS that showed the presence of 19 compounds. The major compound in the extract was 9,19-cyclolanost-24-en-3-ol, (3β)- (24.62%) followed by 9,19-cyclolanostan-3-ol, 24-methylene-, (3β)- (19.51%) and γ-sitosterol (12.51%). Other compounds were phytol (8.11%), *n*-hexadecanoic acid (6.15%), 9,12,15-octadecatrienoic acid, (Z,Z,Z)- (4.90%), neophytadiene (4.88%), cyclohexane, 1,3,5-triphenyl- (3.71%), hexadecanoic acid, ethyl ester (3.04%), phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- (2.42%), octadecanoic acid, ethyl ester (2.24%), octacosane (2.11%), 1-heptacosanol (1.71%), stigmasta-5,24(28)-dien-3-ol, (3β,24Z)- (1.71%), 9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (1.29%), oxirane, hexadecyl- (1.21), 9,12-octadecadienoic acid (Z,Z)- (1.05%), Z-8-methyl-9-tetradecenoic acid (1.01%), and 9-octadecyne (0.76%). Literature survey revealed that eight compounds possess antibacterial, antifungal, nematicidal and/or insecticidal properties. Most of the pesticidal compounds were antibacterial in nature.

Keywords: Euphorbiaceae, GC-MS analysis, Pakistan, Prostrate spurge, Weed.

INTRODUCTION

In recent years, the use of synthetic agrochemicals has become an important component of the modern agriculture systems worldwide (Galt and Asprooth, 2021). These chemicals not only disrupt a wide variety of pathogens, but some have also been developed against specific pests or pathogens resulting in increased crop production. They interrupt the physiological activities of the targeted organisms leading towards their reduced vitality (Ahemad and Khan, 2013; Verheyen and Stoks, 2019). However, the excessive use of these pesticides has attained a high impact on agricultural productions with increased environment contamination (Tleuova *et al.,* 2020). Many of these agrochemical residues are sufficient sources to pollute soils, aquatic and terrestrial ecosystems. These have the characteristics of bioaccumulation, lipophilicity, and long shelf-life, consequently, have toxic effects on human and nonhuman biota even after many years of application (Daam *et al.,* 2019; Lupi *et al.,* 2019). The persistent usage of these products has caused chronic poisoning to the applicators, frame workers, and thus the consumers are compelled to adopt some eco-friendly alternative means (Ahmed *et al.,* 2019; Ali *et al.,* 2020; Jabeen *et al.,* 2021). One of the most significant alternatives is the use of botanical pesticides, which is now being considered as an efficient mean to replace synthetic pesticides (Mfarrej and Rara, 2019; Banaras *et al.,* 2021; Javed *et al.,* 2021).

Submitted: September 24, 2021
Revised: November 13, 2021
Accepted for Publication: November 19, 2021
* Corresponding Author:
Email: arshad.iags@pu.edu.pk

These plants based natural products aid in pest control as well as in increasing agriculture yield (Vurro *et al.,* 2019; Banaras *et al.,* 2020; Khan and Javaid, 2020a, b).

Euphorbia prostrata Aiton, commonly known as prostrate spurge, belongs to family Euphorbiaceae (Kengni *et al.,* 2013). It is a small, prostrate, annual herb, which is abundantly found in Asia and Africa (Pahlevani and Akhani, 2011). This plant contains tannins, flavonoids and phenolic compounds and was demonstrated to have analgesic, antimicrobial, anti-inflammatory, larvicidal, haemostatic, insecticidal, nematicidal and cytotoxic properties (Chudasama *et al.,* 2018; Alsaffar *et al.,* 2021). It is also used for the treatment of diarrhea, dropsy, bronchitis, cancer, jaundice, hemorrhoids, dysentery, cardiac dysfunction, typhoid and paratyphoid fevers (Ahmad *et al.,* 2011; Ashwin *et al.,* 2015; Tchuenguem *et al.,* 2017). Moreover, it has been traditionally used as a remedy against venomous insect bites and digestive system disorders (Özbilgin and Çitoğl, 2012). The present study was carried out to determine various phytochemical compounds in *E. prostrata* through GC-MS and to identify the possible pesticidal compounds through literature survey.

MATERIALS AND METHODS

Collections of plant material: The whole plants of *E. prostrata* were uprooted from a natural habitat from Lahore. The plants were placed into a paper bag to be shifted to the lab for further procedures. The plant material was dried under shade and then placed in an oven at 40 °C to evaporate all the moisture contents.

Preparation of ethyl acetate extract: The completely dried plant material was grounded into very fine powder, and 50 g of that material was placed into a conical flask by completely soaking it with HPLC grade ethyl acetate. That mixture was placed at room temperature for 21 days for maximum extraction of chemical compounds into the solvent. After that, the solvent mixture was filtered by Whatman filter paper and the solution was taken into a glass vial and shifted to lab for GC-MS analysis.

GC-MS analysis: GC-MS analysis was performed to identify the possible pesticidal compounds from the ethyl acetate extract of *E. prostrata* following the procedure given by Ferdosi *et al.* (2020). The GC machine used for the identification of compounds was made by Agilent Technologies; United States of America. The machine model was 7890B whereas helium was used as carrier gas. Injection volume was 1 µL; DB-5ms column (30 m × 0.25 µm × 0.25 µm) was used to run the sample. Total run time for the sample was 28 min with 3 minutes solvent delay time; inlet temperature was 280 °C while oven ramping initial temperature was 80 °C, raising 10 °C per minute up to maximum of 300 °C.

The sample was scanned up to 28 min with 3 min of solvent delay time in Mass spectroscopy machine model 5977A, Agilent, USA. The source temperature was 230 °C whereas the Quadrupole temperature was 150 °C, Scan 50-500 m/z. The compounds were identified by comparing their spectra with NIST 2017 library and were tabulated in ascending order of their retention times and indices.

Literature survey: A thorough literature hunting was done to find the reports of pesticidal activities of organic compounds extracted from the ethyl acetate extract of *E. prostrata*. The structures of these bio-pesticidal compounds were drawn by using ChemDraw Pro 8.0 software of Informer Technologies, Inc.

RESULTS AND DISCUSSION

GC-MS chromatogram of ethyl acetate extract of *E. prostrata* is shown in Figure 1 that indicates 19 peaks of different compounds. Names, formulae, molecular weights, retention times and peak area percentages of these compounds are tabulated in Table 1. The predominant compound in the extract was 9,19-cyclolanost-24-en-3-ol, (3β)- with peak area 24.62%. Compounds namely phytol (8.11%), *n*-hexadecanoic acid (6.15%), 9,12,15-octadecatrienoic acid, (Z,Z,Z)-(4.90%), neophytadiene (4.88%), cyclohexane, 1,3,5-triphenyl- (3.71%), and hexadecanoic acid, ethyl ester (3.04%) were recognized as moderately abundant ones. Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-methyl- (2.42%), octadecanoic acid, ethyl ester (2.24%), octacosane (2.11%), 1-heptacosanol (1.71%), stigmasta-5,24(28)-dien-3-ol, (3β,24Z)- (1.71%), 9,12,15-octadecatrienoic acid, ethyl ester, (Z,Z,Z)- (1.29%), oxirane, hexadecyl- (1.21), 9,12-octadecadienoic acid (Z,Z)- (1.05%), and Z-8-methyl-9-tetradecenoic acid (1.01%) were categorized as less abundant compounds. The least abundant compound was 9-octadecyne (0.76%).

Table 1. Compounds identified in ethyl acetate extract of *Euphorbia351rostrata* through GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	Neophytadiene	$C_{20}H_{38}$	278.51	13.452	4.88
2	9-Octadecyne	$C_{18}H_{34}$	250.46	13.704	0.76
3	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	14.785	6.15
4	Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284.47	15.031	3.04
5	Phytol	$C_{20}H_{40}O$	296.5	16.158	8.11
6	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280.44	16.444	1.05
7	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	$C_{18}H_{30}O_2$	278.42	16.484	4.90
8	9,12,15-Octadecatrienoic acid, ethyl ester, (Z,Z,Z)-	$C_{20}H_{34}O_2$	306.48	16.679	1.29
9	Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	312.53	16.902	2.24
10	Z-8-Methyl-9-tetradecenoic acid	$C_{15}H_{28}O_2$	240.38	17.818	1.01
11	Phenol, 2,2’-methylenebis[6-(1,1-dimethylethyl)-4-methyl-	$C_{23}H_{32}O_2$	340.49	18.813	2.42
12	Cyclohexane, 1,3,5-triphenyl-	$C_{24}H_{24}$	312.44	19.157	3.71
13	Octacosane	$C_{28}H_{58}$	394.76	23.740	2.11
14	1-Heptacosanol	$C_{27}H_{56}O$	396.73	23.791	1.71
15	Oxirane, hexadecyl-	$C_{18}H_{36}O$	268.47	24.736	1.21
16	γ-Sitosterol	$C_{29}H_{50}O$	414.70	25.445	12.51
17	Stigmasta-5,24(28)-dien-3-ol, (3β,24Z)-	$C_{29}H_{48}O$	412.69	25.560	1.71
18	9,19-Cyclolanost-24-en-3-ol, (3β)-	$C_{30}H_{50}O$	426.71	26.160	24.62
19	9,19-Cyclolanostan-3-ol, 24-methylene-, (3β)-	$C_{31}H_{52}O$	440.74	26.681	19.51

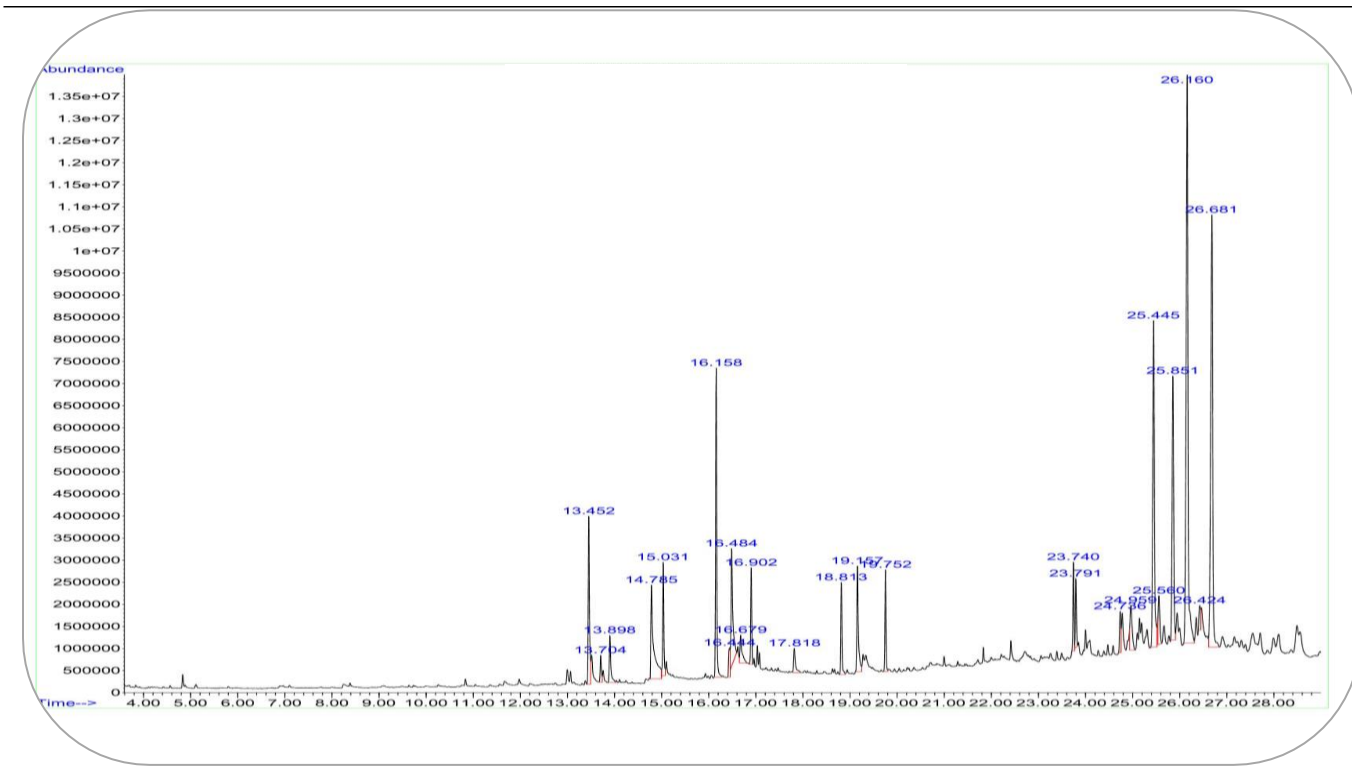

Figure 1. GC-MS chromatogram of ethyl acetate extract of *Euphorbia prostrata.*

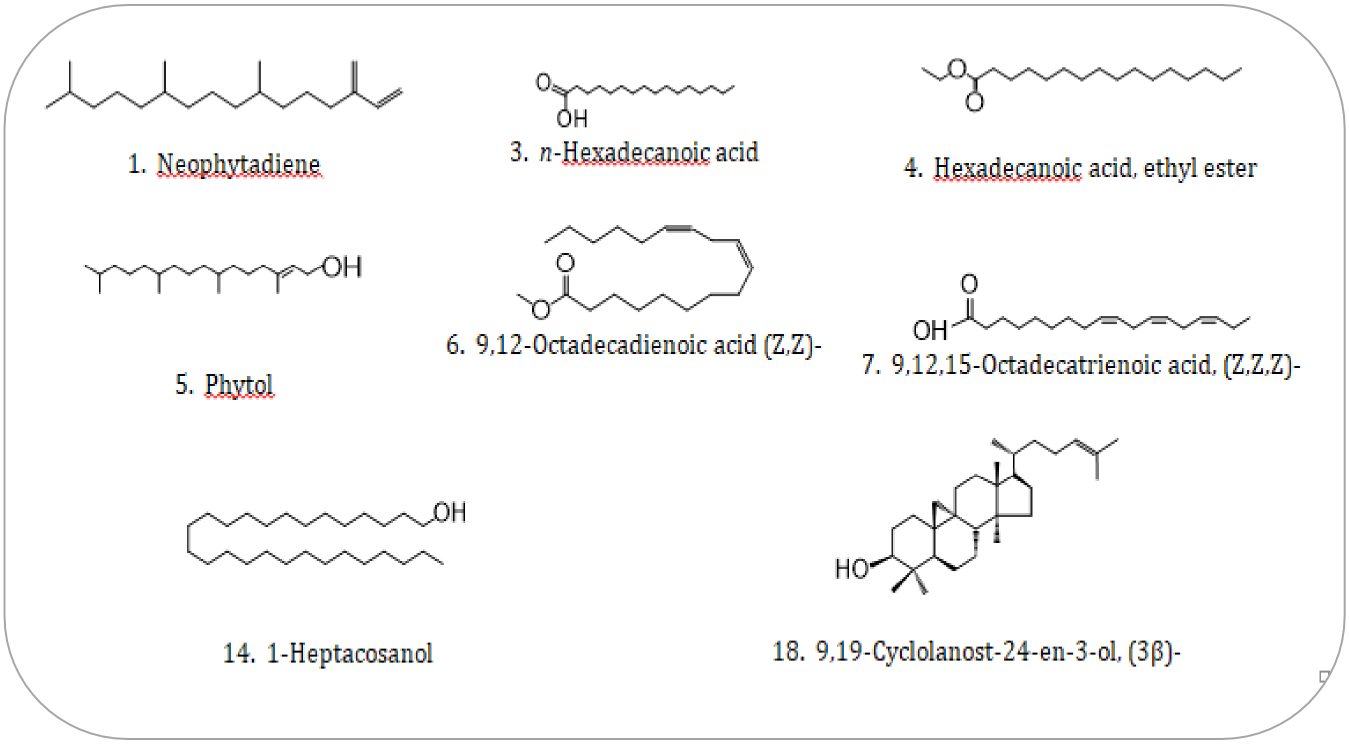

Figure 2. Structures of pesticidal compounds in ethyl acetate extract of *Euphorbia prostrata.* Structures were drawn using ChemDraw Pro 8.0 software of Informer Technologies, Inc.

Table 2. Pesticidal activities of components of ethyl acetate extract of *Euphorbia rostrata*.

Sr. No.	Names of compounds	Pesticidal activity	Reference
1	Neophytadiene	Antibacterial	Ceyhan-Güvensen and Keskin (2016)
3	*n*-Hexadecanoic acid	Antibacterial, mosquito larvicide, nematicide	Kumar *et al.* (2010); Rahuman *et al.* (2000); Yff *et al.* (2002)
4	Hexadecanoic acid, ethyl ester	Nematicide	Kumar *et al.* (2010)
5	Phytol	Antibacterial, antifungal	Ghaneian *et al.* (2015); Lee *et al.* (2016); Saha and Bandyopadhyay (2020)
6	9,12-Octadecadienoic acid (Z,Z)-	Antibacterial	Casillas-Vargas *et al.* (2021)
7	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	Antibacterial, nematicide, insectifuge	Kumar *et al.* (2010); Jung *et al.* (2015)
14	1-Heptacosanol	Antimicrobial	Sharma and Menghani (2017)
18	9,19-Cyclolanost-24-en-3-ol, (3β)-	Antibacterial, antifungal	Ragasa *et al.* (2004); Hasan *et al.* (2014)

Literature survey revealed that some of the compounds identified in *E. prostrata* possess a variety of biological activities including antifungal, antibacterial, larvicidal and nematicidal (Table 2). The most abundant compound 9,19-cyclolanost-24-en-3-ol, (3β)- (24.62%), also known as cycloartenol, was previously identified from a fraction of propolis of *Trigona* spp. in very high concentration (49.91%). This fraction showed profound antibacterial activity against *Escherichia coli* that could be attributed due to activity of this compound (Hasan *et al.,* 2014). Ragasa *et al.* (2004) isolated this compound from *Artocarpus heterophyllus* and reported its antifungal activity against *Candida albicans* and *Aspergillus niger*. It is a triterpenoid belongs to sterol class and is the first point in the synthesis of nearly all plant steroids (Schaller, 2003). The second most abundant compound was 9,19-cyclolanostan-3-ol, 24-methylene-, (3β)- (19.51%), also known as 24-methylenecycloartanol. Although a number of bioactivities including anti-inflammatory (Tsuno *et al.,* 2016), lipase inhibitor, antiobesity (Shaaban *et al.,* 2018) and anticancer (Baniadam *et al.,* 2014) are known, however, it does not have any pesticidal activity. The two major compounds identified in this study were also found as the major constituents in the extract of *Garcinia kola*. Although in purified form these compounds were unable to control bacterial growth, however, they showed synergistic effects with other compounds in the extract and showed antibacterial activity against *Listeria* strains (Penduka *et al.,* 2014). Moderately abundant compound neophytadiene was previously identified in methanolic leaf extract of *Mentha pulegium* as the most abundant compound (69.95%). Antibacterial activity of this leaf extract against *Staphylococcus aureus* and *Salmonella typhimurium* was supposed due to neophytadiene (Ceyhan-Güvensen and Keskin, 2016). *n*-Hexadecanoic acid, also called as palmitic acid, has been reported in various plant species including *Chenopodium murale* (Naqvi *et al.,* 2020) and *Chenopodium quinoa* (Khan and Javaid, 2020b). *n*-Hexadecanoic acid isolated from *Feronia limonia* showed larvicidal activity against fourth instar larvae of *Aedes aegypti, Anopheles stephensi* and *Culex quinquefasciatus* (Rahuman *et al.,* 2000). In addition to that its nematicidal and antibacterial activities are also known (Yff *et al.,* 2002; Kumar *et al.,* 2010). Likewise, hexadecanoic acid, ethyl ester also possesses nematicidal activity (Kumar *et al.,* 2010). Phytol has been found in many plant species including *Ageratum conyzoides* (Ferdosi *et al.,* 2021). It showed antibacterial activity against *Pseudomonas aeruginosa* by inducing oxidative cell death. Cells treated with phytol showed transient NADH depletion and increased reactive oxygen species (Lee *et al.,* 2016). Likewise, phytol isolated from *Adhatoda vasica* controlled *in vitro* and *in vivo* growth of *Bacillus licheniformis* and reduced the mortality rate of goldfish *Carassius auratus* (Saha and Bandyopadhyay, 2020). It also showed antifungal activity against *Candida albicans* and *Aspergillus niger* (Ghaneian *et al.,* 2015). 9,12-Octadecadienoic acid (Z,Z)- or linoleic acid and 9,12,15-octadecatrienoic acid, (Z,Z,Z)- or linolenic acid are unsaturated fatty acids. Such compounds are known for their antibacterial activity and are prepared by algae and higher plants to protect themselves against bacterial invasions (Jung *et al.,* 2015; Casillas-Vargas *et al.,* 2021). 1-Heptacosanol, a fatty alcohol has been found in plants and algae (Murugan and Iyer, 2014), and possesses potent antimicrobial properties (Sharma and Menghani, 2017).

CONCLUSION

This study concludes that ethyl acetate extract of *E. prostrata* contains eight compounds with pesticidal properties. Most of these compounds possess antibacterial property while few of them have antifungal, nematicidal and insecticidal properties.

REFERENCES

Ahemad, M. and M. S. Khan. 2013. Pesticides as antagonists of rhizobia and the legume-Rhizobium symbiosis: a paradigmatic and mechanistic outlook. Biochemistry & Molecular Biology, 1: 63-75.

Ahmad, M., R. A. Khan, F. U. Khan, N. A. Khan, M. S. Shah and M. R. Khan. 2011. Antioxidant and antibacterial activity of crude methanolic extract of *Euphorbia prostrata* collected from District Bannu (Pakistan). African Journal of Pharmacy and Pharmacology, 5: 1175-1178.

Ahmed, S., M. A. Siddique, M. Rahman, M. L. Bari and S. Ferdousi. 2019. A study on the prevalence of heavy metals, pesticides, and microbial contaminants and antibiotics resistance pathogens in raw salad vegetables sold in Dhaka, Bangladesh. Heliyon, 5: e01205.

Ali, A., A. Javaid, A. Shoaib and I. H. Khan. 2020. Effect of soil amendment with *Chenopodium album* dry biomass and two Trichoderma species on growth of chickpea var. Noor 2009 in *Sclerotium rolfsii* contaminated soil. Egyptian Journal of Biological Pest Control, 30: 1-9.

Alsaffar, D. F., A. Yaseen, R. Mahmud and N. H. K. A. Aziz. 2021. Wound Healing Studies of Selected Euphorbia Species: A Review. Annals of the Romanian Society for Cell Biology, 25: 15542-15555.

Ashwin, P., K. Kunal and J. Sagar. 2015. *Euphorbia Prostrata*-a clinically proven drug in hemorrhoids-multiple pharmacological actions targeting pathological processes. International Journal of Medical and Health Sciences, 4: 269-273.

Banaras, S., A. Javaid and A. Shoaib. 2020. Non-Chemical Control of Charcoal Rot of Urdbean by *Sonchus oleraceous* Application. Planta Daninha, 38: e020216088.

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*. International Journal of Agriculture & Biology, 25: 761-767.

Baniadam, S., M. R. Rahiminejad, M. Ghannadian, H. Saeidi, A. M. Ayatollahi and M. Aghaei. 2014. *Cycloartane triterpenoids* from *Euphorbia macrostegia* with their cytotoxicity against MDA-MB48 and MCF-7 cancer cell lines. Iranian Journal of Pharmaceutical Research, 13: 135.

Casillas-Vargas, G., C. Ocasio-Malavé, S. Medina, C. Morales-Guzmán, R. G. Del Valle, N. M. Carballeira and D. J. Sanabria-Ríos. 2021. Antibacterial fatty acids: An update of possible mechanisms of action and implications in the development of the next generation of antibacterial agents. Progress in Lipid Research: 101093.

Ceyhan-Güvensen, N. and D. Keskin. 2016. Chemical content and antimicrobial properties of three different extracts of *Mentha pulegium* leaves from Mugla Region, Turkey. Journal of Environmental Biology, 37: 1341-1346.

Chudasama, R., N. Dhanani, R. Amrutiya, R. Chandni, G. Jayanthi and K. Karthikeyan. 2018. Screening of selected plants from semi-arid region for its phytochemical constituents and antimicrobial activity. Journal of Pharmacognosy and Phytochemistry, 7: 2983-2988.

Daam, M. A., S. Chelinho, J. C. Niemeyer, O. J. Owojori, P. M. C. De Silva, J. P. Sousa, C. A. van Gestel and J. Römbke. 2019. Environmental risk assessment of pesticides in tropical terrestrial ecosystems: test procedures, current status and future perspectives. Ecotoxicology and Environmental Safety, 181: 534-547.

Ferdosi, M. F., I. Haider Khan, A. Javaid, T. Sattar and A. Munir. 2021. Identification of antimicrobial constituents in essential oil from *Paulownia fortunei* flowers. Mycopath, 18: 53-57.

Ferdosi, M. F., I. Khan, A. Javaid and M. F. Fardosi. 2020. Bioactive components in methanolic flower extract of *Ageratum conyzoides*. Pakistan Journal of Weed Science Research, 27: 181-190.

Galt, R. E. and L. Asprooth. 2021. The effects of agrochemicals on humans, Handbook on the Human Impact of Agriculture. Edward Elgar Publishing. pp. 297-332.

Ghaneian, M. T., M. H. Ehrampoush, A. Jebali, S. Hekmatimoghaddam and M. Mahmoudi. 2015. Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environmental Health Engineering and Management Journal, 2: 13-16.

Hasan, A., I. Artika and T. G. Kuswandi. 2014. Analysis of active components of *Trigona spp*. *propolis* from *Pandeglang Indonesia*. Global Journal of Biology, Agriculture & Health Science, 3: 215-219.

Jabeen, N., A. Javaid, A. Shoaib and I. H. Khan. 2021. Management of southern blight of bell pepper by soil amendment with dry biomass of *Datura metel*. Journal of Plant Pathology: 1-13.

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*. Scientific Reports, 11: 8417.

Jung, S. W., S. Thamphiwatana, L. Zhang and M. Obonyo. 2015. Mechanism of antibacterial activity of liposomal linolenic acid against *Helicobacter pylori*. PloS One, 10: e0116519.

Kengni, F., D. S. Tala, M. N. Djimeli, S. P. Fodouop, N. Kodjio, H. N. Magnifouet and D. Gatsing. 2013. *In vitro* antimicrobial activity of *Harungana madagascriensis* and *Euphorbia prostrata* extracts against some pathogenic *Salmonella sp.* International Journal of Biological and Chemical Sciences, 7: 1106-1118.

Khan, I. H. and A. Javaid. 2020. Antifungal activity and GC-MS analysis of n-butanol extract of quinoa (*Chenopodium quinoa* Willd) leaves. Bangladesh Journal of Botany, 49: 1045-1051.

Khan, I. H. and A. Javaid. 2020. Comparative antifungal potential of stem extracts of four quinoa varieties against *Macrophomina phaseolina*. International Journal of Agriculture & Biology, 24: 441-446.

Kumar, P. P., S. Kumaravel and C. Lalitha. 2010. Screening of antioxidant activity, total phenolics and GC-MS study of *Vitex negundo*. African Journal of Biochemistry Research, 4: 191-195.

Lee, W., E. R. Woo and D. G. Lee. 2016. Phytol has antibacterial property by inducing oxidative stress response in *Pseudomonas aeruginosa*. Free Radical Research, 50: 1309-1318.

Lupi, L., F. Bedmar, D. A. Wunderlin and K. S. B. Miglioranza. 2019. Levels of organochlorine pesticides in soils, mesofauna and stream water from an agricultural watershed in Argentina. Environmental Earth Sciences, 78: 1-9.

Mfarrej, M. F. B. and F. M. Rara. 2019. Competitive, sustainable natural pesticides. Acta Ecologica Sinica, 39: 145-151.

Murugan, K. and V. V. Iyer. 2014. Antioxidant activity and gas chromatographic-mass spectrometric analysis of extracts of the marine algae, *Caulerpa peltata* and *Padina gymnospora*. Indian Journal of Pharmaceutical Sciences, 76: 548-552.

Naqvi, S. F., I. H. Khan and A. Javaid. 2020. Hexane soluble bioactive components of *Chenopodium murale* stem. Pakistan Journal of Weed Science Research, 26: 425-432.

Özbilgin, S. and G. S. ÇİTOĞL. 2012. Uses of some euphorbia species in traditional medicine in turkey and their biological activities. Turkish Journal of Pharmaceutical Sciences, 9: 241-255.

Pahlevani, A. H. and H. Akhani. 2011. Seed morphology of Iranian annual species of *Euphorbia* (Euphorbiaceae). Botanical Journal of the Linnean Society, 167: 212-234.

Penduka, D., L. Buwa, B. Mayekiso, A. Basson and A. Okoh. 2014. Identification of the antiListerial constituents in partially purified column chromatography fractions of *Garcinia kola* seeds and their interactions with standard antibiotics. Evidence-Based Complementary and Alternative Medicine, 2014: 850347.

Ragasa, C. Y., K. Jorvina and J. A. Rideout. 2004. Antimicrobial compounds from *Artocarpus heterophyllus*. Philippine Journal of Science, 133: 97-101.

Rahuman, A. A., G. Gopalakrishnan, B. S. Ghouse, S. Arumugam and B. Himalayan. 2000. Effect of *Feronia limonia* on mosquito larvae. Fitoterapia, 71: 553-555.

Saha, M. and P. Bandyopadhyay. 2020. In vivo and in vitro antimicrobial activity of phytol, a diterpene molecule, isolated and characterized from *Adhatoda vasica nees*. (Acanthaceae), to control severe bacterial disease of ornamental fish, *Carassius auratus*, caused by *Bacillus licheniformis* PKBMS16. Microbial Pathogenesis, 141: 103977.

Schaller, H. 2003. The role of sterols in plant growth and development. Progress in Lipid Research, 42: 163-175.

Shaaban, M., M. Ali, M. F. Tala, A. Hamed and A. Z. Hassan. 2018. Ecological and phytochemical studies on *Euphorbia retusa* (Forssk.) from Egyptian Habitat. Journal of Analytical Methods in Chemistry, 2018: 9143683.

Sharma, A. and E. Menghani. 2017. Antibiotic potentials and isolation of metabolomes from microorganisms of mesophilic soil of Rajasthan, India. African Journal of Microbiology Research, 11: 335-344.

Tchuenguem, R. T., J.-R. Kuiate and J. P. Dzoyem. 2017. In vivo Anticandidal Activity of *Euphorbia prostrata*. Journal of Complementary and Alternative Medical Research, 4: 1-10.

Tleuova, A. B., E. Wielogorska, V. P. Talluri, F. Štěpánek, C. T. Elliott and D. O. Grigoriev. 2020. Recent advances and remaining barriers to producing novel formulations of fungicides for safe and sustainable agriculture. Journal of Controlled Release, 326: 468-481.

Tsuno, T., T. Niwano and Y. Kakui. 2016. Anti-Inflammatory agent containing sterol esters for use in cosmetics, quasi-drugs, pharmaceutical and health food. Jpn. Kokai Tokkyo Koho, JP, 2016196418.

Verheyen, J. and R. Stoks. 2019. Current and future daily temperature fluctuations make a pesticide more toxic: Contrasting effects on life history and physiology. Environmental Pollution, 248: 209-218.

Vurro, M., C. Miguel-Rojas and A. Pérez-de-Luque. 2019. Safe nanotechnologies for increasing the effectiveness of environmentally friendly natural agrochemicals. Pest management science, 75: 2403-2412.

Yff, B. T., K. L. Lindsey, M. B. Taylor, D. G. Erasmus and A. K. Jäger. 2002. The pharmacological screening of *Pentanisia prunelloides* and the isolation of the antibacterial compound palmitic acid. Journal of Ethnopharmacology, 79: 101-107.

Contribution of Authors:

Malik F. H. Ferdosi	:	Collection of plant materials and GC-MS analysis
Iqra H. Khan	:	Prepared structures of compounds and contributed in writing and formatting of the manuscript
Arshad Javaid	:	Supervised the work, contributed in writing and finalized the paper
Muhammad Nadeem	:	Contributed in write up
Ayesha Munir	:	Interpreted GC-MS data