Bioactive metabolites of nodule associated microbes for enhanced drought tolerance and biocontrol control activity in horsegram

Abstract:

In the present study, the potential bioactive compounds detected in the ethyl acetate microbial extract of root nodules of horsegram were determined using the gas chromatography-mass spectroscopy (GC-MS). The bioactive metabolites of nodule associated microbes (NAM) revealed the existence of several soluble metabolites which includes phthalic acid, butyl hex-3-yl ester, 2,4-Di-tert-butylphenol,Dodecyl acrylate, pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro, 1-Nonadecene, octadecane, 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester, hexadecanoic acid, methyl ester, dibutyl phthalate, diisooctyl phthalate, 1-docosene, heptadecane, 9-hexyl, pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl), n-Tetracosanol-1, ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'(phenylmethyl)-, (5'à,10à), eicosanal, ethanol, 2-(9-octadecenyloxy), E-15-heptadecenal, octatriacontyl pentafluoropropionate, etc.,. Endophytic bacteria produces bioactive substances that provides resistance against phytopathogens as well as nutrient solubilization during prolonged drought periods in order to overcome biotic and abiotic stresses. Several antibacterial, antifungal, antiviral and antioxidant properties were brought under spotlight to realise the beneficial aspects of nodule associated microbes of horsegram. Understanding the roles of metabolites would enrich the crop growth under stressed environment by promoting the eco-friendly agriculture practices.

Keywords: Metabolites, Bioactive compounds, Antioxidants, Antimicrobials, Rootnodules, Horsegram.

1.Introduction:

Secondary metabolites are low molecular weight organic compound involved in communication between microbial communities, host plants and their resourceful niches unearthing the potential of many defense molecules, growth hormones and stress tolerants and protectants [1]. They can counteract the abiotic stresses caused due to adverse environments by acting as osmoprotectants, ROS scavengers and antioxidants [2]. Studies on endophytes revealed the functional genes for the synthesis of soluble metabolites that stimulates the growth and productivity of crops against biotic and abiotic stresses and thus maintain a symbiotic relationship with plants [3]. Changing the orientation of roots, stomatal regulation, adjustment of osmotic potential and maintaining the homeostasis condition of ROS mechanisms are some of the adaptive techniques by plants against drought conditions [4]. The presence of functional and regulatory genes involved in signalling pathways exhibits an indirect mechanism to tackle drought conditions imposed on plants by regulating oxygen-related metabolisms, activating the expression of osmoregulatory compounds, namely proline and betaine [5].

Bringing forth the massive production of exopolysaccharides, upregulating the gene expression for 1- aminocyclopropane-1-carboxylate (ACC) deaminase, induction of stress tolerant metabolites the enhanced drought tolerance by plant growth growth promoting rhizobacteria [6]. Low molecular organic compounds interactions of various beneficial microorganisms and host plants differ based on phenotypic characteristics, developmental stages of plants lead to the colonization of diverse microbes in below ground and above ground plants [7].

This study focuses on the stress-tolerant properties, osmoregulatory factors, and defensive roles of microbial metabolites extracted from the nodule-associated microbes of horse gram. Understanding the crop-specific metabolic functions may give rise to a significant formulation of metabolites for legume crop production and protection by reducing the use of threatful chemical fertilizers.

2.Materials and Methods:

2.1 Preparation of microbial culture and extraction process:

A total of 10 nodule-associated microbes, namely HGR1, HGB1, HGB2, HGB3, HGB4, HGB5, HGB6, HGB7, HGY1, HGY2 were isolated from horse gram crop. Microbial cultures were grown in Erlenmeyer flask containing Luria-Bertani broth (bacteriological tryptone 10g, yeast extract 5g, NaCl 10g, distilled water 1000ml) incubated in rotary shaker at 110 rpm at 28 °C until it reached the late log phase for metabolite production. The inoculated broth is filtered and extracted using ethyl acetate in a separating funnel [8]. The concentrated metabolites extracted from the inoculated broth were dissolved in 1 mL HPLC grade methanol and used for GC-MS analysis (Perkin Elmer®, USA).

2.2 Characterization of soluble metabolites through GC-MS analysis:

The identification of soluble metabolites present in the different nodule-associated microbial isolates was analyzed with a GC-MS (Gas chromatograph Clarus®, 680 and Mass spectrometer Clarus® SQ8C. The GC-MS studies were performed using a 30 m X 0.32 m capillary column with a cross-band of 5% diphenyl - 95% dimethyl polysiloxane and a 70 eV ionization energy (Elite 5). The oven was set to a temperature of 40 °C for 2 minutes, followed by a 10 °C min^{-1} run to 250 °C, which was held for 2 minutes, and the detector temperature was set to 320 °C, with the input and source lines set to 230 °C. Helium (He) was used as a carrier gas at a linear flow rate of 20 cm^{s-1}. The scanning mode of the MS technique was chosen, with a solvent delay of 0.00 to 0.50 minutes. The GC approach was selected with a 20-minute run duration [9].The MS detector was set to 200 °C, and the substances found were identified using a mass spectral database National Institute of Standards and Technology library (NIST).

3. Result and Discussion:

Ten nodule associated microbes, namely HGR1, HGB1, HGB2, HGB3, HGB4, HGB5, HGB6, HGB7, HGY1, HGY2 isolated from the root nodules of horse gram crop revealed the presence of many bioactive compounds as analyzed through Gas chromatography and mass spectroscopy (**Fig. 1**). Several bioactive metabolites such as phthalic acid, butyl hex-3-yl ester, 2,4-Di-tert-butylphenol,Dodecyl acrylate, pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro, 1-Nonadecene, octadecane, 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester, hexadecanoic acid, methyl ester, dibutyl phthalate, diisooctyl phthalate, 1-Docosene, heptadecane, 9-hexyl, Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl), n-Tetracosanol-1, ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'(phenylmethyl)-, (5'à,10à), eicosanal, ethanol, 2-(9-octadecenyloxy), E-15-Heptadecenal,

octatriacontyl pentafluoropropionate were identified in different peak area percentages and retention time in each microbial isolates (**Fig. 2**). Many of the metabolites with chemical formula and retention time (**Table.1**) were known for renowned of being antioxidant, ROS scavenger, antibacterial, antifungal, antiviral, nematicidal, insecticidal, and drug resistance functions (**Table.2**).

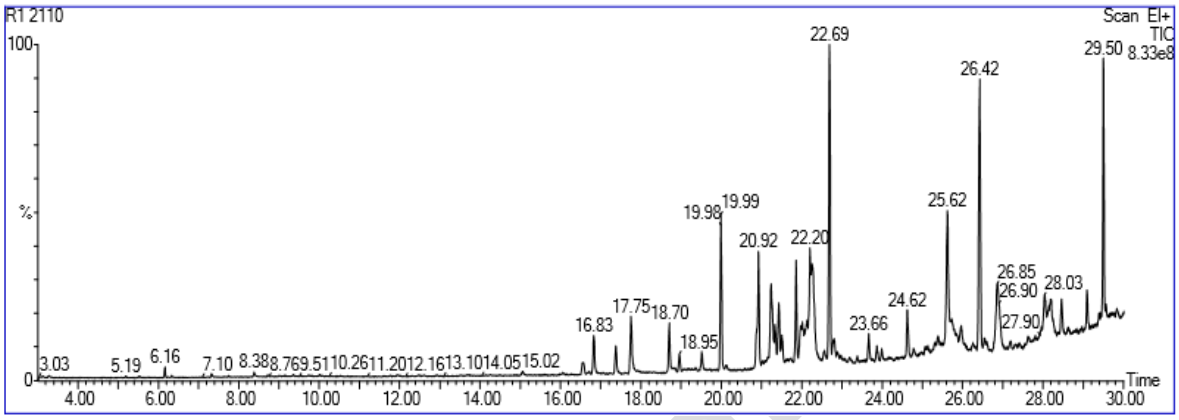

Figure. 1A. GC-MS Chromatogram of HGR1 culture extract

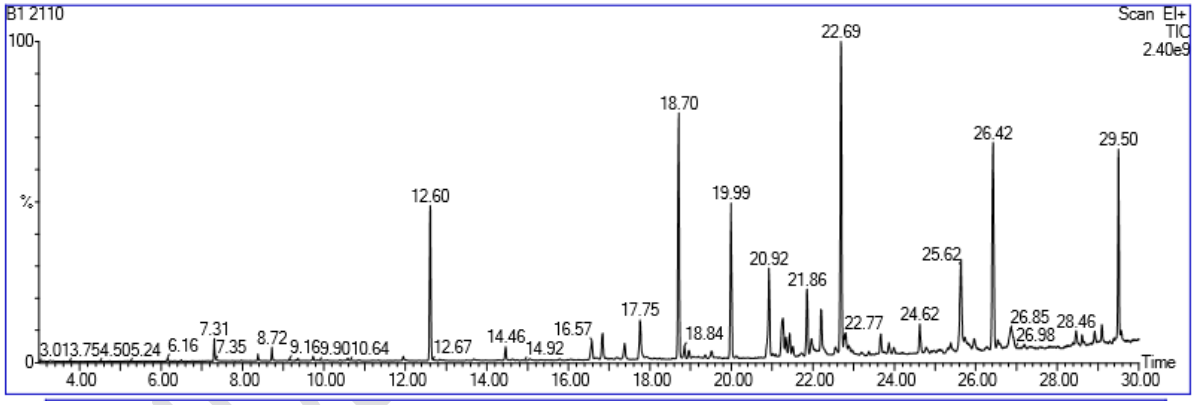

Figure. 1B. GC-MS Chromatogram of HGB1 culture extract

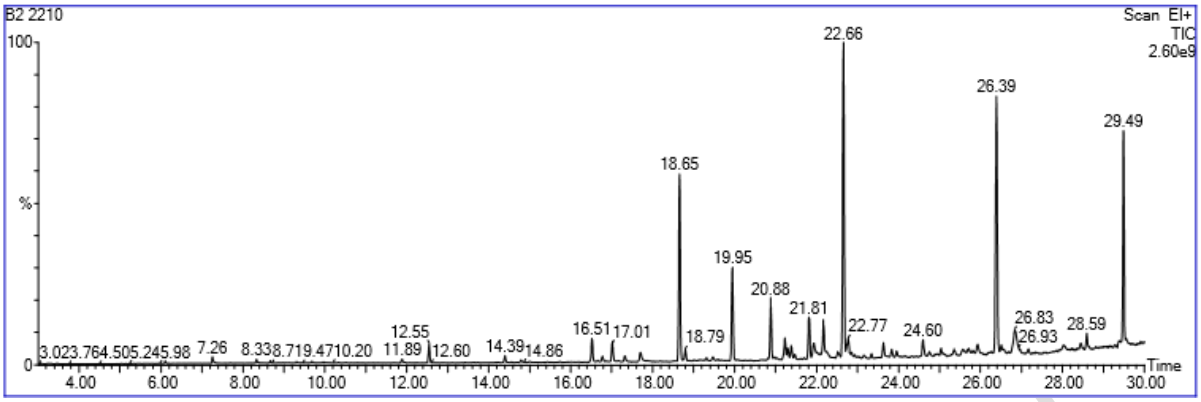

Figure. 1C. GC-MS Chromatogram of HGB2 culture extract

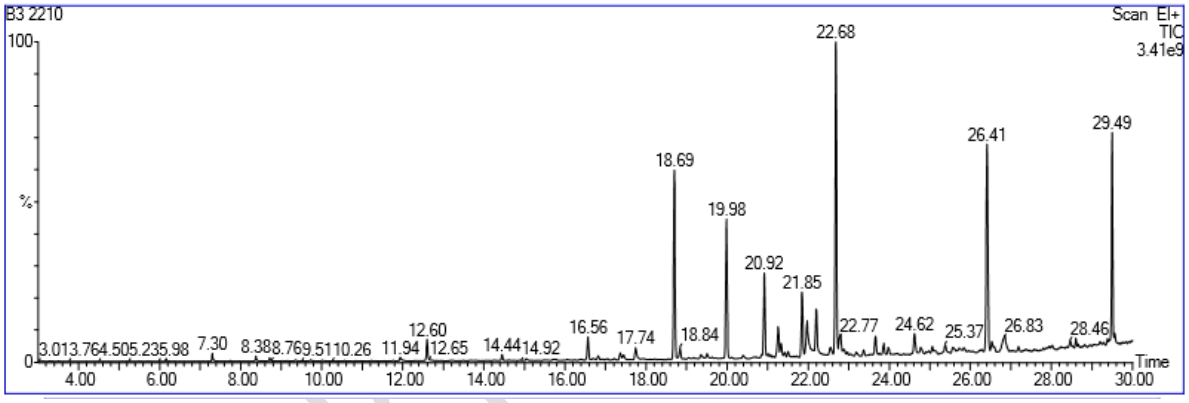

Figure 1D. GC-MS Chromatogram of HGB3 culture extract

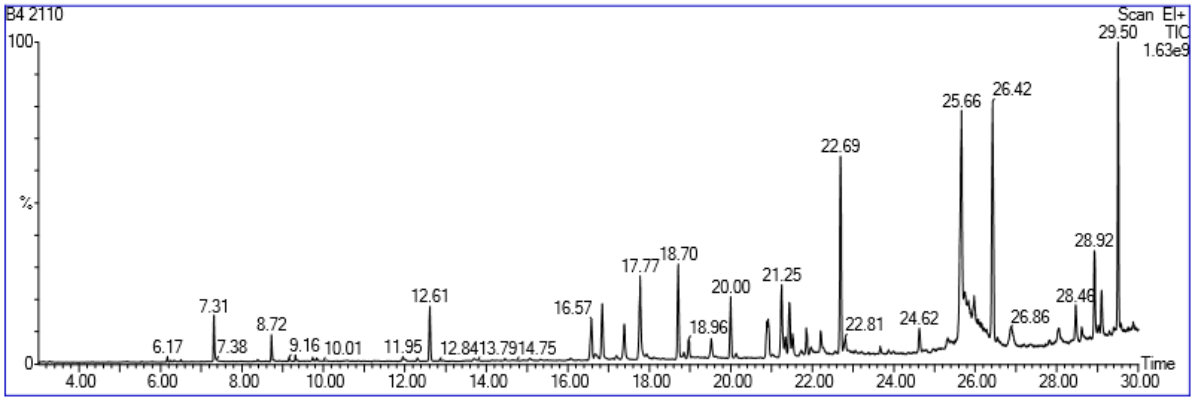

Figure 1 E. GC-MS Chromatogram of HGB4 culture extract

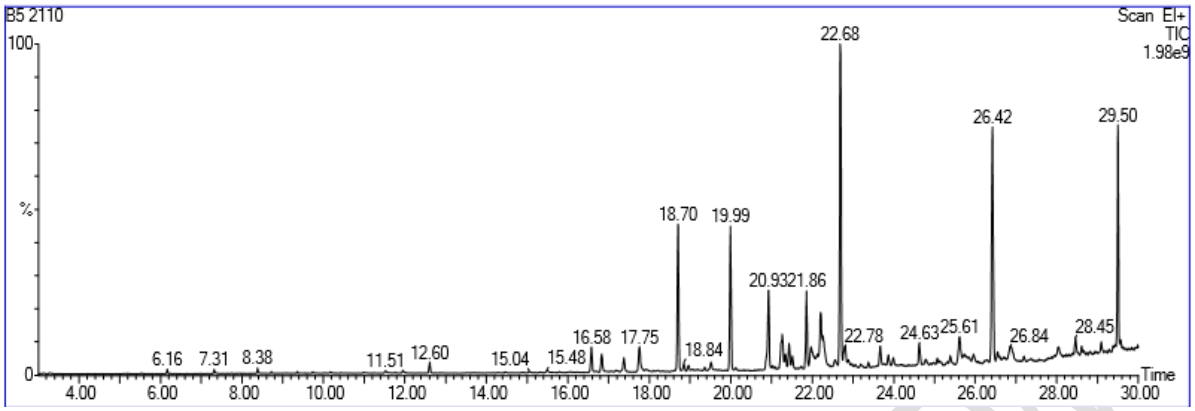

Figure 1F. GC-MS Chromatogram of HGB5 culture extract

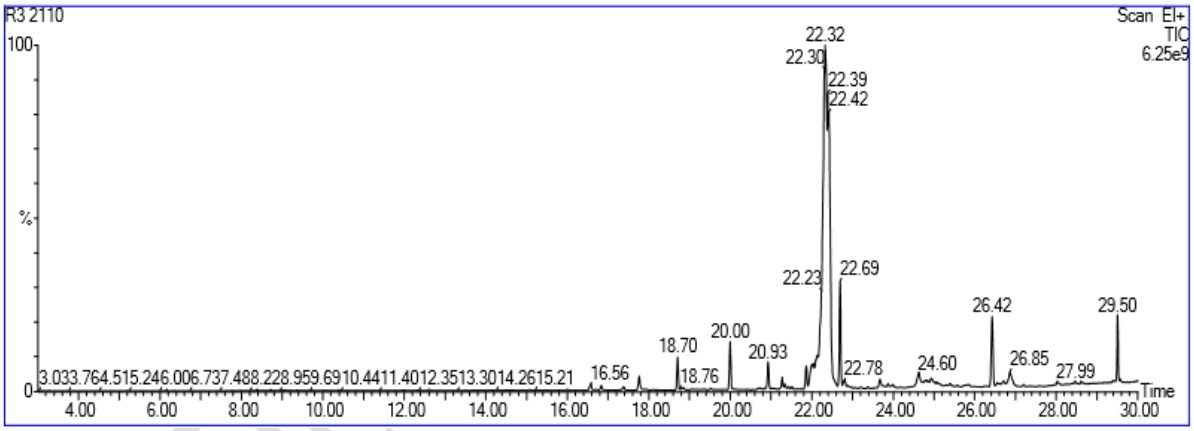

Figure 1G. GC-MS Chromatogram of HGB6 culture extract

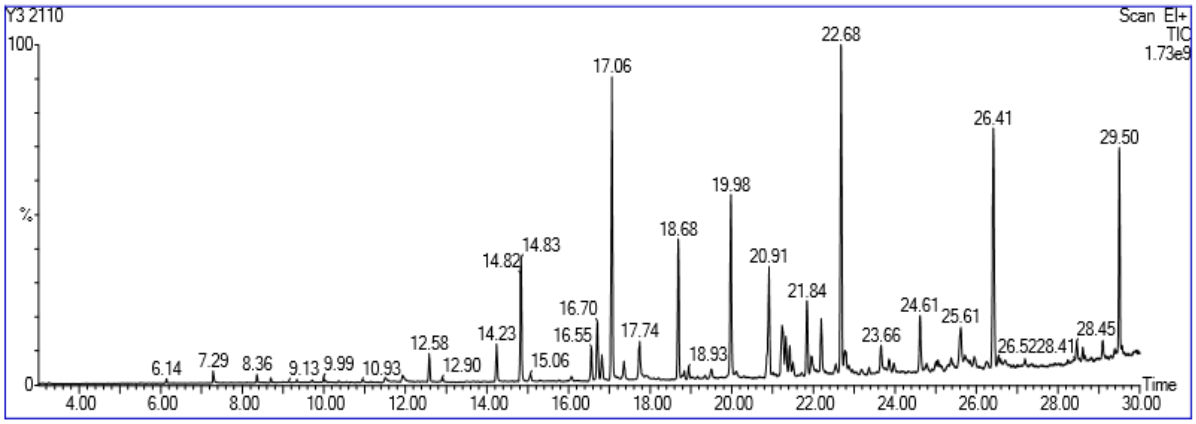

Figure 1H. GC-MS Chromatogram of HGB7 culture extract

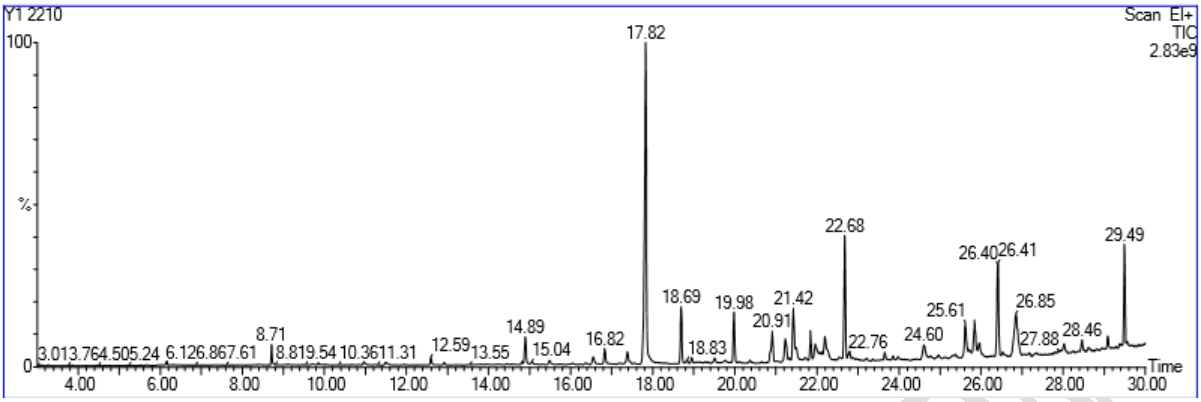

Figure 1I. GC-MS Chromatogram of HGY1 culture

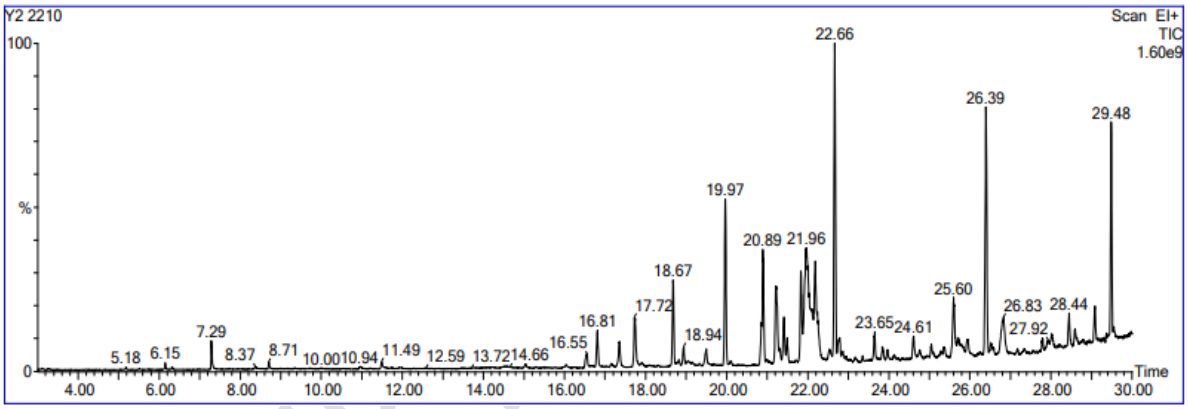

Figure 1J. GC-MS Chromatogram of HGY2 culture extract

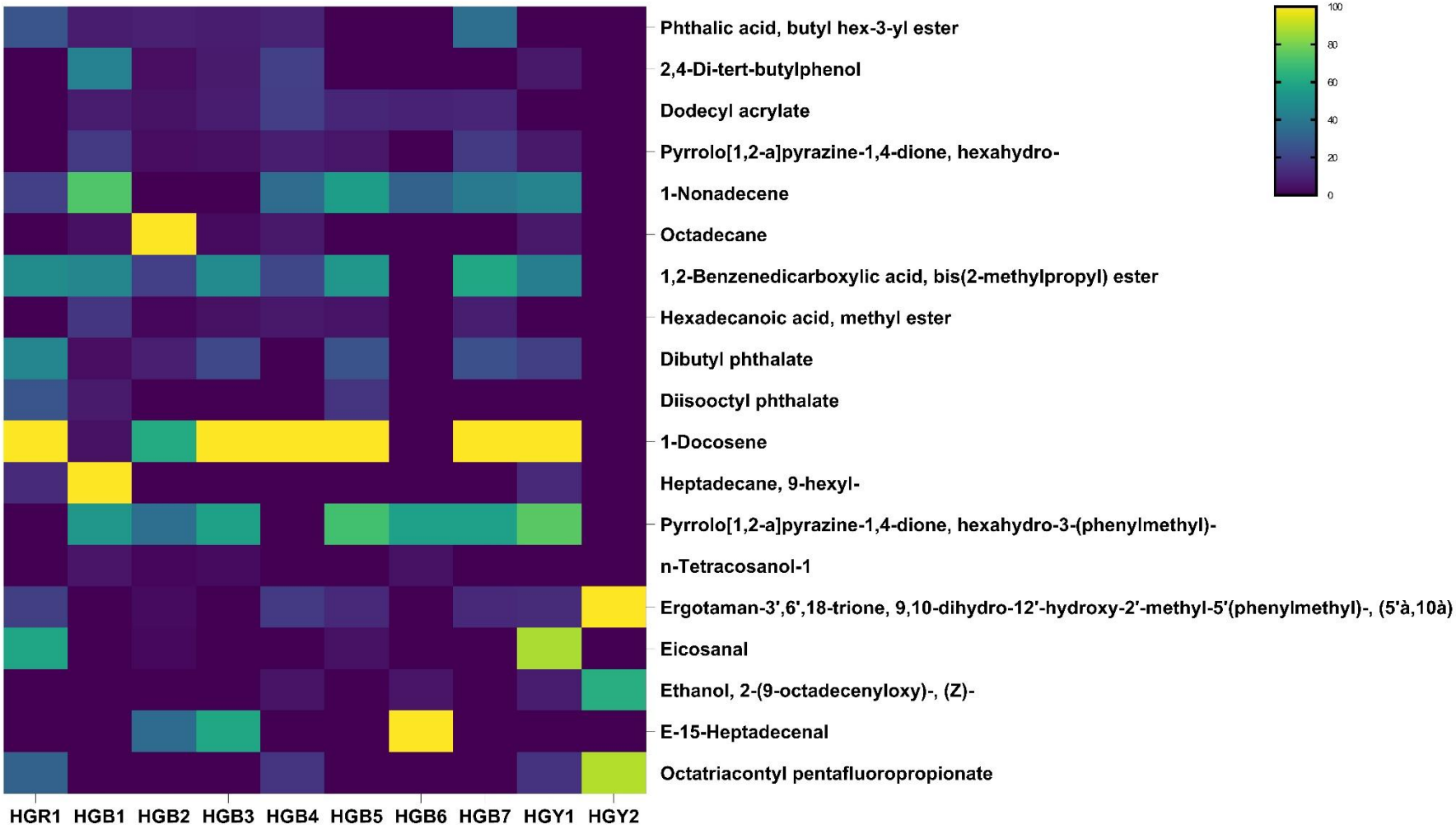

Figure 2: Heatmap representing the relative amount of soluble metabolites present in nodule associated microbes isolated from horsegram crop through GC-MS analysis.

Table 1: Overview of characterized soluble metabolites found in the nodule-associated microbial extract of horsegram crop.

Metabolites	Molecular formula	Retention time
Phthalic acid, butyl hex-3-yl ester	$C_{19}H_{28}O_4$	23.661
2,4-Di-tert-butylphenol	$C_{14}H_{22}O$	12.602
Dodecyl acrylate	$C_{15}H_{28}O_2$	16.569
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	$C_{10}H_{16}N_2O_2$	17.699
1-Nonadecene	$C_{19}H_{38}$	18.705
Octadecane	$C_{18}H_{38}$	18.795
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	19.945
Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	21.291
Dibutyl phthalate	$C_{16}H_{22}O_4$	22.766
Diisooctyl phthalate	$C_{24}H_{38}O_4$	22.566
1-Docosene	$C_{22}H_{44}$	22.686
Heptadecane, 9-hexyl-	$C_{23}H_{48}$	22.796
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	$C_{14}H_{16}N_2O_2$	28.458
n-Tetracosanol-1	$C_{24}H_{50}O$	29.499

Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'(phenylmethyl)-, (5'à,10à)	$C_{33}H_{35}N_5O_5$	28.458
Eicosanal	$C_{20}H_{40}O$	26.868
Ethanol, 2-(9-octadecenyloxy)-, (Z)-	$C_{20}H_{40}O_2$	28.594
E-15-Heptadecenal	$C_{17}H_{32}O$	18.655
Octatriacontyl pentafluoropropionate	$C_{41}H_{77}F_5O_2$	28.043

Table 2: Bioactive metabolites and its properties observed in the nodule-associated cell free extract of horsegram crop.

Metabolites	Properties
Phthalic acid, butyl hex-3-yl ester	Antifungal, antiviral, antibacterial
2,4-Di-tert-butylphenol	Antioxidant and antifungal properties
Dodecyl acrylate	Antimicrobial activity
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	Scavanges ROS members
1-Nonadecene	Antibacterial, antifungal, antioxidant
Octadecane	Nematicidal activity, Antibacterial and antifungal
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	Antibacterial
Hexadecanoic acid, methyl ester	Antibacterial drug resistance
Dibutyl phthalate	Antimicrobial activity
Diisooctyl phthalate	Allelopathic, antimicrobial, insecticidal
1-Docosene	Potent antioxidant
Heptadecane, 9-hexyl-	Antifungal
Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-3-(phenylmethyl)-	Potential antifungal compound.

n-Tetracosanol-1	Antibacterial activity
Ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'(phenylmethyl)-, (5'à,10à)	Antimicrobial (antifungal)
Eicosanal	Antioxidant
Ethanol, 2-(9-octadecenyloxy)-, (Z)-	Antioxidant
E-15-Heptadecenal	Antibacterial compound
Octatriacontyl pentafluoropropionate	Antimicrobial effects

This is the first study to explore the bioactive compounds produced by nodule-associated microbes in horse gram crops. Plants can produce bioactive compounds through natural metabolism, but to increase the amount of low molecular weight compounds, the beneficial eco-friendly plant growth-promoting rhizobacteria are inoculated to enhance the crop growth and production simultaneously bringing down the amendment of hazardous chemical fertilizers [10]. Profiling bioactive peptides produced by rhizobacteria has impacted a visible difference in the yield of potato and maize crops grown in open fields [11]. Some of the bioactive metabolites produced by *P. aeruginosa* PM 105 exhibited antibacterial activity. Additionally, the seeds bacterized with this rhizobacteria increased the germination percentage of seeds, root length, and shoot length and enhanced the secretion of plant growth hormones, namely auxins, gibberellins, and cytokinins [12].

Normal metabolism engages the production of free radicles, but a higher concentration of free radicles leads to oxidative stress as they are highly reactive [13]. Organic metabolite 2, 4-Di-tert-butylphenol produced by *Lactococcus* sp. inhibits the oxidation of biomolecules in the presence of phenolics by neutralizing free radicles to prevent oxidative damage to plants during adverse conditions [14]. ROS production increases due to exogenous factors (environmental change, usage of drugs, harmful radiation) and endogenous factors (generation of ROS in the electron transport chain, oxidation of metabolites, mechanism of

phagocytosis) thus, the presence of microbial antioxidants shields and repairs the oxidative damage by scavenging the immense amount of free radicals [15]. A bioactive compound Glycyl-L-proline extracted from the s*treptomyces* MUM292 has been found to be a potential antioxidant through various bioassays such as ABTS radical scavenging Aactivity, superoxide anion scavenging ctivity, DPPH radical scavenging activity, lipid peroxidation assay, and metal-chelating activities assays [16]. In the present study, we observed [1,2,4]Oxadiazole, 5-benzyl-3-(thiophen-2-yl) [17], hexahydropyrrolizin-3-one [18], glycine, N-(2-amino-1,4-dioxononyl)-, methyl ester [19], tryptophol [20], phenethylpropanamide [21], ticlopidine [22], L-Proline, N-valeryl-, decyl ester [23], trolox [24], phorbol [25] are some of the exclusively present antioxidants extracted by ethyl acetate in horse gram crop.

To withstand biotic and abiotic stresses, endophytic microorganisms produce bioactive compounds that confer resistance against phytopathogens and also render nutrient solubilization and water uptake during extreme drought conditions [26]. Interaction between PGPM and host plant is mediated through signalling compounds that resist a wide range of pathogens as antimicrobial, antifungal, and antiviral molecules [27]. Turning down the need for toxic chemicals against plant pathogens results in an environmentally friendly approach through the production of secondary metabolites [28]. Some of the effective antibacterial bioactive compounds are 5-thiazoleethanol, 4-methyl [29], 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester [30], hexadecanoic acid, methyl ester [31], docosane [32], n-Tetracosanol-1 [33], octadecane, 3-ethyl-5-(2-ethylbutyl) [34], E-15-Heptadecenal [35], Eicosane [36], oxirane, tetradecyl [37], 1H-Carbazole, 2,3,4,9-tetrahydro [38], 1-Nonadecene [39].

Some of the antifungal activity exhibiting organic compounds are 2-Piperidinone [40], heptadecane, 9-hexyl [41], ergotaman-3',6',18-trione, 9,10-dihydro-12'-hydroxy-2'-methyl-5'(phenylmethyl)-, (5'à,10à) [42], androst-4-en-3-one, 17-methoxy-, 3-methoxime [43]. It is interesting to note that several antiviral compounds such as behenic alcohol [44], tetrapentacontane, 1,54-dibromo [45], Dasycarpidan-1-methanol, acetate [46] are produced by nodule associated microbes in horse gram. Despite these concerns, numerous soluble metabolites' physiological and biological importance remains largely unexplored. This is because determining the roles of some bioactive compounds in the microbial ecology is more difficult than determining the roles of antibiotically active natural products. As a result, future studies will focus on identifying the genetic features and mechanisms involved in generating

bioactive metabolites by microorganisms associated with the root nodules of horse gram crop.

4. Conclusion:

The present investigation found that GC-MS analysis of the ethyl acetate extract of ten isolates from root nodules revealed the presence of multiple bioactive constituents responsible for coping against biotic and abiotic stresses. We believe this is the first report of GC-MS analysis of root nodule extract depicting the significant role of bioactive compounds in plant growth promotion. Successful agricultural practices would be encouraged upon formulating ecofriendly soluble metabolites responsible for beneficial effects on plant growth under extreme environments and constructing inbuilt resistance against phytopathogens.

DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge.

References:

1. Sharrar AM, Crits-Christoph A, Méheust R, Diamond S, Starr EP, Banfield JF. Bacterial secondary metabolite biosynthetic potential in soil varies with phylum, depth, and vegetation type. Mbio. 2020;11(3):e00416-20.

2. Arbona V, Manzi M, Ollas CD, Gómez-Cadenas A. Metabolomics as a tool to investigate abiotic stress tolerance in plants. International journal of molecular sciences. 2013;14(3):4885-911.

3. Wang S, Wang J, Zhou Y, Huang Y, Tang X. Prospecting the plant growth–promoting activities of endophytic bacteria Cronobacter sp. YSD YN2 isolated from *Cyperus esculentus* L. var. sativus leaves. Annals of Microbiology. 2022;72(1):1-15.

4. Khan A, Pan X, Najeeb U, Tan DKY, Fahad S, Zahoor R, et al. Coping with drought: stress and adaptive mechanisms, and management through cultural and molecular alternatives

in cotton as vital constituents for plant stress resilience and fitness. Biological research. 2018;51.
5. Yang X, Lu M, Wang Y, Wang Y, Liu Z, Chen S. Response mechanism of plants to drought stress. Horticulturae. 2021;7(3):50.
6. Vurukonda SSKP, Vardharajula S, Shrivastava M, SkZ A. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. Microbiological research. 2016;184:13-24.
7. Kanchiswamy CN, Malnoy M, Maffei ME. Chemical diversity of microbial volatiles and their potential for plant growth and productivity. Frontiers in plant science. 2015;6:151.
8. Tait E, Perry JD, Stanforth SP, Dean JR. Identification of Volatile Organic Compounds Produced by Bacteria Using HS-SPME-GC–MS. Journal of Chromatographic Science. 2013;52(4):363-73. doi: 10.1093/chromsci/bmt042.
9. Narayanasamy S, Uthandi S. Impact of Moisture Stress and *Bacillus altitudinis* FD48 on Physiological Modulation and Seed Germination in Rice (Oryza sativa L.). Madras Agricultural Journal. 2020;107.
10. Rizvi A, Ahmed B, Khan MS, El-Beltagi HS, Umar S, Lee J. Bioprospecting Plant Growth Promoting Rhizobacteria for Enhancing the Biological Properties and Phytochemical Composition of Medicinally Important Crops. Molecules. 2022;27(4):1407.
11. Mülner P, Schwarz E, Dietel K, Junge H, Herfort S, Weydmann M, et al. Profiling for Bioactive Peptides and Volatiles of Plant Growth Promoting Strains of the Bacillus subtilis Complex of Industrial Relevance. Frontiers in Microbiology. 2020;11. doi: 10.3389/fmicb.2020.01432.
12. George E, Kumar SN, Jacob J, Bommasani B, Lankalapalli RS, Morang P, et al. Characterization of the bioactive metabolites from a plant growth-promoting rhizobacteria and their exploitation as antimicrobial and plant growth-promoting agents. Applied biochemistry and biotechnology. 2015;176(2):529-46. doi: 10.1007/s12010-015-1593-3.
13. Tonisi S, Okaiyeto K, Hoppe H, Mabinya LV, Nwodo UU, Okoh AI. Chemical constituents, antioxidant and cytotoxicity properties of *Leonotis leonurus* used in the folklore management of neurological disorders in the Eastern Cape, South Africa. 3 Biotech. 2020;10(3):141-. doi: 10.1007/s13205-020-2126-5.
14. Varsha KK, Devendra L, Shilpa G, Priya S, Pandey A, Nampoothiri KM. 2,4-Di-tert-butyl phenol as the antifungal, antioxidant bioactive purified from a newly isolated *Lactococcus* sp. International Journal of Food Microbiology. 2015;211:44-50. doi: 10.1016/j.ijfoodmicro.2015.06.025.

15. Rani A, Saini KC, Bast F, Mehariya S, Bhatia SK, Lavecchia R, et al. Microorganisms: A potential source of bioactive molecules for antioxidant applications. Molecules. 2021;26(4):1142.
16. Tan LT-H, Chan K-G, Chan CK, Khan TM, Lee L-H, Goh B-H. Antioxidative Potential of *Streptomyces* sp. MUM292 Isolated from Mangrove Soil. BioMed Research International. 2018;2018:4823126. doi: 10.1155/2018/4823126.
17. Siwach A, Verma PK. Therapeutic potential of oxadiazole or furadiazole containing compounds. BMC Chemistry. 2020;14(1):70. doi: 10.1186/s13065-020-00721-2.
18. Druzian SP, Pinheiro LN, Susin NMB, Dal Prá V, Mazutti MA, Kuhn RC, et al. Production of metabolites with antioxidant activity by *Botryosphaeria dothidea* in submerged fermentation. Bioprocess and Biosystems Engineering. 2020;43(1):13-20. doi: 10.1007/s00449-019-02200-y.
19. Yang X, Cui X, Zhao L, Guo D, Feng L, Wei S, et al. Exogenous Glycine Nitrogen Enhances Accumulation of Glycosylated Flavonoids and Antioxidant Activity in Lettuce (*Lactuca sativa L.*). Frontiers in Plant Science. 2017;8. doi: 10.3389/fpls.2017.02098.
20. Herraiz T, Galisteo J. Endogenous and dietary indoles: a class of antioxidants and radical scavengers in the ABTS assay. Free radical research. 2004;38(3):323-331. doi: 10.1080/10611860310001648167.
21. Oloyede GK, Odeja OO, Bamkole OA, Akanmu WA, Onocha PA. Hispanamide: an antioxidant phenolic propanamide from the leaves of Acalypha hispida (burn. f). J Nat Sci Res. 2020;11(14):11-6.
22. Lapenna D, de Gioia S, Ciofani G, Bruno C, Porreca E, Pierdomenico SD, et al. Antioxidant properties of ticlopidine on human low density lipoprotein oxidation. FEBS letters. 1998;436(3):357-60. doi: 10.1016/s0014-5793(98)01166-1.
23. Said SM, Obuid-Allah AH, El-Shimy NA-E, Ali RS, Mahbob MA-E. Analysis of amino acids, fatty acids and neurotoxins using gas chromatography-mass spectrometry in four scorpions species inhabiting New Valley Governorate, Egypt. TURKISH JOURNAL OF ZOOLOGY. 2021;45(6):442-54.
24. Orlo E, Russo C, Nugnes R, Lavorgna M, Isidori M. Natural Methoxyphenol Compounds: Antimicrobial Activity against Foodborne Pathogens and Food Spoilage Bacteria, and Role in Antioxidant Processes. Foods (Basel, Switzerland). 2021;10. doi: 10.3390/foods10081807.
25. Shahab S, Sheikhi M. Antioxidant properties of the phorbol: a DFT approach. Russian Journal of Physical Chemistry B. 2020;14(1):8-15.

26. Fadiji AE, Babalola OO. Elucidating Mechanisms of Endophytes Used in Plant Protection and Other Bioactivities With Multifunctional Prospects. Frontiers in Bioengineering and Biotechnology. 2020;8. doi: 10.3389/fbioe.2020.00467.
27. Lugtenberg B, Rozen DE, Kamilova F. Wars between microbes on roots and fruits. F1000Research. 2017;6.
28. Mohamad OAA, Li L, Ma J-B, Hatab S, Xu L, Guo J-W, et al. Evaluation of the Antimicrobial Activity of Endophytic Bacterial Populations From Chinese Traditional Medicinal Plant Licorice and Characterization of the Bioactive Secondary Metabolites Produced by *Bacillus atrophaeus* Against *Verticillium dahliae*. Frontiers in Microbiology. 2018;9. doi: 10.3389/fmicb.2018.00924.
29. Lau SK, Lam CW, Curreem SO, Lee KC, Chow WN, Lau CC, et al. Metabolomic profiling of *Burkholderia pseudomallei* using UHPLC-ESI-Q-TOF-MS reveals specific biomarkers including 4-methyl-5-thiazoleethanol and unique thiamine degradation pathway. Cell & bioscience. 2015;5:26. doi: 10.1186/s13578-015-0018-x.
30. Zhao J, Jiang L, Tang X, Peng L, Li X, Zhao G, et al. Chemical Composition, Antimicrobial and Antioxidant Activities of the Flower Volatile Oils of *Fagopyrum esculentum*, *Fagopyrum tataricum* and *Fagopyrum Cymosum*. Molecules. 2018;23(1). doi: 10.3390/molecules23010182.
31. Shaaban MT, Ghaly MF, Fahmi SM. Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. Journal of basic microbiology. 2021;61(6):557-68. doi: 10.1002/jobm.202100061.
32. Abdali E, Javadi S, Akhgari M, Hosseini S, Dastan D. Chemical composition and biological properties of Satureja avromanica Maroofi. J Food Sci Technol. 2017;54(3):727-734.
33. Faridha Begum I, Mohankumar R, Jeevan M, Ramani K. GC-MS Analysis of Bio-active Molecules Derived from Paracoccus pantotrophus FMR19 and the Antimicrobial Activity Against Bacterial Pathogens and MDROs. Indian journal of microbiology. 2016;56(4):426-432.
34. Wang M-R, Li W, Luo S, Zhao X, Ma C-H, Liu S-X. GC-MS Study of the Chemical Components of Different *Aquilaria sinensis* (Lour.) Gilgorgans and Agarwood from Different Asian Countries. Molecules. 2018;23(9):2168.
35. Supardy NA, Ibrahim D, Sulaiman SF, Zakaria NA. Inhibition of *Klebsiella pneumoniae* ATCC 13883 cells by hexane extract of *Halimeda discoidea* (Decaisne) and the identification of its potential bioactive compounds. Journal of microbiology and biotechnology.

2012;22(6):872-881. 36. Tonisi S, Okaiyeto K, Hoppe H, Mabinya LV, Nwodo UU, Okoh AI. Chemical constituents, antioxidant and cytotoxicity properties of *Leonotis leonurus* used in the folklore management of neurological disorders in the Eastern Cape, South Africa. 3 Biotech. 2020;10(3):141.

37. Musa AM, Ibrahim MA, Aliyu AB, Abdullahi MS, Tajuddeen N, Ibrahim H, et al. Chemical composition and antimicrobial activity of hexane leaf extract of *Anisopus mannii* (Asclepiadaceae). Journal of intercultural ethnopharmacology. 2015;4(2):129-33. doi: 10.5455/jice.20150106124652.

38. Padmavathi S, Tajne MR. Design, synthesis, molecular docking studies and anti-microbial activity of novel 1, 2, 3, 4-tetrahydrocarbazole derivatives. International Current Pharmaceutical Journal. 2016;5(9):73-8.

39. Khan I, Javaid DA. Antifungal, antibacterial and antioxidant components of ethyl acetate extract of quinoa stem. 2019;3:125-30. doi: 10.33804/pp.003.03.0150.

40. Lawson S, Arumugam N, Almansour AI, Suresh Kumar R, Thangamani S. Dispiropyrrolidine tethered piperidone heterocyclic hybrids with broad-spectrum antifungal activity against *Candida albicans* and *Cryptococcus neoformans*. Bioorganic chemistry. 2020;100:103865. doi: 10.1016/j.bioorg.2020.103865.

41. Amudha P, Jayalakshmi M, Pushpabharathi N, Vanitha V. Identification of bioactive components in *Enhalus acoroides* seagrass extract by gas chromatography–mass spectrometry. Asian Journal of Pharmaceutical and Clinical Research. 2018;11(10):131-7.

42. Lulamba TE, Green E, Serepa-Dlamini MH. *Photorhabdus* sp. ETL Antimicrobial Properties and Characterization of Its Secondary Metabolites by Gas Chromatography–Mass Spectrometry. Life. 2021;11(8):787.

43. Bhutani N, Maheshwari R, Kumar P, Suneja P. Bioprospecting of endophytic bacteria from nodules and roots of *Vigna radiata*, *Vigna unguiculata* and *Cajanus cajan* for their potential use as bioinoculants. Plant Gene. 2021;28:100326.

44. Agbidye IG, Msughter AQ, Chinelo NJ, Iortyom SD. Phytochemical Screening and Antimicrobial Analysis of Hymenocardia acida.

45. Enema O, Umoh U, Thomas P, Adesina S, Eseyin O. Phytochemical and antioxidant studies of leaf of *Tetrapleura Tetraptera* (Schum and thon) Taubert (Mimosaceae). Brifish Journal of Pharmaceufical and Medicinal Research. 2019;4(3):1865-1875.

46. Al-Gara NI, Abu-Serag NA, Shaheed KAA, Al Bahadly ZK. Analysis of bioactive phytochemical compound of (*Cyperus alternifolius* L.) by using gas chromatography–mass

spectrometry. IOP Conference Series: Materials Science and Engineering: IOP Publishing; 2019. p. 012047.