Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Acoustics & Biochemistry

Comparative GC-MS Analysis of Phytochemicals from *Clitorea ternatea* L. (White Flower Variety) Leaves Treated with Planetary Sounds (Planet Earth and Planet Jupiter) and Estimation of Bioactive Compounds

Aditya Kumar Dash[1], Durgamadhab Rath[1], Sanhita Padhi[2*]

[1]Acoustics & Biochemistry Laboratory, PG Department of Botany and Biotechnology, Ravenshaw University, Cuttack, Odisha 753003, India
[2]Professor in Botany, Acoustics & Biochemistry Laboratory, PG Department of Botany and Biotechnology, Ravenshaw University, Cuttack, Odisha 753003, India

DOI: 10.36347/sajb.2023.v11i06.002 | **Received:** 03.05.2023 | **Accepted:** 12.06.2023 | **Published:** 18.06.2023

***Corresponding author:** Sanhita Padhi
Professor in Botany, Acoustics & Biochemistry Laboratory, PG Department of Botany and Biotechnology, Ravenshaw University, Cuttack, Odisha 753003, India

Abstract **Original Research Article**

The butterfly pea (*Clitoria ternatea* L.) is a plant with various potential health benefits due to the presence of many bioactive compounds. The leaves contain triterpenoids, flavonoids, glycosides, and alkaloids. In the present experimental work, the leave extracts treated with planetary sound (Sound of Earth and Sound of Jupiter) classified as Medium-low frequency sound waves (200 Hz to 1 KHz), in methanol were checked for bioactive components through GC-MS and the third set was kept as control. Different bioactive compounds identified, were further estimated by using Lupeol (Merck, India) as an external standard. In compounds common among plants treated with Earth's sound and control, 3-O-Methyl-d-glucose from plants treated with Earth's sound was highest at 1.20 PPM. Compounds common among plants treated with Jupiter's sound and control, 3-Hexenal from plants treated with Jupiter's sound was found to be highest with 1.87 PPM, Similarly, compounds common among plant's treated with Earth's sound and Jupiter's sound, Gamma sitosterol from leaves of plants treated with Earth's sound was found to have highest value i.e, 1.27 PPM. Compounds that were common for all the three groups, plants treated with Earth's sound was found to have increased Squalene with 1.23 PPM. A general trend of phytochemicals from plants treated with planet Earth's sound had an elevated value. This suggests that the treatment of planet Earth's sound has tremendous impact on the production of secondary metabolites in the referred species.
Keywords: Butterfly pea, Medium-low frequency, Lupeol, GC-MS, Planetary Sound.

INTRODUCTION

Phytoacoustics is a newly emerging field of research that explores the ability of plants to emit sounds and respond to sounds in their environment. Plants have been shown to sense and respond to various stimuli such as light, volatiles, touch, and sound (Khait *et al*., 2019).

Sound reception can provide significant advantages for plants as it is a fast-traveling sensory input that is naturally present in the environment. Sounds carry valuable information about the presence of pollinators, herbivores, frugivores, weather conditions, and essential resources like water. Therefore, it is expected that natural selection would favour plants with even basic abilities to respond to sounds, leading to improved variety on continuous applications.

Recent studies have demonstrated that plants exhibit physiological responses to sound stimuli. They have also revealed morphological adaptations in plants that are related to acoustic signalling. Furthermore, the emission of sound by plants themselves has been documented (Khait *et al*., 2023). Sound-induced changes in calcium ion (Ca2+) signatures and reactive oxygen species (ROS) concentrations, as well as differential gene expression, have been observed in Arabidopsis (Monshausen *et al*., 2009).

Overall, the study of Phytoacoustics provides insights into how plants perceive and interact with their acoustic environment. Understanding the adaptive significance of sound responsiveness in plants can shed light on their ecological roles and interactions with other organisms.

During the Voyager I mission, a device known as the "Plasmawave recorder" was utilized to capture and convert different forms of radiation and waves into audible sounds. This instrument was specifically designed to investigate various plasma waves and their interactions in the vastness of deep space. By converting these phenomena into audible sounds, scientists and researchers could study and analyse the complex patterns and behaviours of plasma waves and their effects in the interstellar environment. This provided valuable insights into the nature of electromagnetic phenomena, radio waves, and other interactions occurring within the planetary magnetosphere, ionosphere, and solar winds. Additionally, the instrument allowed for the detection and recording of natural occurrences such as earthquakes, lightning storms, chemical rain, volcanic eruptions, and the rotational movements of planetary bodies. The availability of these planetary sounds collected by NASA offers a unique opportunity to explore and appreciate the diverse and dynamic nature of our cosmic surroundings and this can be used to understand the underline of a favourable environment and finding the existance of life hence, leading to Human colonization (Dash *et al.*, 2009).

Clitoria ternatea L., commonly known as butterfly pea or blue pea flower, is a perennial climber belonging to the Plantae kingdom, Tracheophyta phylum, Magnoliopsida class, and Fabaceae family. It is grown as an ornamental plant and is distributed in various regions, including India, the Philippines, and tropical Asian countries. The plant has blue flowers and different flower colour variations. It is also used as a food colorant in Southeast Asia. Butterfly pea is known for its ability to suppress weeds, enrich soil through nitrogen fixation, and is considered a nootropic herb in Ayurvedic medicine. The plant prefers full sunlight or partial shade and has pinnate leaves and elongated seed pods. The seeds are oval-shaped and can be consumed when tendered (Jeyaraj *et al*., 2020).

Reports on C. ternatea flowers contain protein, fibre, carbohydrates, and fats, along with high moisture content. They are also rich in minerals such as calcium, magnesium, potassium, zinc, sodium, and iron. The flowers contain various bioactive compounds, including anthocyanins (ternatins), flavonols, fatty acids, phytosterols, tocols, and other identified components (Kalmankar *et al*., 2020). Through RNA-Seq data and de novo transcriptome assembly, the researchers identified 71 cyclotide precursor genes in C. ternatea. Among these genes, 51 sequences displayed unique cyclotide domains, including 26 novel cyclotide sequences, which were found in four different tissues of the plant. The cyclotide diversity was confirmed through MALDI-TOF mass spectrometry analysis of tissue extracts coupled with the precursor protein sequences obtained from the transcriptomic data (Villareal *et al*., 2013).

MATERIALS AND METHODS

Soil Collection

In this experiment, two materials were used: alluvial soil and coco peat. The alluvial soil was collected from the riverbank of Mahanadi River Basin at Jobra, Cuttack, with the coordinates 20°28.0199'N 85°53.7779'E. The collection took place in December 2017. On the other hand, coco peat was procured from the Regional Plant Resource Center (RPRC) located at CRP Ekamra Kanana Road, IRC Village, Nayapalli, Bhubaneswar, Odisha. For soil preparation, alluvial soil was 40 % and coco peat used was 60% (Oguis *et al*., 2019). The two materials were mixed thoroughly to ensure proper blending. The resulting mixture was then packed into trays with dimensions of L=33cm, B=17cm, and H=14cm.

Seed Collection

The seeds of Clitoria ternatea L. (White Variety), specifically the Aparajita variety, were collected from the "Simlipal National Bio-reserve Forest" located in the Mayurbhanj district of Odisha, India. The collection site has coordinates 21°50′N 86°20′E and is situated in the northern part of Odisha.

To facilitate further experiments, a larger quantity of seeds, amounting to three kilograms, was procured in the summer month of January 2018. These seeds were carefully inspected, tested, and approved by a senior scientist at the Regional Plant Resource Center (RPRC) located at CRP Ekamra Kanana Road, IRC Village, Nayapalli, Bhubaneswar, Odisha. The procurement process ensured that the seeds met the necessary quality standards for conducting subsequent experiments.

Sound sourcing

To provide the planetary sound stimulus, a Philips SPA8140B/94 40 W 4.1 Channel music system was employed. The system played the planetary sound for three hours a day, with a one-hour gap after each application, for the plant system to absorb the stimulus. The sound recordings of both Earth and Jupiter were obtained from NASA's official website [23]. The sound files were further analysed using Audacity© software, specifically version 3.2.3.

The analysis revealed that the sound of Jupiter had a frequency of 44100 Hz, while the sound of Earth had a frequency of 22050 Hz. Both sound waves fell within the medium-high frequency range of audible sound.

Seeding and growing of Plants

In this study, a total of 20 pots were used and divided into two groups: Control and Planetary sounds (Earth and Jupiter). Each pot was then used to sow *Clitoria ternatea* L. (White variety), Aparajita seeds. The seeds were arranged in three rows and five columns within each pot, resulting in a total of 15 seeds placed in a single pot. After planting the Aparajita seeds, all the trays were marked and divided into three batches: Control, seeds treated with Planet Earth's sound, and seeds treated with Planet Jupiter's sound. Each batch consisted of 20 pots, and each pot contained 15 seeds.

The trays from all three batches were placed in a controlled environment with a room temperature of about 28°C to 30°C (Purnadurga et al, 2018). The humidity and light conditions were also controlled, and the plants were exposed to light for a duration of 4 to 5 hours. This controlled environment ensured that the germination and growth conditions were consistent across all batches, allowing for a more accurate comparison of the effects of the different treatments on the Aparajita seeds.

For the experimental setup, three identical rooms were allocated to house the different batches: the control group, seeds growing under the influence of Planet Jupiter's sound, and seeds growing under the influence of Planet Earth's sound. Each room had the same dimensions, measuring 548.7 cm (length) x 335.3 cm (width) x 305 cm (height). In each room, a table was placed at the center, with dimensions of 72 cm (length) x 165 cm (width) x 85.34 cm (height) (L x B x H). This table served as the base for the setup.

All three setups, including the control and the two experimental groups, were subjected to the same physiological conditions. This ensured that the environmental factors such as temperature, humidity, and light were consistent across all setups. By maintaining these conditions, any observed differences in the growth and development of the Aparajita seeds could be attributed to the specific treatment received (control, Jupiter's sound, or Earth's sound) rather than variations in the physiological conditions.

Sampling of leaves

After two months of growth, plant leaves were taken from each setup, dried in dehydrator at 35°C for 24 hours. The dried matter was crushed and the plant extract prepared using absolute alcohol, Methanol using Soxhlet apparatus. The extract was further dried using rotary vacuum evaporator. The dried plant extract was stored at 4 ° C for further use.

Gas Chromatography Mass Spectrometry (GC-MS) Analysis

In the process of analysing the methanolic extract of control and treated samples, several steps were followed. After the initial processing, which involved the addition of 250 mg/ml sodium sulfate, the extract underwent filtration using PTFE syringe filters with a pore size of 0.2 microns and a diameter of 13mm. This filtration process ensured the removal of any particulate matter or impurities from the samples.

Once filtered, the samples were transferred to GC vials for further analysis. The GC-MS (Gas Chromatography-Mass Spectrometry) analysis was performed using a Shimadzu TQ-8040 system with an SH-Rtx-5MS column. The following conditions were employed during the analysis:

Sample runtime: Approximately 45 minutes in mass spectrum, Column: SH-Rtx-5MS

Each component was identified by analyzing their retention time and the percentage of the peak area of each compound. The identification was done using the NIST structural library [7]. Furthermore, the quantification of bioactive metabolites was carried out using lupeol as the external analytical standard, obtained from Sigma. Lupeol is a reference compound used to quantify the target compounds present in the samples. Overall, the GC-MS analysis allowed for the identification and quantification of various compounds present in the methanolic extract, providing insights into the composition and potential bioactive metabolites of the control and treated samples.

The GC-MS analysis of the methanolic extract was performed using the following conditions:

Table 1: Showing GC-MS conditions implemented during a run

Ion source temperature	200°C
Interface temperature	280°C
Solvent cut time	2 minutes
Column oven temperature	50°C
Injection temperature	250°C
Injection mode	Splitless
Sampling time	1.00 minute
Carrier gas	Helium
Pressure	100.1 kPa
Column flow	1.69 ml/min
Column thickness	0.25 µm
Column length	30 m
Column diameter	0.25 µm

These conditions were set to ensure optimal separation, retention, and analysis of the compounds present in the methanolic extract. The specific temperature settings, injection mode, sampling time, carrier gas, pressure, column flow, and dimensions of the column were chosen to achieve accurate and reliable results in the GC-MS analysis.

RESULT

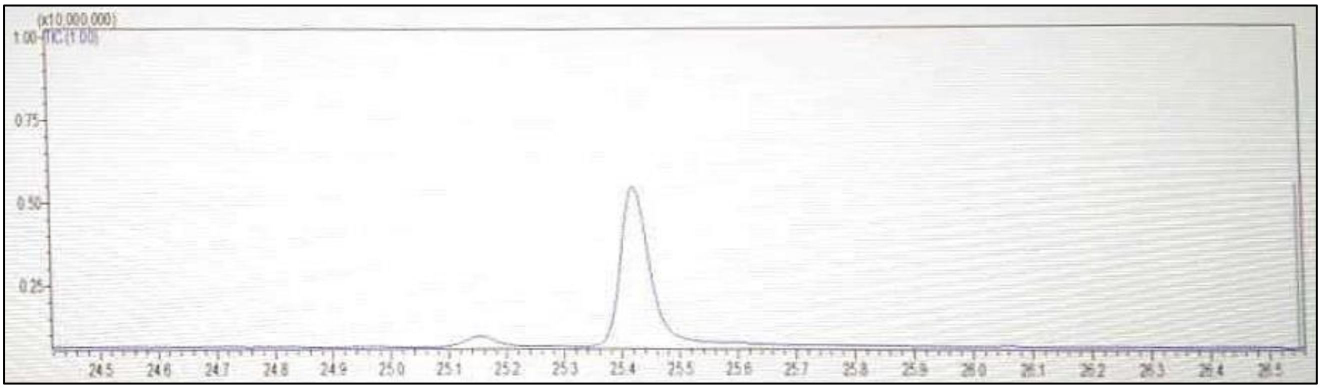

Figure 1: Chromatogram of 2.5 PPM Lupeol injection

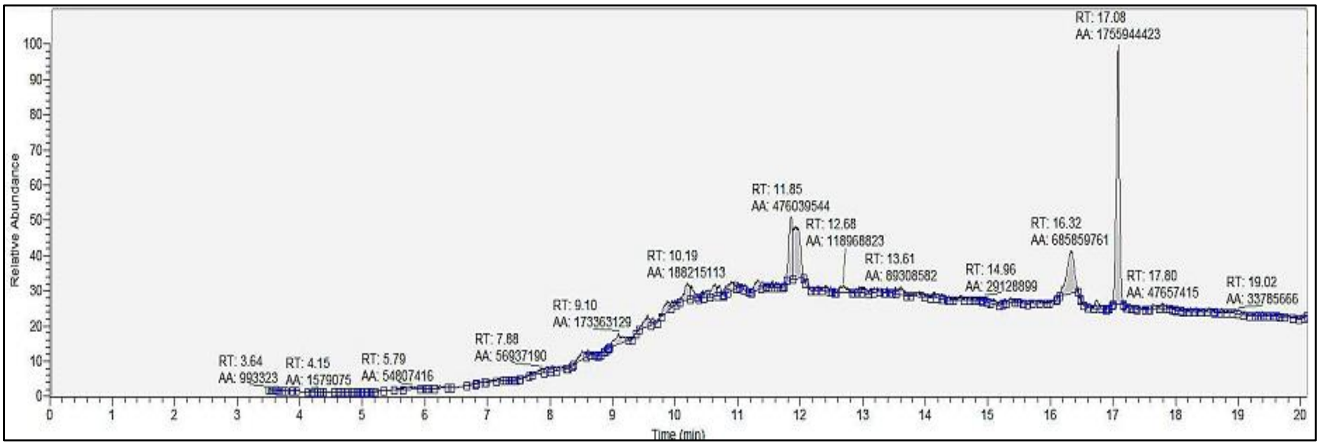

Figure 2: Chromatogram of 14 PPM Lupeol injection

Table 2: Standard grid for Lupeol with peak area and concentration

Sl. No	Sample	Molecular Formula	Molecular Weight	Peak Area	Concentration (PPM)
1	Lupeol	$C_{30}H_{50}O$	426.7 g/mol	0	0
2	Lupeol	$C_{30}H_{50}O$	426.7 g/mol	16372881	2.5
3	Lupeol	$C_{30}H_{50}O$	426.7 g/mol	1755944423	14

Quantification of Bio active compounds

To prepare the standard solutions, Lupeol (Sigma) was dissolved in LC-MS grade methanol (99.99%) at concentrations of 2.5 ppm and 14 ppm. These standard solutions were then injected into the GC-MS system, maintaining the previously mentioned conditions (Table 1).

During the analysis, the elution time for Lupeol was found to be 25 minutes for the 2.5-ppm standard and 17.08 minutes for the 14-ppm standard (Table 2). Both standards exhibited a single peak in the chromatogram, as shown in Figure 1 and Figure 2.

A linear progression standard graph was constructed by plotting the peak area of the Lupeol standard solutions against their respective concentrations (ppm). From this Figure 3, the line formula was derived, allowing for the calculation of the concentration of all the bioactive compounds present in the samples, as outlined in

The standard graph provides a quantitative relationship between the peak area and the concentration of the bioactive compounds, enabling the determination of their concentrations in the analysed samples.

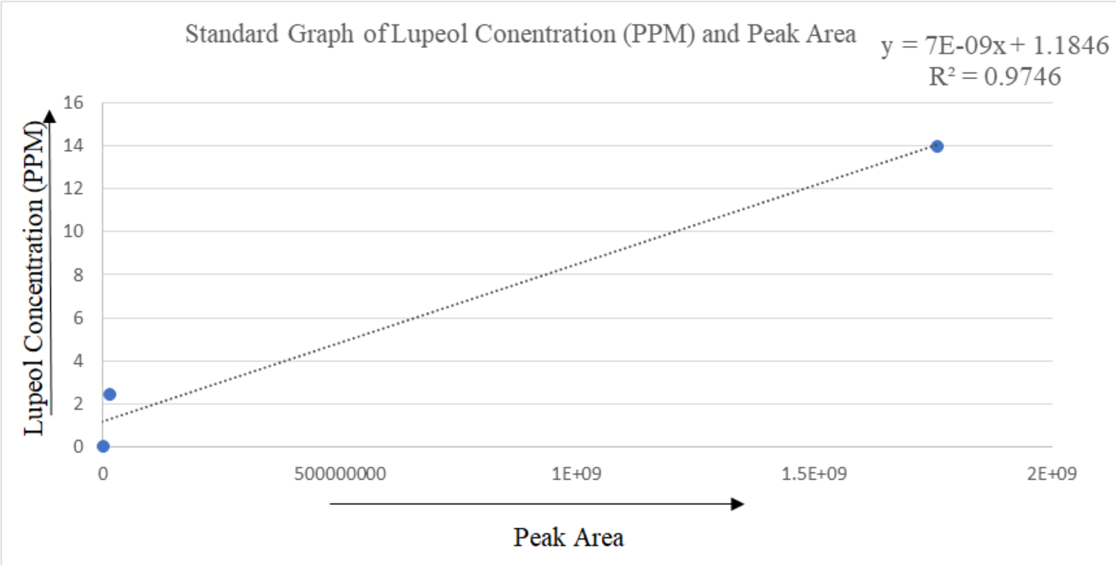

Figure 3: Standard plot of Lupeol as external standard

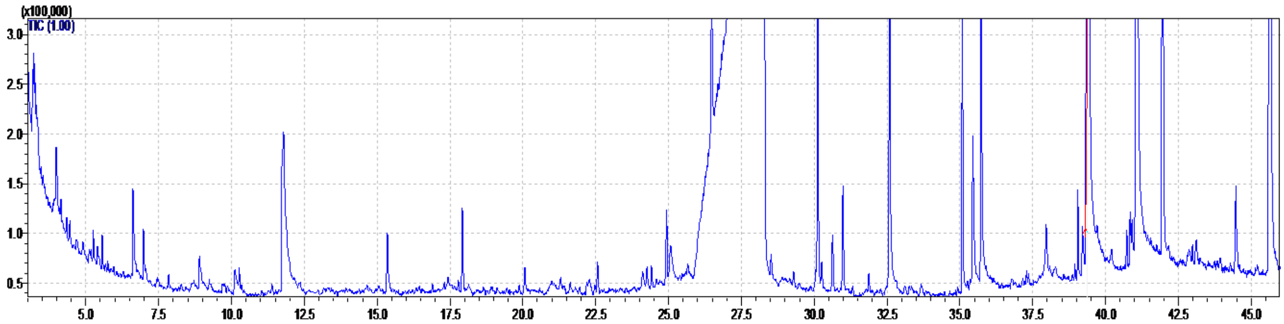

Figure 4: GC-MS Chromatogram of methanol extract made from leaves of control plants

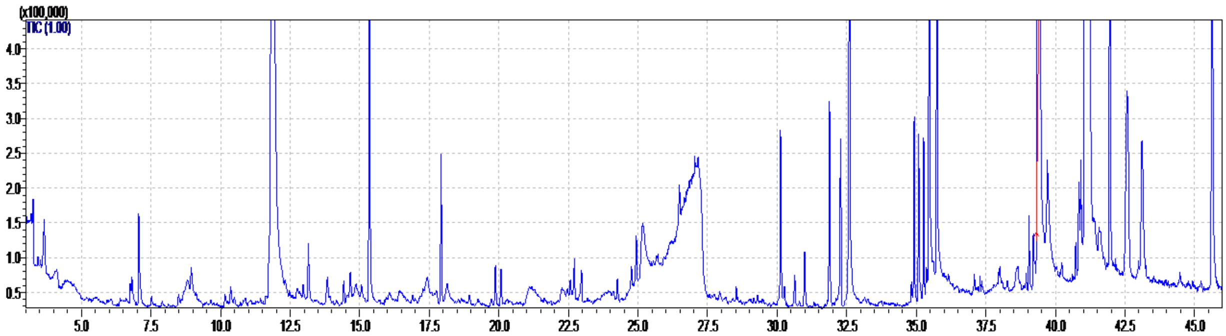

Figure 5: GC-MS Chromatogram of methanol extract made from leaves of plants treated with planet Earth's Sound

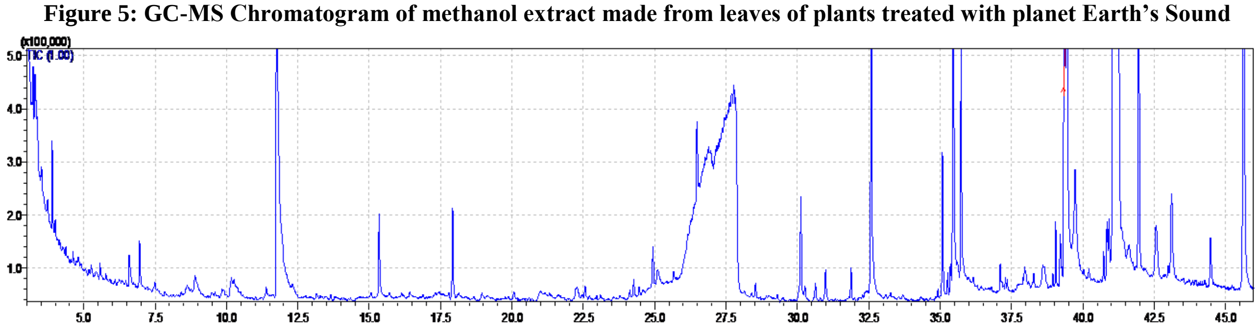

Figure 6: GC-MS Chromatogram of methanol extract made from leaves of plants treated with planet Jupiter's Sound

Table 3: Compounds from GC-MS analysis found common in plants treated with Earth's sound and control, Jupiter's sound and control, and plants treated with Jupiter's and Earth's Sound

Sl. No	Compounds Present only in Treated (the Earth's Sound) and in Control	Chemical Group	Canonical SMILES String	Biological Activity/Uses	Reference
1	Benzaldehyde, 2-methyl-	benzoyl derivatives	CC1CCCCC1C=O	Plant Metabolite, Flavouring agent	(Masoudi *et al.*, 2009) (Dionisio *et al.*, 2018) (NCBI, 2023)
2	4-O-Methylmannose	Polysaccharides	COC1C(CO)OC(O)C (O)C1O	Antigenic	(Masuzawa, *et al.*, 1996) (NCBI, 2023)
3	3-O-Methyl-d-glucose	D-aldohexose	COC(C(C=O)O)C(C (CO)O)O	Marker to assess glucose transport	(NCBI, 2023)
Sl No	**Compounds Present in one of the treated (Jupiter's sound) and control**	**Chemical Group**	**Canonical SMILES String**	**Biological Activity/Uses**	**Reference**
1	3-Hexenal	unsaturated aldehyde	CCC=CCC=O	Flavoring Agents	(Dionisio *et al*., 2018) (NCBI, 2023)
2	Decane, 1-bromo-2-methyl-	branched alkanes	CCCCCCCCC(C)CBr	Natural Pheromone	(Francke *et al*., 2000) (NCBI, 2023)
Sl No	**Compound present only in Treated (Control's and Earth's Sound)**	**Chemical Group**	**Canonical SMILES String**	**Biological Activity/Uses**	**Reference**
1	Linoelaidic acid	Linoleic acid and derivatives	CCC=CCC=CCC= CCCCCCCCC(=O)O	a omega-3 fatty acid, micronutrient, a nutraceutical and a mouse metabolite	(NCBI, 2023) (Azam *et al*., 2021) (Nomura *et al*., 2023)
2	Gamma-Sitosterol	stigmastanes and derivatives	CCC(CCC(C)C1CCC2C1 (CCC3C2CC=C4C3(CCC (C4)O)C)C)C(C)C	Phytosterol biosynthesis by-product, a plant metabolite and a marine metabolite	(Sanyal, 2021) (NCBI, 2023)
3	Lupeol	triterpenoids	CC(=C)C1CCC2 (C1C3CCC4C5 (CCC(C(C5CCC4 (C3(CC2)C)C)(C)C)O)C)C	Anticancer, anti-inflammatory, melangenic agent, antioxidant, anti-microbial and antiprotozoal activity	(NCBI, 2023) (Rath *et al*., 2023)

Table 4: Compounds from GC-MS analysis found common in plants treated with Earth's sound, Jupiter's Sound and Control Plants

Sl No.	Compounds Common in all treated Samples and the control	Chemical Group	Canonical SMILES String	Biological Activity/Uses	Reference
1	1-Butanol, 3-methyl-, acetate	Isoamyl Acetate	CC(C)CCOC(=O) C	small molecule agonists of the thyroid stimulating hormone receptor (TSHR) signalling pathway, Flavoring Agents and Cosmetics	(Lewis, 2007) (NCBI, 2023)
2	2-Methoxy-4-viny lphenol	Phenols and Phenolics	COC1=C(C=CC(= C1)C=C)O	Cytochrome P450 Family 3 Subfamily Intermediate antagonistic molecule, in Sonic Hedgehog pathway, Food additives, Plant Growth Promoter, Pesticide, Antioxidant	(Hapsari *et al*., 2023) (Nagrale *et al*., 2023) (NCBI, 2023)
3	Neophytadiene	Diterpene	CC(C)CCCC(C)C CCC(C)CCCC(=C) C=C	anti-inflammatory agent, an antimicrobial agent, a plant metabolite and an algal metabolite	(NCBI, 2023) (Toh *et al*., 2023)
4	1-Octadecyne	Aromatic hydrocarbons	CCCCCCCCCCC CCCCCC#C	Volatile by-product of Cyanobacteria	(Armstrong *et al*., 2019) (NCBI, 2023)
5	Phytol	Acyclic diterpenoids	CC(C)CCCC(C)C CCC(C)CCCC(=C	a plant metabolite, a schistosomicide drug and an algal metabolite	(Song *et al*., 2023) (Angupale

Sl No.	Compounds Common in all treated Samples and the control	Chemical Group	Canonical SMILES String	Biological Activity/Uses	Reference
			CO)C		*et al*., 2023) (NCBI, 2023)
6	Octadecanoic acid	Saturated fatty acid	CCCCCCCCCCCCCCCCCC(=O)O	Antimicrobial, Antioxidant, hardens soap, Softens Plastic, used in cosmetics, plant metabolite, a human metabolite, a Daphnia magna metabolite and an algal metabolite	(NCBI, 2023) (Akpuaka *et al*., 2013) (Keawsa-Ard *et al*., 2012)
7	Squalene	Isoprenoid compound	CC(=CCCC(=CCCC(=CCCC=C(C)CCC=C(C)CCC=C(C)C)C)C)C	anticancer, antioxidant, drug carrier, detoxifier, skin hydrating, and emollient activity, intermediates of cholesterol synthesis, Skin protection, a human metabolite, a plant metabolite, a Saccharomyces cerevisiae metabolite and a mouse metabolite	(Kim & Karadeniz, 2012) (Huang *et al*., 2009) (NCBI, 2023)
8	Vitamin E	Methylated phenols	CC1=C(C2=C(CCC(O2)(C)CCCC(C)CCCC(C)CCCC(C)C)C(=C1O)C)C	an antioxidant, a nutraceutical, an antiatherogenic agent, an EC 2.7.11.13 (protein kinase C) inhibitor, an anticoagulant, an immunomodulator, an antiviral agent, a micronutrient, an algal metabolite and a plant metabolite	(NCBI, 2023)

Table 5: Compounds from GC-MS analysis found common in plants treated with Earth's sound and control, Jupiter's sound and control, and plants treated with Jupiter's and Earth's Sound and their estimation using standard plot (Figure 1)

Sl. No	CAS	Compounds Present only in Treated (the Earth's Sound) and in Control	Molecular formula	Molecular weight	Peak area (%) Control Plants	Peak area (%) Plants treated with Earth's Sound	Concentration of Control Plants (PPM)	Concentration of Plants treated with Earth's Sound (PPM)
1	529-20-4	Benzaldehyde, 2-methyl-	C_8H_8O	120 g/mol	164366	427479	1.185750562	1.187982353
2	27552-11-0	4-O-Methylmannose	$C_7H_{14}O_6$	194 g/mol	593660	1572462	1.18875562	1.195607234
3	3370-81-8	3-O-Methyl-d-glucose	$C_7H_{14}O_6$	194.18 g/mol	1137758	3434114	1.192564306	1.208638798
Sl. No	**CAS**	**Compounds Present in one of the treated (Jupiter's sound) and control**	**Molecular formula**	**Molecular weight**	**Peak area (%) Plants treated with Jupiter's Sound**	**Peak area (%) Control Plants**	**Concentration of Plants treated with Jupiter's Sound (PPM)**	**Concentration of Control Plants (PPM)**
1	69112-21-6	3-Hexenal	$C_6H_{10}O$	98 g/mol	275619	196142	1.186529333	1.185972994
2	127839-47-8	Decane, 1-bromo-2-methyl-	$C_{11}H_{23}Br$	235 g/mol	187386	185551	1.185911702	1.185898857
Sl. No	**CAS**	**Compound present only in Treated (Control's and Earth's Sound)**	**Molecular formula**	**Molecular weight**	**Peak area (%) Plants treated with Jupiter's Sound**	**Peak area (%) Plants treated with Earth's Sound**	**Concentration of Plants treated with Jupiter's Sound (PPM)**	**Concentration of Plants treated with Earth's Sound (PPM)**
1	463-40-1	Linoelaidic acid	$C_{18}H_{30}O_2$	278 g/mol	109181	156932	1.185364267	1.185698524
2	83-47-6	Gamma Sitosterol	$C_{29}H_{50}O$	414 g/mol	5044279	11311159	1.219909953	1.263778113
3	545-47-1	Lupeol	$C_{30}H_{50}O$	426 g/mol	730863	2836138	1.189716041	1.204452966

Table 6: Compounds from GC-MS analysis found common in plants treated with Earth's sound, Jupiter's Sound and Control Plants, and their estimation using standard plot (Figure 1)

Sl. No	CAS	Compounds Common in all the control and Treated Sample	Molecular formula	Molecular weight	Peak area (%) Plants treated with Jupiter's Sound	Peak area (%) Plants treated with Earth's Sound	Peak area (%) Control Plants	Concentration of Plants treated with Jupiter's Sound (PPM)	Concentration of Plants treated with Earth's Sound (PPM)	Concentration of Control Plants (PPM)
1	123-92-2	1-Butanol, 3-methyl-, acetate	$C_7H_{14}O_2$	130 g/mol	3808921	6856798	1161879	1.211262447	1.232597586	1.192733153
2	7786-61-0	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150 g/mol	400678	501282	198568	1.187404746	1.188108974	1.185989976
3	504-96-1	Neophytadiene	$C_{20}H_{38}$	278.5 g/mol	643486	831223	419933	1.189104402	1.190418561	1.187539531
4	629-89-0	1-Octadecyne	$C_{18}H_{34}$	250.5 g/mol	327311	405522	135143	1.186891177	1.187438654	1.185546001
5	150-86-7	Phytol	$C_{20}H_{40}O$	296.5 g/mol	643227	737259	603462	1.189102589	1.189760813	1.188824234
6	57-11-4	Octadecanoic acid	$C_{18}H_{36}O_2$	284.5 g/mol	1150686	1947110	1080749	1.192654802	1.19822977	1.192165243
7	111-02-4	Squalene	$C_{30}H_{50}$	410 g/mol	5601076	6677662	2630871	1.223807532	1.231343634	1.203016097
8	59-02-9	Vitamin E	$C_{29}H_{50}O_2$	430 g/mol	4684437	6559614	2165793	1.217391059	1.230517298	1.199760551

DISCUSSION

Table 3 and Table 4 describes the phytochemicals that were found in common plants treated with Earth's sound and control, Jupiter's sound and control, and plants treated with Jupiter's and Earth's Sound groups. Not all compounds were common for all the three groups. This fluctuation of compounds presence and absence may be attributed to the type of treatment provided to the plants.

In Table 5 and Table 6 all the compounds that have uses and bioactivity described earlier in Table 3 and Table 4 have been estimated using Lupeol as external standard (Table 2 and Figure 3). A trend of higher concentration of phytochemicals in plants treated with planet Earth sound can be observed followed by plants treated with planet Jupiter's sound and finally control plants. This may be due to the type of treatment. Planet earth's sound has a frequency of 22050 Hz and Planet Jupiter's sound has a frequency of 44100 Hz. The sound frequency from planet Earth's sound may have a effect on certain compounds being formed that are absent in control and plants treated with planet Jupiter's sound. Also, among the compounds common among across all the groups, they are produced in higher amounts in plants treated by planet Earth's sound.

CONCLUSION

The pharmaceutical industry is focusing on developing plant-based drugs from traditional medicines. *Clitoria ternatea* L. extracts from leaves exhibit a range of pharmacological activities (Table 3 and Table 4). Important bioactive compounds can be isolated from this plant and their expression levels increase with the treatment of planetary sounds. The trend in compounds variation due to frequency variance can be observed. *Clitoria ternatea* L is a goldmine of a plant and our research will further establish evidence regarding secondary metabolites, bioavailability, pharmacokinetics, clinical trials and potential in medicine.

ACKNOWLEDGMENT

The authors express their sincere gratitude to the Department of Botany, Ravenshaw University, for their support and necessary facilities during the research. They extend special thanks to their supervisor, Dr. (Prof.) Sanhita Padhi, for her invaluable support and timely guidance. Additionally, they acknowledge Dr. Totan Adhak, Senior Scientist at the Pesticide Residue Laboratory, ICAR-National Rice Research Institute, for his genuine assistance in conducting the GC-MS experiments to identify the bioactive compounds.

REFERENCES

- Akpuaka, A., Ekwenchi, M. M., Dashak, D. A., & Dildar, A. (2013). Biological activities of characterized isolates of n-hexane extract of Azadirachta indica A. Juss (Neem) leaves. *Nature and science*, 11(5), 141-147.
- Angupale, J. R., Tusiimire, J., & Ngwuluka, N. C. (2023). A review of efficacy and safety of Ugandan anti-malarial plants with application of RITAM score. *Malaria journal*, 22(1), 97. https://doi.org/10.1186/s12936-023-04486-6
- Armstrong, L., Vaz, M. G. M. V., Genuário, D. B., Fiore, M. F., & Debonsi, H. M. (2019). Volatile compounds produced by cyanobacteria isolated from

mangrove environment. *Current microbiology*, 76, 575-582.

- Azam, A., Ejaz, R., Qadeer, S., Irum, S., Ul-Husna, A., Ullah, S., Shahzad, Q., Akhtar, T., & Akhter, S. (2021). Synergistic impact of α-linolenic acid and α-tocopherol on in vitro maturation and culture of buffalo oocytes. *Brazilian journal of biology Revista brasleira de biologia*, 84, e253514. https://doi.org/10.1590/1519-6984.253514
- Dash, A. K., Rath, D., Padhi, S. (2023, April 15). Effect of planetary sound (Earth & Jupiter) on germination of Clitorea ternatea L. (asian pigeonwings) white variety. *RJLBPCS*, 9(1), 11-29. doi. 10.26479/2023.0902.02
- Dionisio, K. L., Phillips, K., Price, P. S., Grulke, C. M., Williams, A., Biryol, D., Hong, T., & Isaacs, K. K. (2018, July 10). The Chemical and Products Database, a resource for exposure-relevant data on chemicals in consumer products. *Scientific Data*, 5(1). https://doi.org/10.1038/sdata.2018.125
- Francke, W., Plass, E., Zimmermann, N., Tietgen, H., Tolasch, T., Franke, S., & Woodcock, C. M. (2000). Major sex pheromone component of female herald moth Scoliopteryx libatrix is the novel branched alkene (6Z, 13)-methylheneicosene. *Journal of chemical ecology*, 26, 1135-1149.
- Hapsari, Y., Rachman, F., Septiana, E., & Simanjuntak, P. (2023, March). Identification of Antioxidant Active Compounds from Watercress (Nasturtium officinale R. Br). In 1st International Conference for Health Research–BRIN (ICHR 2022) (pp. 374-384). Atlantis Press.
- Huang, Z. R., Lin, Y. K., & Fang, J. Y. (2009). Biological and pharmacological activities of squalene and related compounds: potential uses in cosmetic dermatology. *Molecules*, 14(1), 540-554.
- Jeyaraj, E. J., Lim, Y. Y., & Choo, W. S. (2020, September 1). Extraction methods of butterfly pea (Clitoria ternatea) flower and biological activities of its phytochemicals. *Journal of Food Science and Technology*, 58(6), 2054–2067. https://doi.org/10.1007/s13197-020-04745-3
- Kalmankar, N. V., Venkatesan, R., Balaram, P., & Sowdhamini, R. (2020, July 29). Transcriptomic profiling of the medicinal plant Clitoria ternatea: identification of potential genes in cyclotide biosynthesis. *Scientific Reports*, 10(1). https://doi.org/10.1038/s41598-020-69452-7
- Keawsa-Ard, S., Liawruangrath, B., Liawruangrath, S., Teerawutgulrag, A., & Pyne, S. G. (2012). Chemical constituents and antioxidant and biological activities of the essential oil from leaves of Solanum spirale. *Natural product communications*, 7(7), 1934578X1200700740.
- Khait, I., Lewin-Epstein, O., Sharon, R., Saban, K., Goldstein, R., Anikster, Y., Zeron, Y., Agassy, C., Nizan, S., Sharabi, G., Perelman, R., Boonman, A., Sade, N., Yovel, Y., & Hadany, L. (2023, March). Sounds emitted by plants under stress are airborne and informative. *Cell*, 186(7), 1328-1336.e10. https://doi.org/10.1016/j.cell.2023.03.009.
- Khait, I., Obolski, U., Yovel, Y., & Hadany, L. (2019, August). Sound perception in plants. *Seminars in Cell & Developmental Biology*, 92, 134–138. https://doi.org/10.1016/j.semcdb.2019.03.006
- Kim, S. K., & Karadeniz, F. (2012). Biological importance and applications of squalene and squalane. *Advances in food and nutrition research*, 65, 223-233.
- Lewis, R. J. Sr. (2007). Hawley's Condensed Chemical Dictionary 15th Edition. John Wiley & Sons, Inc. New York, NY, p. 703.
- Masoudi, S., Aghajani, Z., Rustaiyan, A., Feizbakhsh, A., & Kakhky, A. M. (2009, January). Volatile Constituents of Teucrium persicum Boiss, Thymus caucasicus Willd. ex Ronniger subsp.grossheimii(Ronniger) Jalas. and Marrubium crassidensBoiss. Three Labiatae Herbs Growing Wild in Iran. *Journal of Essential Oil Research*, 21(1), 5–7. https://doi.org/10.1080/10412905.2009.9700093
- Masuzawa, T., Nakamura, R., Beppu, Y., & Yanagihara, Y. (1996). Immunochemical characteristics and localization on cells of protective antigen (PAg) prepared from Leptospira interrogans serovar lai. *Microbiology and immunology*, 40(3), 237–241. https://doi.org/10.1111/j.1348-0421.1996.tb03340.x
- Monshausen, G. B., Bibikova, T. N., Weisenseel, M. H., & Gilroy, S. (2009, August 1). Ca2+ Regulates Reactive Oxygen Species Production and pH during Mechanosensing in Arabidopsis Roots. *The Plant Cell*, 21(8), 2341–2356. https://doi.org/10.1105/tpc.109.068395
- Nagrale, D. T., Chaurasia, A., Kumar, S., Gawande, S. P., Hiremani, N. S., Shankar, R., Gokte-Narkhedkar, N., Renu, & Prasad, Y. G. (2023). PGPR: the treasure of multifarious beneficial microorganisms for nutrient mobilization, pest biocontrol and plant growth promotion in field crops. *World journal of microbiology & biotechnology*, 39(4), 100. https://doi.org/10.1007/s11274-023-03536-0.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 10722, 2-Methylbenzaldehyde. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/2-Methylbenzaldehyde.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 345716, 4-O-Methylmannose. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/4-O-Methylmannose.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 8973, 3-O-Methyl-d-glucose. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/3-O-Methyl-d-glucose.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 643139, 3-Hexenal. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/3-Hexenal.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 545631, 1-Bromo-2-methyldecane. Retrieved June 2, 2023 from

https://pubchem.ncbi.nlm.nih.gov/compound/1-Bromo-2-methyldecane.

- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 5280934, Linolenic Acid. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Linolenic-Acid.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 457801, Clionasterol. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Clionasterol.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 259846, Lupeol. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Lupeol.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 31276, Isoamyl acetate. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Isoamyl-acetate.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 332, 2-Methoxy-4-vinylphenol. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/2-Methoxy-4-vinylphenol.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 10446, Neophytadiene. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Neophytadiene.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 69425, 1-Octadecyne. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/1-Octadecyne.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 5280435, Phytol. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Phytol.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 5281, Stearic Acid. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Stearic-Acid.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 638072, Squalene. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Squalene.
- National Center for Biotechnology Information. (2023). PubChem Compound Summary for CID 14985, Vitamin E. Retrieved June 2, 2023 from https://pubchem.ncbi.nlm.nih.gov/compound/Vitamin-E.Eff
- Nomura, M., Tanaka, K., Banno, Y., Hara, R., Asami, M., Otsuka, T., & Tomata, Y. (2023). Polyunsaturated fatty acids and risk of anorexia nervosa: A Mendelian randomization study. *Journal of affective disorders*, 330, 245–248. https://doi.org/10.1016/j.jad.2023.03.016
- Oguis, G. K., Gilding, E. K., Jackson, M. A., & Craik, D. J. (2019). Butterfly pea (*Clitoria ternatea*), a cyclotide-bearing plant with applications in agriculture and medicine. *Frontiers in plant science*, 10, 645.
- Purnadurga, G., Lakshmi Kumar, T. V., Koteswara Rao, K., Rajasekhar, M., & Narayanan, M. S. (2018). Investigation of temperature changes over India in association with meteorological parameters in a warming climate. *International Journal of Climatology*, 38(2), 867-877.
- Rath, D., Dash, A. K., & Padhi, S. (2023). Wheat: A potential Bioreactor for Lupeol Production. *International Journal of Current Science*, 13(2), 713-723.
- Sanyal, R., M, M., Pandey, S., Nandi, S., Biswas, P., Dewanjee, S., Gopalakrishnan, A. V., Jha, N. K., Jha, S. K., Joshee, N., Pandey, D. K., Dey, A., & Shekhawat, M. S. (2023). Biotechnological interventions and production of galanthamine in Crinum spp. *Applied microbiology and biotechnology*, 107(7-8), 2155–2167. https://doi.org/10.1007/s00253-023-12444-0
- Song, J., Jiang, M., Jin, Y., Li, H., Li, Y., Liu, Y., Yu, H., & Huang, X. (2023). Phytol from Faeces Bombycis alleviated migraine pain by inhibiting Nav1.7 sodium channels. *Journal of ethnopharmacology*, 306, 116161. https://doi.org/10.1016/j.jep.2023.116161
- Toh, S. C., Lihan, S., Bunya, S. R., & Leong, S. S. (2023). In vitro antimicrobial efficacy of Cassia alata (Linn.) leaves, stem, and root extracts against cellulitis causative agent Staphylococcus aureus. *BMC complementary medicine and therapies*, 23(1), 1-17.
- Villareal, M., Han, J., Matsuyama, K., Sekii, Y., Smaoui, A., Shigemori, H., & Isoda, H. (2013, February 13). Lupenone from Erica multiflora Leaf Extract Stimulates Melanogenesis in B16 Murine Melanoma Cells through the Inhibition of ERK1/2 Activation. *Planta Medica*, 79(03/04), 236–243. https://doi.org/10.1055/s-0032-1328189