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Effect of harmonic octave consonants (Classical musical notes) on the different phytochemical characteristics in sweet leaf *viz*., *Stevia rebaudiana* (Bertoni)

Sanhita Padhi, Rageshree Swain and Pragyan P Rout

Abstract
The present research work is aimed at finding the unique impact of the harmonic octave consonants in Indian classical instrumental music exhibited through various Raga as on the different phytochemical characteristics in sweet leaf *viz*., *Stevia rebaudiana* (Bertoni). The calculation of mean, variance and standard deviation was done to know the difference between various series and to make a comparative study of the variability of two or more series using the statistical software SPSS (Statistical Package for Social Sciences). The content of total phenols, flavonoids and tannins were individually analyzed and presented as mean ± SEM. The total phenolic content in treated and control plants were found to be 154.98±0.06 and 68.44±0.05 respectively. The total tannin content in treated plant sample was calculated as 681.04±4.893 and in control, it was 669.09±6.54. In treated plants, the total flavonoid content was found to be 2.96±0.065 and it was evaluated to be 1.94±0.107 for control.

Keywords: Sound, music, Indian classical ragas, harmonic octave consonants, strings and closed-pipe, physiognomic study, biochemical study

1. Introduction
Sound is what we can consider as a unique form of energy. It is a compressional wave triggered by vibrations (Wood, 1976) [24]. The vibrations distressing the air around us can be easily considered as equivalent to the vibrations generated from a speaker. It cannot be seen as well but sound can be heard as it reaches our ears in the form of waves produced when particles in the medium vibrates (Rigden, 1977) [17]. Therefore, sound is produced due to vibration of a body.

Music has been proved to provide calming effect as it is composed of rhythmic and periodic vibrations, while, noise has been considered as exasperating because it has no rhythm (Lapp, 2002) [11]. Music for about a thousand of years have been a source of harmony for people. Music is a soothing and tranquilizing form that is produced from musical instrument. Music is a fine art of sound and is basically prearranged by people for many reasons like, to dance to, to speak a story, to make other people feel a certain way, or just to sound pretty or be amusing (Padhi *et al*., 2022) [14].

Music certainly has many dimensions and so it is successful in stirring various physical, psychological, spiritual and social stages of awareness (Kneafsey, 1997) [10]. Nowadays, positive music that is known to be potentially beneficial as in treating negative thoughts and thus can used as a therapy for people in stress. In contrast, adverse responses, frustration, resentment, desolation, hatred and fright can be stimulated by negative music (Ekici *et al*. 2007) [4].

An important concept related to music is known to be a doubling in frequency that is for instance, 40 Hz (one octave greater than 20 Hz) and is technically called as an octave that has been arranged on the basis of audible sound range (Shah, 2001). Certain mathematical principles, suggest to classify an individual octave into intervals. The categorization of an octave has been done into twelve parts along with the adjacent frequencies having a constant ratio of twelfth root of two. In Indian Classical Music, Saptak is similar to octave. Saptak is a component of twenty-two tones called as 'Shruti'. 'Shuddha swaras' are the seven pure notes found in Indian Classical music and show distinct frequency range. An undergraduate Dorothy Retallack carried out one of the first experiments in 1973 [16] to study the relationship between plants and music. Retallack used many genres of music in her experiment. She realized that the plants displayed a dislike to Led Zeppelin and Jimi Hendrix music.

However, Bach organ music and jazz fascinated them. Her eventual experiments with North Indian classical music played on the sitar proved to be valuable for plant's growth (Rettalack, 1973).

All living beings be it animals or plants all respond to some sort of external stimuli. The idea of perception of sound waves by plants was originally proposed in 1977, by an internationally commended, a renowned Indian physicist, a plant physiologist and a Nobel prize winner, Sir Jagdish Chandra Bose. He subjected the plants to pleasant music and observed them growing more quickly. However, he also noticed poor growth in plants that were exposed to harsh music and loud speech (Padhi *et al.*, 2022) [14].

The vibrations in a sound, stimulate different phytochemical biochemical reaction which in turn helps in the developmental processes of plant. When sounds of variable frequencies and amplitude reaches our ear drum, they are immediately received by the brain cells that helps us in differentiating sound types. An analogous mechanism is believed to be occurring in plants, where such vibrations are thought to be perceived by their protoplasm. Numerous studies related to the effects of subjecting seeds and plants to sound waves, which is generally stated to as sonication, has been seen in scientific literature. The effect of music on 30 Rose (*Rosa chinensis)* plants taken in separate pots was studied (Chivukula and Ramaswamy, 2014) [2]. The plants exposed to Indian classical music exhibited the highest internode elongation, which evidently displayed that exposing the plants to Vedic chants or Indian classical music stimulates the growth of plants in comparison to the control group or the plants that were subjected to Western classical or Rock music. The note *Shadja* (Sa) generates a total of 240 vibrations. The other notes and the vibrations produced by them are: *Rishab* (Re)-270, *Gandhar (*Ga)-300, *Madhyam* (Ma)-320, *Pancham* (Pa)-360, *Dhaivat* (Dha)-400 and *Nishad* (Ni)-450 (Thakkar *et al*. 2014) [22].

2. Materials and methods
2.1. Materials
2.1.1 Acoustic chamber
All experiments were done in an acoustical chamber which was provided with a sound system to produce the sound of known frequency. The light, humidity, temperature and the distance of the experimental plant materials from the source of the sound were maintained so as to give a healthy environment for the growth of the plants.

2.1.2. Plant material
The plant commonly called as sweetleaf (Marcinek and Krejpcio, 2015) [12] with the scientific name *Stevia rebaudiana* (Bertoni) was purchased from medicinal garden at Patrapada, Bhubaneswar in large number.

2.1.3. Glassware and Equipment
Mortar and pestle, measuring cylinders, test tubes, beakers, petri plates, pipette, conical flask, aluminium foil, centrifuge tubes, cuvette, centrifuge machine, analytical balance, vortex machine, hot plate, water bath, thermometer, spectrophotometer, Millipore water purifier etc. were required to conduct the biochemical analysis of the leaf samples.

2.1.4. *Ragaas*
- The application of the Indian Classical Instrumental music notes (Octaves) through different *Ragaas* were applied to our experimental plant set in the following time sequence: -
- Raga Kedar- Flute (closed pipe) by Pandit Pannalal Ghosh (38:14) during early morning between 6am-8am, followed by-
- Raga Kedar- Santoor (stringed instrument) by Pandit Shiv Kumar Sharma (23:14) in the Morning time between 10am-12pm,
- Raga Rageshree- Sitar (stringed instrument) by Nikhil Banerjee (29:15) in the evening between 4pm-6pm followed by-
- Raga Bhairavi- Flute (closed pipe) by Pandit Hari Prasad Chaurasia (9:50)

2.2 Methodology
The plants were treated with harmonic sound frequencies for 4 hours at frequent intervals daily.

2.2.1 Qualitative phytochemical analysis
To perform the experiments, two sets of sweetleaf (*Stevia rebaudiana*) plants were taken, one set with three plants for control and the other set with three plants for experiments. The leaves were macerated in 4 pure solvents the extract was subjected to different phytochemical tests.

Pure solvents Required
a) Methanol
b) Ethanol
c) Acetone
d) Deionized water

Preparation of extract

1 gram of leaf sample was taken and was macerated using 10 ml of solvent. Total of 8 samples (4 treated and 4 control) were prepared. All the samples were centrifuged for 3 hours at 300 rpm. Extracts were then allowed to stand for 12 hours in the dark. After the period of 12 hours all the samples were centrifuged at 3000 rpm for 15 mins. Supernatants were collected and the residues were redisposed off with 10 ml of freshly prepared solvents. The extracts were then centrifuged at 3000 rpm for 15 mins. These samples can be stored now for 2 days.

Detection of alkaloids was done through Mayer's test using Mayer's reagent, hydrochloric acid (HCl).

Detection of tannins was done through Ferric chloride test using ferrous chloride (FeCl) solution and distilled Water.

Detection of Glycosides was done through Alkaline reagent test using sodium chloride (NaOH) solution and distilled water.

Detection of Saponins was performed by Froth test using dilute hydrochloric acid (HCl). Detection of Anthocyanins was done using deionized water.

Quantitative analysis of phytochemicals

Assessment of phytochemicals in both treated and control plant sample was done by using UV-Vis spectroscopy at a range of 350nm to 700nm. The statistical analysis for quantitative data of phytochemicals was done using the statistical software SPSS (Statistical Package for Social Sciences).

Quantitative analysis of total phenolic content.

Total phenolic content of the extract was evaluated with Folin-Ciocalteu method (Uddin *et al*., 2015) [23]. Samples containing polyphenols are reduced by the Folin-Ciocalteu reagent there by producing blue colored complex. The phenolic concentration of extracts was evaluated from a gallic acid calibration curve. To prepare a calibration curve, 0.5mL aliquots of 12.5, 25, 50, 100, 200 and 400 µg/mL methanolic gallic acid solutions were mixed with 2.5 mL Folin-Ciocalteu reagent (diluted ten-fold) and 2.5 mL (75 g/L) sodium carbonate. After incubation at 25°C for 30 min, the quantitative phenolic estimation was performed at 765 nm against reagent blank by UV Spectrophotometer 1650. The calibration curve was constructed by putting the value of absorbance vs. concentration. A similar procedure was adopted for the extract as above described in the preparation of calibration curve. All determinations were performed in triplicate. Total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per gm of extract.

Quantitative analysis of total tannin content

The tannins were determined by Folin-Ciocalteu method (Tambe and Bhambar, 2014) [21]. About 0.1 ml of the sample extract was added to a volumetric flask (10 ml) containing 7.5 ml of distilled water and 0.5 ml of Folin Ciocalteu phenol reagent, 1 mi of 35% sodium carbonate solution and dilute to 10 ml with distilled water. The mixture was shaken well and kept at room temperature for 30 min. a set of reference standard solutions of tannic acid (20, 40, 60, 80, 100 µg/ ml) were prepared in the same manner as described earlier. Absorbance for test and standard solutions were measured against the blank at 700 nm with an UV/ Visible spectrophotometer. The estimation of the tannin content was carried out in triplicate. The tannin content was expressed in terms of mg of tannic acid equivalents/ g of dried sample.

Quantitative analysis of total flavonoid content

Total flavonoid content of ethanol extract was evaluated with method of Jiao (Jiao & Wang, 2000) [8]. One ml of the plant leaf extract or standard of different concentrations was taken in a test tube and 3 ml of methanol was added. Then 200 µl of 10% aluminium chloride solution was added into the same test tube followed by the addition of 200 µl of 1M potassium acetate. Finally, 5.6 ml of distilled water was mixed with the reaction mixture. The reaction mixture was then incubated for 30 min at room temperature to complete the reaction. Then the absorbance of the solution was measured at 415 nm using a spectra photometer against blank. Methanol served as blank. The Total content of flavonoid compounds in the plant extract was expressed in mg/gm quercetin equivalent (QE).

3. Results

The phytochemicals give the fruits their characteristic colour while some give a distinctive aroma. Several studies have proved that there is an association between sound vibrations and plants and that both are profoundly interconnected to each other (Rettalack, 1973). While sound with a low amplitude improves plant growth, likewise, any type of sound vibrations with large amplitude values for e.g., rock music declines plant growth (Rettalack, 1973). The results of both the qualitative and quantitative phytochemical analysis in pure solvents is represented in the form of tables and figures. The content of total phenols, flavonoids and tannins were presented as mean ± SEM.

3.1 Results for Qualitative Analysis of Phytochemicals

The results for qualitative analysis of phytochemicals are given in tables (table 2 and table 3).

Table 2: Qualitative analysis (presence and absence) of phytochemicals in the leaves of *Stevia rebaudiana* (Bertoni) (control) in pure solvents

Solvent	Alkaloids	Tannins	Glycosides	Saponins	Anthocyanins
Methanol	++	++	++	-	-
Ethanol	+	+	+	-	-
Acetone	++	++	+	-	-
Petroleum ether	-	-	-	+	-
Deionized water	+	-	+	+	-

Key: +++ = High concentration, ++ = medium concentration, + = low concentration, - = absent

Table 3: Qualitative analysis (presence and absence) of phytochemicals in the leaves of *Stevia rebaudiana* (Bertoni) (experimental) in pure solvents

Solvent	Alkaloids	Tannins	Glycosides	Saponins	Anthocyanins
Methanol	+++	+++	++	-	-
Ethanol	+++	++	+	-	-
Acetone	+++	++	+	-	-
Petroleum ether	+	+	+	+++	-
Deionized water	+	+	-	+	-

Key: +++ = High concentration, ++ = medium concentration, + = low concentration, - = absent

3.2 Results for Quantitative Analysis of Phytochemicals

The results for quantitative analysis of phytochemicals are shown in tables (table 4, table 5, table 6, table 7, table 8, table 9 and table 10) and figures (figure 1, figure 2 and figure 3).

Table 4: Results for statistical analysis for quantitative data on phytochemicals (both control and treated) in ethanol in sweet leaf (*Stevia rebaudiana*)

Descriptive Statistics ethanol									
	N	Range	Minimum	Maximum	Sum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
ce	2	.111	2.537	2.648	5.185	2.59250	.055500	.078489	.006
ce	2	.19	2.48	2.68	5.16	2.5785	.09650	.13647	.019
ce	2	.46	2.05	2.50	4.55	2.2735	.22850	.32315	.104
ce	2	.14	2.08	2.21	4.29	2.1440	.06900	.09758	.010
ce	2	.28	2.04	2.32	4.36	2.1780	.14200	.20082	.040
ce	2	.21	2.09	2.29	4.38	2.1875	.10250	.14496	.021

ce	2	.29	2.18	2.46	4.64	2.3205	.14350	.20294	.041
ce	2	.40	2.12	2.52	4.64	2.3210	.19800	.28001	.078
ce	2	.26	2.12	2.38	4.50	2.2515	.12850	.18173	.033
ce	2	.38	2.00	2.38	4.38	2.1885	.18750	.26517	.070
ce	2	.35	2.00	2.35	4.34	2.1710	.17400	.24607	.061
ce	2	.34	1.99	2.33	4.33	2.1635	.16950	.23971	.057
ce	2	1.59	1.19	2.78	3.97	1.9830	.79300	1.12147	1.258
ce	2	1.37	.97	2.34	3.31	1.6570	.68600	.97015	.941
ce	2	.38	.51	.89	1.41	.7030	.19000	.26870	.072
ce	2	.29	.40	.69	1.09	.5425	.14550	.20577	.042
ce	2	.08	.76	.84	1.61	.8040	.04000	.05657	.003
ce	2	.07	.30	.36	.66	.3305	.03250	.04596	.002
ce	2	.04	.46	.50	.97	.4835	.01950	.02758	.001
ce	2	.44	.23	.67	.90	.4480	.22200	.31396	.099
ce	2	.17	.38	.54	.92	.4605	.08250	.11667	.014
ce	2	.33	.31	.64	.95	.4760	.16400	.23193	.054
ce	2	.28	.36	.64	.99	.4960	.13900	.19658	.039
ce	2	.59	.81	1.40	2.21	1.1060	.29500	.41719	.174
ce	2	.24	.19	.43	.62	.3085	.12150	.17183	.030
ce	2	.05	.13	.18	.32	.1580	.02600	.03677	.001
Valid N (listwise)	2								

Table 5: Results for statistical analysis for quantitative data on phytochemicals (both control and treated) in acetone in sweet leaf (*Stevia rebaudiana*)

Descriptive Statistics acetone									
	N	**Range**	**Minimum**	**Maximum**	**Sum**	**Mean**		**Std. Deviation**	**Variance**
	Statistic	**Statistic**	**Statistic**	**Statistic**	**Statistic**	**Statistic**	**Std. Error**	**Statistic**	**Statistic**
ce	2	.022	2.238	2.260	4.498	2.24900	.011000	.015556	.000
ce	2	.08	2.41	2.49	4.90	2.4505	.03850	.05445	.003
ce	3	20.68	1.32	22.00	25.13	8.3757	6.81357	11.80145	139.274
ce	2	.15	1.20	1.35	2.56	1.2775	.07450	.10536	.011
ce	2	.28	1.05	1.33	2.38	1.1910	.14000	.19799	.039
ce	2	.42	.92	1.33	2.25	1.1225	.20750	.29345	.086
ce	2	.82	1.00	1.82	2.81	1.4065	.40950	.57912	.335
ce	2	.84	.86	1.70	2.56	1.2815	.41950	.59326	.352
ce	2	.91	.83	1.74	2.58	1.2880	.45600	.64488	.416
ce	2	.06	.66	.72	1.38	.6910	.03000	.04243	.002
ce	2	.07	.71	.78	1.48	.7420	.03600	.05091	.003
ce	2	.04	.70	.74	1.44	.7220	.02000	.02828	.001
ce	2	.01	.62	.63	1.26	.6275	.00450	.00636	.000
ce	2	.05	.43	.48	.92	.4580	.02500	.03536	.001
ce	2	.02	.37	.40	.77	.3840	.01100	.01556	.000
ce	2	.02	.34	.36	.70	.3520	.00800	.01131	.000
ce	2	.01	.33	.34	.67	.3365	.00650	.00919	.000
ce	2	.04	.37	.40	.77	.3845	.01850	.02616	.001
ce	2	.01	.31	.32	.62	.3110	.00500	.00707	.000
ce	2	.14	.15	.29	.44	.2205	.06750	.09546	.009
ce	2	.16	.13	.29	.42	.2085	.07750	.10960	.012
ce	2	.14	.13	.27	.39	.1970	.07100	.10041	.010
ce	2	.14	.15	.29	.44	.2215	.06750	.09546	.009
ce	2	.04	.28	.32	.60	.3000	.02000	.02828	.001
ce	2	.03	.28	.31	.59	.2930	.01700	.02404	.001
ce	2	.01	.25	.26	.51	.2555	.00450	.00636	.000
Valid N (listwise)	2								

Table 6: Results for statistical analysis for quantitative data on phytochemicals (both control and treated) in deionized water in sweet leaf (*Stevia rebaudiana*)

Descriptive Statistics deionized water									
	N	**Range**	**Minimum**	**Maximum**	**Sum**	**Mean**		**Std. Deviation**	**Variance**
	Statistic	**Statistic**	**Statistic**	**Statistic**	**Statistic**	**Statistic**	**Std. Error**	**Statistic**	**Statistic**
ce	2	.128	2.477	2.605	5.082	2.54100	.064000	.090510	.008
ce	2	.49	1.67	2.16	3.83	1.9145	.24250	.34295	.118
ce	3	20.87	1.13	22.00	24.53	8.1760	6.91242	11.97266	143.345
ce	2	.31	.96	1.27	2.23	1.1150	.15600	.22062	.049
ce	2	.39	.79	1.18	1.97	.9860	.19300	.27294	.074
ce	2	.44	.67	1.10	1.77	.8860	.21800	.30830	.095
ce	2	.31	.43	.73	1.16	.5795	.15250	.21567	.047
ce	2	.29	.22	.50	.72	.3590	.14300	.20223	.041

ce	2	.22	.19	.41	.60	.3005	.10950	.15486	.024
ce	2	.19	.17	.36	.53	.2665	.09350	.13223	.017
ce	2	.16	.17	.34	.51	.2550	.08100	.11455	.013
ce	2	.14	.12	.27	.39	.1935	.07150	.10112	.010
ce	2	.10	.11	.20	.31	.1565	.04750	.06718	.005
ce	2	.07	.10	.17	.27	.1340	.03500	.04950	.002
ce	2	.17	.14	.32	.46	.2290	.08700	.12304	.015
ce	2	.08	.30	.38	.68	.3390	.04100	.05798	.003
ce	2	.08	.28	.36	.64	.3190	.04100	.05798	.003
ce	2	.05	.26	.31	.56	.2810	.02400	.03394	.001
ce	2	.06	.27	.33	.59	.2960	.03100	.04384	.002
ce	2	.04	.25	.29	.54	.2695	.01950	.02758	.001
ce	2	.05	.24	.29	.53	.2650	.02500	.03536	.001
ce	2	.03	.26	.29	.54	.2715	.01450	.02051	.000
ce	2	.04	.29	.32	.61	.3045	.01950	.02758	.001
ce	2	.11	.24	.35	.59	.2970	.05300	.07495	.006
ce	2	.06	.24	.30	.54	.2690	.03200	.04525	.002
ce	2	.06	.23	.29	.51	.2570	.03200	.04525	.002
Valid N (listwise)	2								

Table 7: Results for statistical analysis for quantitative data on phytochemicals (both control and treated) in methanol in sweet leaf (*Stevia rebaudiana*)

Descriptive Statistics methanol									
	N	**Range**	**Minimum**	**Maximum**	**Sum**	**Mean**		**Std. Deviation**	**Variance**
	Statistic	**Statistic**	**Statistic**	**Statistic**	**Statistic**	**Statistic**	**Std. Error**	**Statistic**	**Statistic**
ce	2	.120	2.415	2.535	4.950	2.47500	.060000	.084853	.007
ce	2	.10	2.54	2.64	5.17	2.5860	.04900	.06930	.005
ce	3	19.40	2.60	22.00	27.31	9.1037	6.44824	11.16869	124.740
ce	2	.11	2.66	2.77	5.43	2.7165	.05450	.07707	.006
ce	2	.11	2.64	2.75	5.39	2.6945	.05350	.07566	.006
ce	2	.11	2.55	2.66	5.20	2.6015	.05550	.07849	.006
ce	2	.04	2.40	2.44	4.84	2.4210	.01800	.02546	.001
ce	2	.06	2.33	2.39	4.72	2.3590	.02800	.03960	.002
ce	2	.06	2.27	2.33	4.60	2.3015	.02850	.04031	.002
ce	2	.26	2.02	2.28	4.30	2.1495	.12750	.18031	.033
ce	2	.08	2.18	2.26	4.45	2.2235	.03950	.05586	.003
ce	2	.12	2.10	2.22	4.32	2.1590	.05800	.08202	.007
ce	2	.19	2.00	2.19	4.19	2.0935	.09250	.13081	.017
ce	2	.23	1.99	2.22	4.21	2.1035	.11250	.15910	.025
ce	2	1.14	.74	1.88	2.62	1.3110	.56900	.80469	.648
ce	2	.21	.43	.63	1.06	.5295	.10250	.14496	.021
ce	2	.15	.30	.45	.75	.3765	.07650	.10819	.012
ce	2	.16	.20	.36	.56	.2780	.08200	.11597	.013
ce	2	.11	.28	.40	.68	.3395	.05550	.07849	.006
ce	2	.19	.16	.34	.50	.2500	.09300	.13152	.017
ce	2	.17	.14	.32	.46	.2300	.08600	.12162	.015
ce	2	.17	.14	.30	.44	.2190	.08400	.11879	.014
ce	2	.16	.12	.28	.41	.2025	.07950	.11243	.013
ce	2	.17	.12	.29	.41	.2035	.08250	.11667	.014
ce	2	.14	.13	.27	.40	.1995	.06850	.09687	.009
ce	2	.17	.12	.29	.41	.2045	.08450	.11950	.014
Valid N (listwise)	2								

Table 8: Result for Total Phenolic Content (Control vs Treated)

Sample type	**Control**	**Treated**
Sample absorbance	0.719	1.394
	0.720	1.395
	0.721	1.396
Calculated conc. (mg/gm) GAE	70	154.85
	70	154.98
	70	155.11
Mean	68.44	154.98
SD	0.102	0.105
Mean±SEM	68.44±0.05	154.98±0.06

Values are the mean of triplicate experiments and represented as mean ± SEM (n=3). SD= Standard Deviation, SEM= Standard Error Mean, GAE=Gallic Acid Equivalent

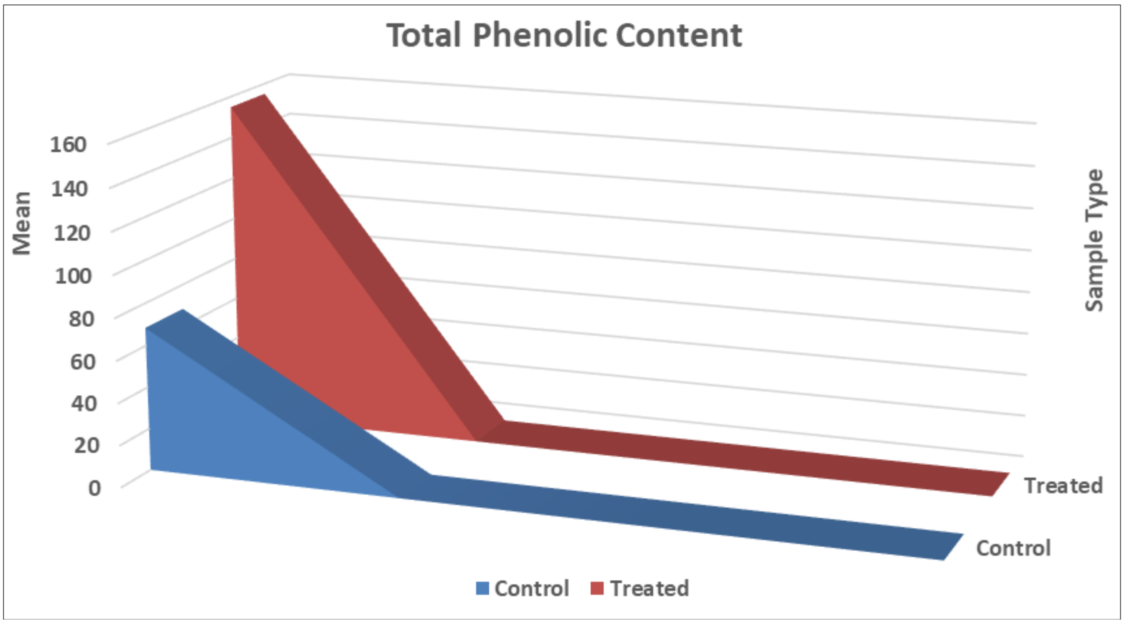

Fig 1: Graph showing total phenolic content (Control vs Treated)

Table 9: Result for Total Tannin Content (Control vs Treated)

Sample type	Control	Experimental
Sample absorbance	0.946	0.961
	0.952	0.977
	0.982	0.990
Calculated conc. (mg/gm) TAE	659	670
	663	681
	685	690
Mean	669.09	681.04
SD	11.34	8.475
Mean±SEM	669.09±6.54	681.04±4.893

Values are the mean of triplicate experiments and represented as mean ± SEM (n=3). SD= Standard Deviation, SEM= Standard Error Mean, TAE= Tannic Acid Equivalent

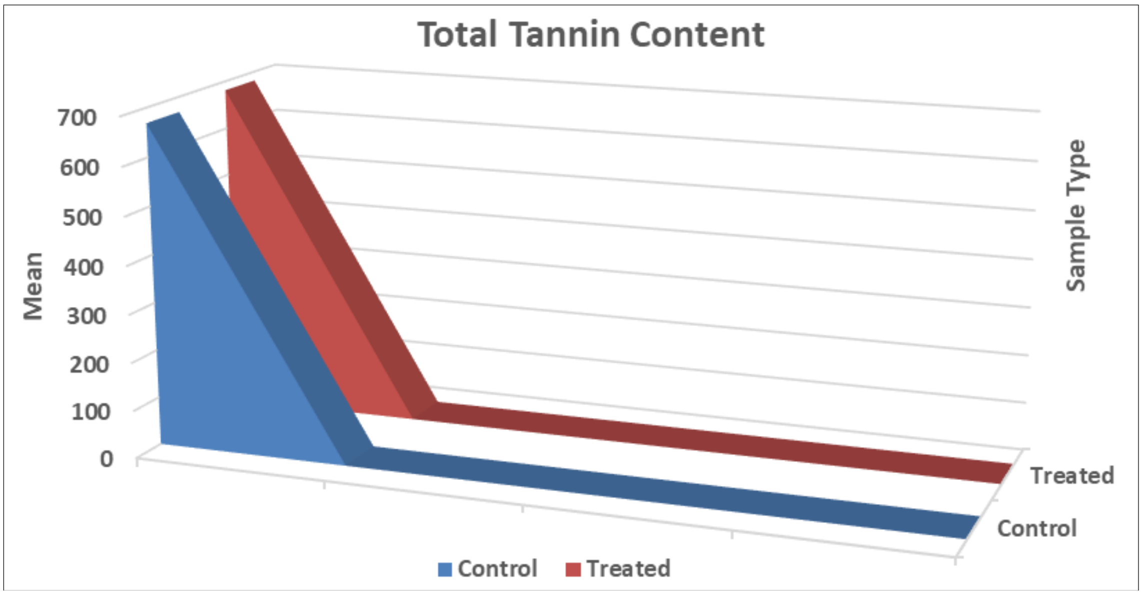

Fig 2: Graph showing total tannin content (Control vs Treated)

Table 10: Result for Total Flavonoid Content (Control vs Treated)

Sample type	Control	Experimental
Sample absorbance	0.026	0.056
	0.024	0.055
	0.021	0.053
Calculated conc. (mg/gm) QE	2.16	3.09
	1.98	3.00
	1.70	2.81
Mean	1.94	2.96
SD	0.187	0.114
Mean± SEM	1.94±0.107	2.96±0.065

Values are the mean of triplicate experiments and represented as mean ± SEM (n=3). SD= Standard Deviation, SEM= Standard Error Mean, QE= Quercetin Equivalent

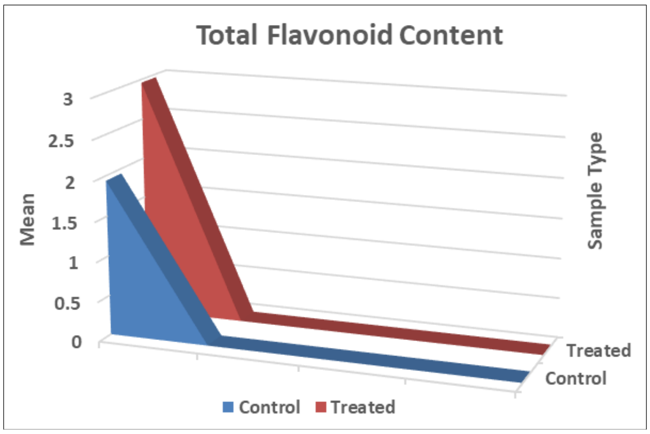

Fig 3: Graph showing total flavonoid content (Control vs Treated)

4. Discussion

There is a worldwide existence of audible sound (20–20000Hz). However, not much effort has been put forth in studying the relation of plant and audible sound (Padhi *et al.*, 2022) [14].

Mechano-sensitive channels have been observed to be found in the membranes of plant cell that aids in the perception of sound vibrations by plant cells. Several experiments on the effect of sound vibrations on plants have suggested that these vibrations have the ability to cause tensions in biological membranes that could perhaps stimulate signaling pathways through the activation of these mechano-sensitive channels (Haswell and Meyerowitz, 2006) [6]. Other experiments carried out showed that certain frequencies of sound vibrations can induce seed germination, root elongation, callus growth, and cell cycling (Gagliano, 2013) [5].

Growth of the plants, including the concentration of various metabolites; e.g., chlorophyll and starch have been observed to be increased by audible harmonic frequencies (Sharma *et al*. 2015) [18]. Also, musical vibration stimulates water molecules within biological systems (Creath and Schwartz, 2002) [3]. As a result, temperature that is a measure of motion of molecules is raised due to vibration of the water molecules. This leads to an increase in the rate of metabolism. Resonances from audible harmonic sound vibration can combine directly into biological systems because they are constituted mostly of water. A crucial plant hormone that helps in plant's growth and development is known to be IAA (Indole Acetic Acid). Studies have found that when six species of vegetable plants were subjected to music, they showed a rise in IAA content in treated plants as compared to the control plants (Jun-ru *et al*. 2011) [9].

The medicinal plants enriched with anti-oxidants and other phytochemicals have the potential to avoid oxidative stress and diseases linked to them (Soory, 2009) [19]. Thus, it has become obligatory for researchers to explore for new, harmless and affordable sources of plants rich in anti-oxidants for humans (Islam *et al.*, 2016) [7]. As the phenolic compounds have a classic structural chemistry that enables them to act as potential scavengers of free radicals, hence, they are found to be very essential as dietary antioxidants. These compounds have also been proved to prevent the oxidative damage of DNA (Reddy *et al.* 2012) [15]. The results obtained by our present study have indicated that the leaves of *Stevia rebaudiana* on treatment with Indian classical *ragaas* displayed substantial quantity of total phenols and flavonoids as compared to the leaves of the control ones. The outcomes also signify the effectiveness of Indian classical *ragaas* on the leaves of Stevia rebaudiana, thus making these plants, an inexpensive source of these beneficial compounds.

5. Conclusion

The standard deviation is a measure that is used to quantify the amount of variation in a set of data. Biological samples are generally huge in number and also biology deviates from the mean. Standard deviation helps us to determine whether our samples are close to the mean or are some of them dispersed widely in each direction. If the value of standard deviation comes out to be zero or nearer to it, it would indicate the data to be very close to the mean. A high standard deviation means that the data points are spread over a wide range of values. Thus, calculation of mean, variance and standard deviation are necessary to know the difference between various series and to make a comparative study of the variability of two or more series. The present study was done to make such comparison of phytochemicals data in both control and treated plant samples using SPSS software.

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