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Effect of synchronized sound waves in the form of Indian Classical Ragas on Phytochemical analysis of *Chamaecostus cuspidatus* (Nees & Mart.) C. Specht & D. W. Stev

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Abstract

Synchronized sound waves in the form of Indian classical ragas have a remarkable impact on the living beings. According to various studies, Indian Classical music has been confirmed to encourage plant growth positively and it is observed that the sounds from metal-rock, rock, rap, pop, and monotonous sounds have a negative impact on plant growth. The present research work was aimed at finding the impact and effect of the synchronized sound waves and their frequencies in different strings and closed-pipe Indian classical instrumental music displayed through various *Ragas, viz*: *Raga- Kedar* (flute), *Raga- Kedar* (santoor), *Raga-Rageshree* (sitar), *Raga-Bhairavi* (flute), *Raga- Shree* (Sarangi), *Raga- Milan kiTodi* (Sarod), *Raga- Ramkali* (Sitar) on phytochemical analysis in the leaves of Insulin (*Chamaecostus cuspidatus*). The standard deviation values in all of the pure solvents indicates that the data were spread over a wider range of values, which further showed that the treated plants have higher concentration phytochemicals when compared to that of the untreated samples. The total phenol content was found to be 207.66±0.33 mg/GAE per gram extract and 113.33±3.33 mg/GAE per gram extract in case of treated and untreated plant samples of *Chamaecostus cuspidatus* respectively. Similarly, the total flavonoid content was found to be 37.66±0.33 mg QE/g extract and 26.66±0.33 mg QE/g extract in case of treated and untreated plant samples respectively and the total tannin content was found to be 843±1.99 mg TAE/g extract and 503±0.57 mg TAE/g extract in case of treated and untreated plant samples respectively.

Keywords: Indian Classical Ragas; Synchronized sound waves; Indian classical music; Instrumental music; Phytochemical analysis

1 Introduction

1.1 Sound

Sound, purely is a form of energy and furthermore is a vibration (a transverse pressure wave) which produces a sensation of hearing in our ears. It is generally produced when a body vibrates [1]. Vibration occurs when an object undergoes rapid back and forth motion about its mean position. The vibration can appear from a tuning fork, a guitar string, the column of air in an organ pipe, the head (or rim) of a snare drum, steam evading from a radiator, the reed on a clarinet, the diaphragm of a loudspeaker, the vocal cords, or nearly anything that vibrates in a frequency range that is audible to a listener (roughly 20 to 20,000 cycles per second, for humans) [2]. The two situations that are necessary for the generation of a sound wave are a vibratory disturbance and an elastic medium, the most common of which is air [2]. Concerning the propagation of sound, it is known that when a body vibrates, it transfers its energy to the surroundings [2]. On receiving this energy, the air molecules around the body also starts vibrating about their mean position and

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thereby transferring the energy to their adjacent molecules [2]. This whole process of transferring energy constitutes a wave such that it carries only the energy from one molecule to another without actually moving them. Sound requires a medium to propagate which may be solid, liquid or gas [2].

1.2 Musical Notes and Octaves

The music and noise are the different forms of sound that differs from each other. Music is a melodious sound which is pleasing to our ears whereas the sound that is not pleasant, known as noise [2]. Music is the result of regular vibrations whereas noise is produced by irregular vibrations [1]. Musical tones are generally (almost) periodic. The fact that these sounds are periodic is the key to their having definite pitches. Sounds which are periodic have a definite pitch and musical sounds are perfectly periodic. Octave which can simply be known as a doubling in frequency (40 Hz is one octave greater than 20 Hz) is one dynamic concept related to music. The audible sound range has been arranged into several 'octaves' [3]. An individual octave has been classified into intervals, on the basis of some mathematical principles. The frequencies regarding Western music are compiled in a geometric series and an octave is categorized into twelve parts with the adjacent frequencies having a constant ratio of twelfth root of two. *Saptak*, which is equivalent to octave, in Indian Classical Music, is composed of twenty-two tones referred to as '*Shruti'.* Indian Classical Music has the seven pure notes called '*shuddha swaras'*. The *'shuddha swaras'* are surrounded by the microtones i.e. *shrutis,* which exhibit different frequency range (Table 1). In addition to these seven notes, the other additional five notes called the *'vikrit swaras'* are known, which are *Re Komal*, *Ga Komal*, *Ma tivra*, *Dha Komal* and *Ni Komal*. While '*Komal'* refers to a decrease in the frequency form the contiguous pure note, a rise in frequency means '*Tivra*'.

Table 1 Frequency ratios of 'shruti' values [3]

Shruti	Frequency ratio (f=v/λ)	Frequency (Hertz)
Sa	1/1	240
Re1	32/31	252.8
Re2	16/15	256
Re3	10/9	266.6
Re4	9/8	270
Ga1	32/27	284.4
Ga2	6/5	288
Ga3	5/4	300
Ga4	81/64	303.7
Ma1	4/3	320
Ma2	27/20	324
Ma3	45/32	337.5
Ma4	64/45	341.3
Pa	3/2	360
Dha1	128/81	379
Dha2	8/5	384
Dha3	5/3	400
Dha4	27/16	405
Ni1	16/9	426.6
Ni2	9/5	432
Ni3	15/8	450
Ni4	31/16	465

*Sa-Shadja, Re-Rishab, Ga-Gandhar, Ma-Madhyam, Pa-Pancham, Dha-Dhaivat, Ni-Nishad [4]; * V is the wave speed, λ is the wavelength of the wave [4].

The tone and scales in music are also an important element in music and the equally related terms are rhythm and speed. Several degrees of speed or beat called as '*Laya*', ranging from '*vilambit*' (very slow) to ultra-fast ('*Drut*'). A total of 240 vibrations per second are created by the note *Shadja* (SA). Likewise, number of vibrations generated by other notes is: *Rishab*(Re)-270, *Gandhar (*Ga)-300,*Madhyam*(Ma)-320, *Pancham*(Pa)-360, *Dhaivat* (Dha)-400 and *Nishad* (Ni)-450 [8]. Hypothetically, the Indian Classical Ragas can also be scientifically classified like the living organisms and such classification can be referred to as Genesis. According to the genesis of Indian Classical Ragas, *Thaat*equals Class, the Ragasare equivalent to Family and the Notes may be synonymous to species (Table 2).

Table 2 Genesis of Indian Classical *Ragaas* [5]

Name of *Thaat* (Class) [4]	**Some well-known Ragasof the corresponding *Thaat*(Family) [4]**	**Properties of *Notes* (species) of that *Ragas* [4]**	**Time of playing**
Bilawal	*Bilawal, Bihag, Durga, Hansdhwani*	All *shuddh* or natural notes	Almost all day
Kafi	*Kafi, Pilu, Bageshri, MiankiMalhar*	Ga, Ni, *Komal*	Almost all the day
Asavari	*Asavari, Jaunpuri, Darbari, Kanada*	Ga, Dha, Ni *Komal*	Noon (10am-12am), Night (10pm-12am), Midnight (12am-2am)
Khamaj	*Khamaj, Jhinjhoti, Desh*	Ni *Komal*	Late evening (8pm-10pm)
Kalyan	*Kalyan, ShuddhKalyan, Aiman*	No *Komal*, only *Teevra* Ma	Almost all day
Bhairav	*Bhairavi, Gauri, Lalit, Jogiya, Ramkali*	Re, Dha*Komal*	Early morning (6am-8am), Morning (8am-10am), Evening (6pm-8pm)
Bhairavi	*Bhairavi, Bilaskhani, Todi, Malkaus*	Re, Ga, Dha, Ni *komal*	Morning (8am-10am), Noon (10am-12am), Midnight (12am-2am)
Marwa	*Marwa, Jait, Vibhas,*	Re *Komal*, Ma *Teevra*	Almost all the day
|Poorvi	*Purvi, Shree, Basant*	Re, Dha*Komal*, Ma *Teevra*	Predawn (2am-4am), Dawn (4am-6am), Dusk (4pm-6pm), Evening (6pm-8pm)
Todi	*Todi, Multani, GurjariTodi*	Re, Ga, Dha*Komal*, Ma *Teevra*	Morning (8am-10am), Dusk (4pm-6pm)

1.3 Effect of music on plants

Synchronized sound waves of Indian classical music is also known to effect or stimulate the seed germination, flowering, fruiting and fruit ripening in several plants [6]. Several works and observations showed that plants respond to different types of music. It can show both detrimental and stimulatory effect in its growth and development. Sound with hard-core vibrations causes major detrimental effects on the plant growth and development [7]. Plants accept Indian classical music and showed an increased growth and development, which is well inferred from our present experimental research work [4]. We observed the effect of Indian classical music on plants at usual basis and could conclude from our observations how aptly our experimental plants responded to the tune of Indian classical music.

2 Material and methods

2.1 Materials used were as follows

- Acoustic chamber [4].
- Music system with the main unit of frequency 50 Hz-200Hz and two satellite units (with a frequency range of 200Hz-1800Hz) [4].

- Selected plant materials (*Chamaecostus cuspidatus*) [4].

2.2 Methods

- Extraction of bioactive compounds was carried out in both the selected control and experimental plant species by modified maceration method [8].
- Phytochemical screening of the extracts was carried out as per the standard methods [9].
- Assessment of the bioactive compounds in the leaf extracts was done by using UV-Vis spectroscopy [10].
- Determination of total phenolic content [11], total flavonoid content in the plant sample [12] and total tannin content [11].

3 Results and discussion

3.1 Results

Leaves of insulin (*Chamaecostus cuspidatus*) were taken to carry out the phytochemical analysis. The leaves were macerated in 9 different solvents and the extract was subjected to different phytochemical tests [8]. The observations are given in tabular forms.

3.1.1 Phytochemical screening of the extracts was carried out as per the standard methods [9].

a) Phytochemical screening of the extracts was carried out as per the standard methods [9] in *Chamaecostus cuspidatus* (control and experimental) in pure solvents- 5 different phytochemicals like alkaloids, tannins, glycosides, saponins and anthocyanins were analyzed using 4 different types of pure solvents like methanol, ethanol, acetone, and deionized water using different procedures [9]. The results are given in a tabular form (Table 3 and Table 4).

Table 3 Presence and absence of phytochemicals in *Chamaecostus cuspidatus* (control) in pure solvents

Solvents	Phytochemicals				
	Alkaloids	**Tannins**	**Glycosides**	**Saponins**	**Anthocyanins**
Methanol	+	+	+	+	-
Ethanol	+	+	+	-	-
Acetone	+	+	+	+	-
Deionized water	+	+	-	+	-

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

Table 4 Presence and absence of phytochemicals in *Chamaecostus cuspidatus* (experimental) in pure solvents

Solvents	Phytochemicals				
	Alkaloids	**Tannins**	**Glycosides**	**Saponins**	**Anthocyanins**
Methanol	+++	+++	+++	++	++
Ethanol	+++	+++	++	+	+
Acetone	+++	++	++	+	+
Deionized water	-	-	-	-	-

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

3.1.2 Assessment of the bioactive compounds in the leaf extracts was done by using UV-Vis spectroscopy [10].

Plant extracts were measured for their absorbance against solvent blanks at a wavelength ranging from 220-700 nm. Statistical analysis was carried out using SPSS software. The results were given in tabular forms (Table 5, Table 6, Table

7 and Table 8). The standard deviation and variance statistic were calculated for the control and experimental absorbance values.

Table 5 Quantitative analysis of the phytochemicals in *Chamaecostus cuspidatus* in methanol

	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
A	2	0.029	2.278	2.307	2.29250	0.014500	0.020506	0.000
B	2	0.046	2.517	2.563	2.54000	0.023000	0.032527	0.001
C	2	0.519	2.464	2.983	2.72350	0.259500	0.366988	0.135
D	2	0.427	2.025	2.452	2.23850	0.213500	0.301935	0.091
E	2	0.445	2.034	2.479	2.25650	0.222500	0.314663	0.099
F	2	0.435	1.978	2.413	2.19550	0.217500	0.307591	0.095
G	2	0.633	1.952	2.585	2.26850	0.316500	0.447599	0.200
H	2	0.430	2.048	2.478	2.26300	0.215000	0.304056	0.092
I	2	0.897	2.151	3.048	2.59950	0.448500	0.634275	0.402
J	2	0.062	3.276	3.338	3.30700	0.031000	0.043841	0.002
K	2	0.111	3.221	3.332	3.27650	0.055500	0.078489	0.006
L	2	0.855	1.901	2.756	2.32850	0.427500	0.604576	0.366
M	2	0.998	1.560	2.558	2.05900	0.499000	0.705693	0.498
N	2	1.780	0.665	2.445	1.55500	0.890000	1.258650	1.584
O	2	1.389	0.509	1.898	1.20350	0.694500	0.982171	0.965
P	2	0.801	0.975	1.776	1.37550	0.400500	0.566393	0.321
Q	2	0.027	1.519	1.546	1.53250	0.013500	0.019092	0.000
R	2	0.044	1.497	1.541	1.51900	0.022000	0.031113	0.001
S	2	0.691	0.554	1.245	0.89950	0.345500	0.488611	0.239
T	2	1.091	0.141	1.232	0.68650	0.545500	0.771453	0.595
U	2	0.820	0.078	0.898	0.48800	0.410000	0.579828	0.336
V	2	0.722	0.066	0.788	0.42700	0.361000	0.510531	0.261
W	2	0.313	0.254	0.567	0.41050	0.156500	0.221324	0.049
X	2	0.316	0.240	0.556	0.39800	0.158000	0.223446	0.050
Y	2	0.232	0.257	0.489	0.37300	0.116000	0.164049	0.027
Z	2	0.193	0.285	0.478	0.38150	0.096500	0.136472	0.019
Valid N (list wise)	2							

Table 6 Quantitative analysis of the phytochemicals in *Chamaecostus cuspidatus* in ethanol

	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
A	2	0.316	2.440	2.756	2.59800	0.158000	0.223446	0.050
B	2	0.223	2.455	2.678	2.56650	0.111500	0.157685	0.025
C	2	0.19	2.27	2.46	2.3625	0.09250	0.13081	0.017
D	2	0.09	2.16	2.25	2.2025	0.04250	0.06010	0.004
E	2	0.33	2.02	2.35	2.1860	0.16400	0.23193	0.054
F	2	0.23	1.97	2.20	2.0870	0.11400	0.16122	0.026
G	2	0.17	2.03	2.20	2.1120	0.08600	0.12162	0.015
H	2	0.13	1.87	2.00	1.9335	0.06450	0.09122	0.008
I	2	0.09	1.79	1.88	1.8330	0.04300	0.06081	0.004
J	2	0.30	2.47	2.77	2.6200	0.14800	0.20930	0.044
K	2	0.54	2.03	2.57	2.2960	0.27100	0.38325	0.147
L	2	0.28	1.80	2.08	1.9385	0.13750	0.19445	0.038
M	2	0.59	1.39	1.99	1.6900	0.29700	0.42002	0.176
N	2	0.40	1.48	1.88	1.6780	0.20100	0.28426	0.081
O	2	0.82	0.95	1.77	1.3585	0.40950	0.57912	0.335
P	2	0.74	0.83	1.57	1.1970	0.37000	0.52326	0.274
Q	2	0.45	0.75	1.20	0.9775	0.22350	0.31608	0.100
R	2	0.47	0.64	1.10	0.8695	0.23250	0.32880	0.108
S	2	0.42	0.58	1.00	0.7880	0.20900	0.29557	0.087
T	2	0.30	0.49	0.79	0.6415	0.14750	0.20860	0.044
U	2	0.21	0.47	0.68	0.5755	0.10250	0.14496	0.021
V	2	0.22	0.45	0.67	0.5570	0.11000	0.15556	0.024
W	2	0.13	0.48	0.61	0.5445	0.06350	0.08980	0.008
X	2	0.06	0.54	0.60	0.5680	0.03000	0.04243	0.002
Y	2	0.08	0.72	0.80	0.7595	0.03850	0.05445	0.003
Z	2	0.10	0.71	0.81	0.7560	0.04900	0.06930	0.005
Valid N (listwise)	2							

Table 7 Quantitative analysis ofthe phytochemicals in *Chamaecostus cuspidatus* in acetone

	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
A	2	0.100	2.368	2.468	2.41800	0.050000	0.070711	0.005
B	2	0.186	2.404	2.590	2.49700	0.093000	0.131522	0.017
C	2	0.120	2.707	2.827	2.76700	0.060000	0.084853	0.007
D	2	0.088	2.630	2.718	2.67400	0.044000	0.062225	0.004
E	2	0.248	2.742	2.990	2.86600	0.124000	0.175362	0.031
F	2	0.136	2.658	2.794	2.72600	0.068000	0.096167	0.009
G	2	0.084	2.439	2.523	2.48100	0.042000	0.059397	0.004
H	2	0.093	2.387	2.480	2.43350	0.046500	0.065761	0.004
I	2	0.101	2.330	2.431	2.38050	0.050500	0.071418	0.005
J	2	0.100	2.272	2.372	2.32200	0.050000	0.070711	0.005
K	2	0.097	2.263	2.360	2.31150	0.048500	0.068589	0.005
L	2	0.103	2.217	2.320	2.26850	0.051500	0.072832	0.005
M	2	0.117	2.186	2.303	2.24450	0.058500	0.082731	0.007
N	2	0.092	2.185	2.277	2.23100	0.046000	0.065054	0.004
O	2	0.253	1.425	1.678	1.55150	0.126500	0.178898	0.032
P	2	0.277	1.393	1.670	1.53150	0.138500	0.195869	0.038
Q	2	0.206	1.361	1.567	1.46400	0.103000	0.145664	0.021
R	2	0.210	1.346	1.556	1.45100	0.105000	0.148492	0.022
S	2	0.150	1.339	1.489	1.41400	0.075000	0.106066	0.011
T	2	0.141	1.337	1.478	1.40750	0.070500	0.099702	0.010
U	2	0.114	0.342	0.456	0.39900	0.057000	0.080610	0.006
V	2	0.016	0.339	0.355	0.34700	0.008000	0.011314	0.000
W	2	0.015	0.337	0.352	0.34450	0.007500	0.010607	0.000
X	2	0.008	0.342	0.350	0.34600	0.004000	0.005657	0.000
Y	2	0.008	0.339	0.347	0.34300	0.004000	0.005657	0.000
Z	2	0.023	0.319	0.342	0.33050	0.011500	0.016263	0.000
Valid N (listwise)	2							

Table 8 Quantitative analysis of the phytochemicals in *Chamaecostus cuspidatus* in deionized water

	N	Range	Minimum	Maximum	Mean		Std. Deviation	Variance
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic
A	2	.091	2.477	2.568	2.52250	0.045500	0.064347	0.004
B	2	.785	1.672	2.457	2.06450	0.392500	0.555079	0.308
C	2	1.271	1.132	2.403	1.76750	0.635500	0.898733	0.808
D	2	1.448	0.954	2.402	1.67800	0.724000	1.023891	1.048
E	2	1.496	0.793	2.289	1.54100	0.748000	1.057832	1.119
F	2	1.619	0.668	2.287	1.47750	0.809500	1.144806	1.311
G	2	1.818	0.427	2.245	1.33600	0.909000	1.285520	1.653
H	2	1.982	0.216	2.198	1.20700	0.991000	1.401486	1.964
I	2	1.798	0.191	1.989	1.09000	0.899000	1.271378	1.616
J	2	1.794	0.173	1.967	1.07000	0.897000	1.268550	1.609
K	2	1.729	0.147	1.876	1.01150	0.864500	1.222588	1.495
L	2	1.723	0.122	1.845	0.98350	0.861500	1.218345	1.484
M	2	1.659	0.109	1.768	0.93850	0.829500	1.173090	1.376
N	2	1.468	0.099	1.567	0.83300	0.734000	1.038033	1.078
O	2	1.140	0.316	1.456	0.88600	0.570000	0.806102	0.650
P	2	1.137	0.298	1.435	0.86650	0.568500	0.803980	0.646
Q	2	0.956	0.278	1.234	0.75600	0.478000	0.675994	0.457
R	2	0.936	0.265	1.201	0.73300	0.468000	0.661852	0.438
S	2	0.651	0.257	0.908	0.58250	0.325500	0.460327	0.212
T	2	0.645	0.254	0.899	0.57650	0.322500	0.456084	0.208
U	2	0.636	0.240	0.876	0.55800	0.318000	0.449720	0.202
V	2	0.422	0.257	0.679	0.46800	0.211000	0.298399	0.089
W	2	0.385	0.285	0.670	0.47750	0.192500	0.272236	0.074
X	2	0.323	0.244	0.567	0.40550	0.161500	0.228395	0.052
Y	2	0.360	0.205	0.565	0.38500	0.180000	0.254558	0.065
Z	2	0.254	0.202	0.456	0.32900	0.127000	0.179605	0.032
Valid N (listwise)	2							

3.1.3 Determination of total phenolic content [11] and total flavonoid content in the plant sample [11].

The total phenolic contents in plant extracts were determined by using Folin-Ciocalteu colorimetric method based on oxidation reduction reaction. All the experiments were repeated for three times and the results in the triplicate was determined. The absorbance was measured in 760nm against blank. The average absorbance values obtained were plotted for the calibration curve. The plant extracts were prepared, total phenolics content of the extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample in dry weight (mg/g). The results are given in tabular forms (Table 9 and Table 10).

Table 9 Total Phenol content in the leaves of *Chamaecostus cuspidatus*

Sample Type	Sample solution (gm/ml)	Weight of dry weight per ml (gm)	Absorbance	GAE concentration (mg/ml)	$C=c_1*v/m$ (mg/gm)	Mean±SEM
Control	1000	0.001	0.368	0.12	120	113.33±3.33
Control	1000	0.001	0.369	0.11	110	
Control	1000	0.001	0.368	0.12	110	

* Total Phenol content in the leaves of *Chamaecostuscuspidatus* (control).

Table 10 Total Phenol content in the leaves of *Chamaecostus cuspidatus*

Sample Type	Sample solution (gm/ml)	Weight of dry weight per ml (gm)	Absorbance	GAE concentration (mg/ml)	C=c1*v/m (mg/gm)	Mean±SEM
Experimental	1000	0.001	0.655	0.207	207	207.66±0.33
Experimental	1000	0.001	0.656	0.208	208	
Experimental	1000	0.001	0.657	0.208	208	

*Total Phenol content in the leaves of *Chamaecostus cuspidatus* (experimental).

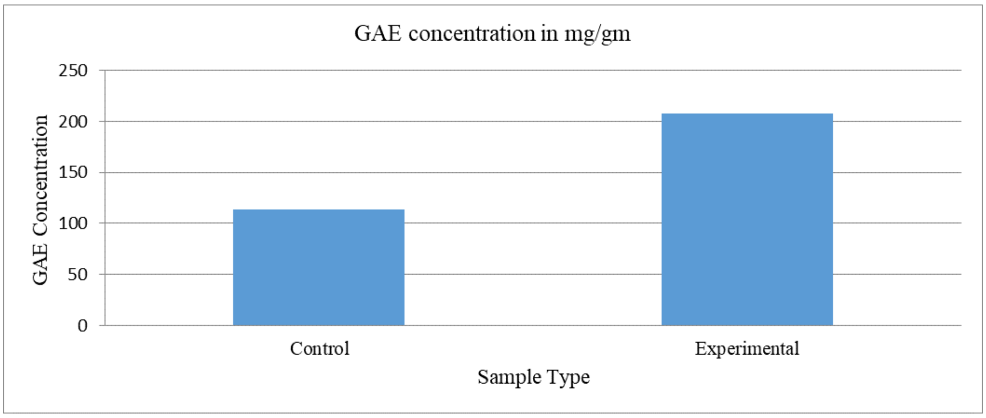

Figure 1 GAE concentration in mg/gm

The total flavonoid contents in plant extracts were determined by using aluminium chloride colorimetric assay. All the experiments were repeated for three times and the results in the triplicate was determined. The absorbance was measured in 510 nm against blank. The average absorbance values obtained were plotted for the calibration curve. The plant extracts were prepared, total flavonoid content of the extracts was expressed as mg quercetin equivalents (QE) per gram of dry extract (mg/g). The results are given in tabular form (Table 11 and Table 12).

Table 11 Total Flavonoid content in leaves of *Chamaecostus cuspidatus*

Sample Type	Sample solution (gm/ml)	Weight of dry weight per ml (gm)	Absorbance	Quercetin concentration (mg/ml)	$C=c_1*v/m$ (mg/gm)	Mean±SEM
Control	1000	0.001	0.051	0.027	27	26.66±0.33
Control	1000	0.001	0.050	0.026	26	
Control	1000	0.001	0.051	0.027	27	

*Total Flavonoid content in leaves of *Chamaecostus cuspidatus* (control).

Table 12 Total Flavonoid content in leaves of *Chamaecostus cuspidatus*

Sample Type	Sample solution (gm/ml)	Weight of dry weight per ml (gm)	Absorbance	Quercetin concentration (mg/ml)	$C=c_1*v/m$ (mg/gm)	Mean±SEM
Experimental	1000	0.001	0.069	0.038	38	37.66±0.33
Experimental	1000	0.001	0.069	0.038	38	
Experimental	1000	0.001	0.068	0.037	37	

*Total Flavonoid content in leaves of *Chamaecostus cuspidatus* (experimental).

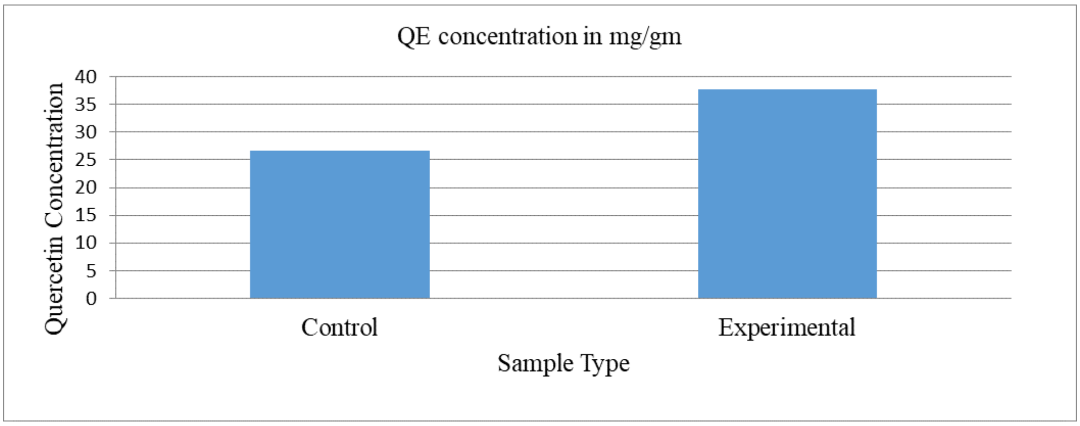

Figure 2 QE concentration in mg/gm

The total tannin contents in plant extracts were determined by using the modified Folin and ciocalteu method. All the experiments were repeated for three times and the results in the triplicate was determined. The absorbance was measured in 725 nm against blank. The average absorbance values obtained were plotted for the calibration curve. The plant extracts were prepared, total tannin content of the extracts was expressed as mg tannic acid equivalents (TAE) per gram of dry extract (mg/g). The results are given in tabular form (Table 13 and Table 14).

Table 13 Total Tannin content in leaves of *Chamaecostus cuspidatus*

Sample Type	Sample solution (gm/ml)	Weight of dry weight per ml (gm)	Absorbance	TAE concentration (mg/ml)	$C=c_1*v/m$ (mg/gm)	Mean±SEM
Control	1000	0.001	0.926	0.502	502	503±0.57
Control	1000	0.001	0.929	0.504	504	
Control	1000	0.001	0.928	0.503	503	

*Total Tannin content in leaves of *Chamaecostus cuspidatus* (control).

Table 14 Total Tannin content in leaves of *Chamaecostus cuspidatus*

Sample Type	Sample solution (gm/ml)	Weight of dry weight per ml (gm)	Absorbance	TAE concentration (mg/ml)	$C=c_1*v/m$ (mg/gm)	Mean±SEM
Experimental	1000	0.001	1.200	0.841	841	843±1.99
Experimental	1000	0.001	1.200	0.841	841	
Experimental	1000	0.001	1.210	0.847	847	

*Total Tannin content in leaves of *Chamaecostus cuspidatus* (experimental).

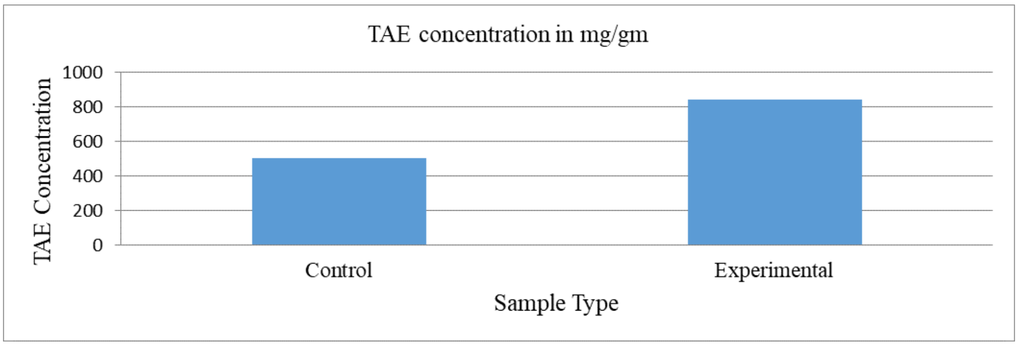

Figure 3 TAE concentration in mg/gm

4 Discussion

In the present research work, the leaves of the treated plant showed the presence of higher concentration of phytochemicals like alkaloids, tannins, glycosides, saponins and anthocyanins when qualitatively examined when treated with Indian classical Instrumental music. Through the assessment of the bioactive compounds in the leaf extracts by using UV-VIS spectroscopy the standard deviation was calculated by taking the absorbance of both control and experimental sample. The standard deviation values in all of the pure solvents that are methanol, ethanol, acetone and deionized water indicates that the data were spread over a wider range of values, which further shows that the treated plants have higher concentration phytochemicals when compared to that of the untreated samples. The total phenol content was found to be 207.66±0.33 mg/GAE per gram extract in case of treated plant samples and 113.33±3.33 mg/GAE per gram extract in case of untreated plant samples of *Chamaecostus cuspidatus*. Similarly, the total flavonoid content was found to be 37.66±0.33 mg QE/g extract in case of treated plant samples and 26.66±0.33 mg QE/g extract in case of untreated plant samples and the total tannin content was found to be 843±1.99 mg TAE/g extract in case of treated plant samples and 503±0.57 mg TAE/g extract in case of untreated plant samples (Figure 1,2 and 3).

5 Conclusion

Plants do absorb and resonate to external frequencies in the form of sound waves as conceptualized and established by Sir Jagdish Chandra Bose a Nobel Prize winner in 1977 [4]. He revealed that plants could feel and were aware of their surroundings [4]. Sound waves are received by the plants and results in the faster protoplasmic movement [6]. Due to the sound waves mechanical vibrations are generated by the charged cell membranes and wall [4]. The mechanochemical enzymes which use chemical energy in the form of ATP to produce mechanical vibrations in cells contribute to the sound wave generation in plant cells [4]. These mechanisms lead to the nanomechanical oscillations of cytoskeletal components of the plant cells that generate both high and low wave frequency vibrations [4]. The synchronized sound waves in the form of Indian classical *ragas* act as potent plant growth stimulant and as plant protectant [4]. Plant growth stimulant as it elicits various effects in the plants [4]. It leads to the enhancement of seed germination in the way showing early seed germination and healthy seedling growth [4]. Synchronized sound waves also showed an earlier fruiting and fruit ripening in terms of increased concentration of metabolites [4]. Musical sound promotes the plant growth by regulating synthesis of phytohormones Indole3-acetic acid and Gibberellic acid in increased manner [4]. Plant growth promotion can also be related to that of the enhancement of photosynthetic activity. The sound waves manipulate the expression of photosynthetic related genes that leads to an increase in the photosynthetic rates in plants [13]. Synchronized sound waves in the form of Indian classical Instrumental Classical music showed an increased in phytochemical contents. The phytochemical compounds have an effect in the biological systems that elicit pharmacological or toxicological effects in humans and animals [8]. The standard deviation values in all of the pure solvents indicates that the data were spread over a wider range of values, which further showed that the treated plants have higher concentration phytochemicals when compared to that of the untreated samples.

Compliance with ethical standards

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Disclosure of conflict of interest

All authors declare that there is no conflict of interest as all authors have contributed equally to the experimental works and preparation of the manuscript.

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