TIME BASED STUDY OF THE INFLUENCE OF MORNING AND EVENING CLASSICAL RAGAS ON PROTEIN, CARBOHYDRATE AND CHLOROPHYLL OF SELECTED MEDICINAL HERBS. APPLICATION OF RAGA - TIME CONCEPT TO INCREASE MEDICINAL VALUE OF SELECTED PLANTS

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ABSTRACT

Classical music is proven to enhance metabolism and gene expression. Objective of this experiment is to study the effect of classical Morning and Evening Ragas on plants and determine if their effect co-related to the time of the day during which it is played and to determine if altering the specified time will antagonize and alter the nutritive value of the plant. 30 kitchen herbs of medicinal value are taken in separate pots. Plants are divided into five groups of six herbs each. Morning Raga is exposed in the morning to first group. Evening Raga is exposed during evening to second group. The timing of the ragas were interchanged and played to the third and fourth groups respectively. The control group was not exposed to any music. Growth was recorded and studied for 15-30 days over a series of three test runs. At the end of experimentation, the plants were harvested and estimated for protein, carbohydrates and chlorophyll. Results show that protein and carbohydrates were higher in the plants exposed to morning rag in the morning and evening rag in the evening as compared to the plants whose music was interchanged or the control. This finding can be used to quantitatively improve the productivity and nutritive value of the medicinal plants. Many Indian ragas are sung or played irrespective of their conventional proper time. But it has not been possible to demonstrate that on account of the wrong time the melody has not had the desired effect. This is why it is difficult to accept the old Raga Samaya (time) theory fully in the present day. This study aims to study the Time-Raga
theory. The main factor considered in this study was time of the day during which the specified time based raga is played.

**KEYWORDS:** Morning Raga, Evening Raga, classical music, antagonize, Protein, Carbohydrates.

**INTRODUCTION**
The universal sound ‘AUM” or the sacred syllable as believed in Hinduism embodies the essence of the universe - it is the hum of the atoms and the music of the planets in space and it represents the primal energy that holds the material world together. It is connected to the kundalini energy.\(^3\) Origin of music is believed to come from nature.\(^1\) The basic sounds are associated with the call of a specific animal or bird.\(^2\) In ancient India, music originated from Sama veda of the Hindu tradition where 2 or more notes were used for hymns. Indian Classical Music is the traditional art music of the Indian subcontinent. It is performed based on particular ragas (melodies) and talas (rhythms). Raga means “color” in sanskrit and is figuratively applied to indicate different moods of harmony. They are a combination of 5 or more notes of either low or high frequencies pitches derived from a parent raga. The way the notes are approached and the musical phrases are arranged defines a specific raga. Thus each raga has a unique emotional quality.\(^3\)

The time theory of Raga is found only in Indian music. Hindustani classical music has a distinctive feature of relating seasons, emotions and time of the day as morning ragas, afternoon ragas, evening, night ragas.\(^{4,5,22}\) Vedic literature also deals with cycles of time. In Ayurveda, there appears to be considerable evidence linking it with musical performance in ancient times. There exists a relation between various scales and the humors of the body. Narada”s Sangeetamakaranda I.23-24 states that wrong usage can reduce lifespan. Time theory is believed to work in the human body in correlation with the 3 Doshas - Kaph, Pitta and Vata. These elements work in a cyclic order of rise and fall during the 24 hour period. It also differ with the seasons. Hence it is said that performing or listening to a raga at the proper allotted time can affect the health of human beings.\(^6\) This concept forms the basis of music therapy or Raga Chikitsa.

Time based classification was given by Vishnu Narayan Bhatkhande (1860-1936). It is based on the impact of a particular note at a particular hour of the day.\(^{7,8}\) Frequencies of 96-1024 Hz can be produced by Indian vocal music, while the human ear is capable of picking up
sound frequencies between 20-20,000 Hz.\textsuperscript{[3]} but the frequencies that plants can hear has not been clearly established.

Plants respond to acoustic stimuli such as music has been established by various experiments.\textsuperscript{[9,10]} The metabolism and protein expression\textsuperscript{[11]} is significantly improved with exposure to classical music over others.\textsuperscript{[12,13,14]} Raga Therapy involves playing, performing and even listening to appropriate ragas can work as a complementary medicine for psychological and physiological ailments.\textsuperscript{[15,16]} This effect is due to correspondence of 7 musical notes to the 7 energy chakras of human body.\textsuperscript{[3]} Early experiments have proven that they respond to gravitational, temperature and electromagnetic changes at the same time. They are believed to have living consciousness.\textsuperscript{[17, 18]}

The objective of this study is to find out if plants are affected by the emotion (Rasa) of a tune based on the Time-Raga relationship by examining the effect of ragas at different times of the day on kitchen herbs used day to day. To determine if the effect co-related to the time of the day during which it is played and study if altering the specified time will antagonize and alter the nutritive value of the plant. This will help to quantitatively increase nutrient value of medicinal plants.

MATERIALS AND METHODS

30 pots (1kg pots) were separated into five sets each containing the 6 herbs, Chick pea (Cicer arietinum) Moong beans (Vigna radiata), Fenugreek or Methi (Trigonella foenum-graecum), Cilantro or Coriander (Coriandrum sativum), Wheat (Triticum aestivum) and Horse gram (Macrotyloma uniflorum). The materials used were standardized. Healthy organic soil with (pH 5.5-6.5) with mineral content (N, P, K, Mg, Cu, B, Zn) was chosen for potting ensuring the amount of mud and moisture content was equal in all pots. Seeds of the selected herbs were soaked overnight in bowls of water. Healthy seeds- those that sink were removed and kept aside. About 12 holes were made with finger tips 3 cm deep with good spacing between each hole. Each hole was numbered. The chosen seeds were placed 2 seeds in each hole and loosely covered with soil the same day. For wheat germ, 3 seeds were placed in each hole.

Morning Raga (Aditya Hrudayam in rag Bibhas/Vibhas also known as Bhupalam in Carnatic music) was played to one set of plants at dawn during sunrise for 2hrs (6.30-8.30 AM) and to another set during night time (9.30-11.30 PM) for 2 hrs regularly each for 15 days. Aditya Hrudayam is a vedic hymn dedicated to Sun God. Similarly Evening Raga
Bhageshri was exposed to one set during sunrise (6.30-8.30 for 2hrs) and another set during night (9.30-11.30 PM) for 2hrs. The pots were placed at a distance of 20cm from the speaker. The Control was not exposed to any music. The pots were watered with 100 ml of water every 3 days. Care was taken to ensure minimal ambient noise and the same amount of sunlight and water for all the plants. During other times all the pots were placed in a well lit and temperature controlled environment of 23 deg C. The music selected was a combination of both vocal (Hindustani classical by Pt. Sanjeev Abhyankar in both the ragas) and instrumental music (keyboard rendition by Adithi Ravichandran in both ragas).

The plants were monitored for 15-30 days over various trials after which the plants were harvested and biochemically tested.

**Estimation of Protein, Carbohydrate and Chlorophyll**

**Extraction of Protein from plants**

Ground 5 mg of each plant sample material to a powder with liquid nitrogen in a mortar and pestle. Added 2.5 mL of Tris pH 8.8 buffered phenol and 2.5 mL of extraction media (0.1 M Tris-HCl pH 8.8, 10 mM EDTA, 0.4% 2-mercaptoethanol, 0.9 M sucrose) and continued grinding for an additional 30 sec in a fume hood. Alternatively, transferred to a 15 or 50 mL Falcon tube and homogenized in polytron homogenizer for one minute. Transferred to Falcon tube and agitated for 30 min at 4 C. Centrifuge 10 min at 4,000 g, 4 C.

Removed phenol phase (should be top phase) and back-extract aqueous phase with 2.5 mL + 2.5 mL of extraction media and phenol by vortexing. Centrifuged and combined with first extraction. Phenol extracted proteins are precipitated by adding 5 volumes of 0.1 M ammonium acetate in 100% methanol (stored at –20 C) to phenol phase. Vortexed and incubated at –20 C for at least 1 h or overnight. The precipitate was collected by centrifugation (20 min, 4,000 g, 4 C). Washed the pellet 2X with 0.1 M ammonium acetate in methanol, 2X with ice-cold 80% acetone and finally 1X with cold 70% ethanol. Completely resuspended the pellet each time with vortexing and sonicated. Placed the resuspended sample at –20 C for at least 15 min between each wash. Stored the last suspended pellet in 80% acetone at –20 C until dry. The last pellet under nitrogen (or at 37 C for 15 min) and stored at -20 C. Resuspended final pellet in 0.5-1 mL of IEF extraction solution (8 M urea, 2 M thiourea, 2% CHAPS, 2% Triton X-100, 50 mM DTT, 0.5% pH 3-10 ampholytes) by pipetting and vortexing at 25-30 C. Incubated sample for 1 h at room temperature with agitation. Precipitate protein sample dissolved with acetone for estimation.
Protein estimation: Bradford method
Bradford assay is used to find the concentration of protein for the given sample.\textsuperscript{[21]}

Preparation of Bradford Reagent
50mg of Coomassie brilliant blue (CBB) was dissolved in 25ml of 95% Ethanol and mixed with 50ml of 85% phosphoric acid and made up to 500ml using distilled water. The mixture was then filtered for 4-5 times using Whatmann filter paper. The filtrate was stored in the absence of sun light.

Preparation of BSA Standard Solution
100 mg of BSA was weighed and dissolved in 100ml of distilled water as a stock solution. 10ml of BSA stock solution was taken and made up to 100ml with distilled water as a standard solution. The concentration of standard solution is 100μg/ml.

The different concentration of standard solution was taken from 10μg-100μg in series of ten test tubes and made up to 5ml with distilled water. Then 5ml of CBB reagent was added to reaction mixture. The readings were measured with colorimeter at 595nm. The Standard graph is plotted with concentration of protein and absorbance (fig 1).

![Figure 1: Standard graph of BSA.](image)

Carbohydrate Analysis
0.5g of macerated sample was placed in 25ml bottle, 5ml of distilled water was then added and shaken vigorously followed by addition of 7.5ml of 52% perchloric acid. This was stirred continuously for 30minutes and the mixture was later filtered using Whatman no1 filter paper. 1 ml of the filtrate was mixed with 2ml of Anthrone reagent in a test tube and the
absorbance of the mixture was measured using spectrophotometer at a wavelength of 620nm. The total soluble carbohydrate was then estimated using the standard curve of Glucose.

![Figure 2: Standard graph of Glucose.](image)

Chlorophyll Analysis: Spectrophotometric Determination of Chlorophyll

**Procedure**

0.10g (100 mg) of the sample material was weighed separately for grinding. The tissue was put into a mortar and 10 ml of 80% acetone (acetone: water 80: 20 v: v) was added. The tissue was ground with a pestle. A few grains of sand was used to pulverize the tissue completely. Leaf sample was thus made homogenous. The leaf homogenate was filtered through the filter paper. The residue was removed by the filter paper (and discarded) and the extract (or filtrate) is collected in a test-tube. Determination of chlorophyll concentration was done by using UV-spectrophotometer at 663 and 645nm.

**RESULTS**

MM- Morning rag in Morning time, ME-Morning rag in Evening time, EM- Evening rag in Morning time, EE-Evening Rag in Evening time.

**Table 1: Bengal Gram: Biochemical properties.**

<table>
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A- Protein (μg/ml), B-Carbohydrate (nm/ml), C-Chlorophyll (mg/g of sample).
Table 2: Coriander: Biochemical properties.

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A- Protein (μg/ml), B-Carbohydrate (nm/ml), C-Chlorophyll (mg/g of sample).

Table 3: Fenugreek: Biochemical properties.

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A- Protein (μg/ml), B-Carbohydrate (nm/ml), C-Chlorophyll (mg/g of sample).

Table 4: Greengram: Biochemical properties.

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A- Protein (μg/ml), B-Carbohydrate (nm/ml), C-Chlorophyll (mg/g of sample).

Table 5: Wheat germ: Biochemical properties.

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A- Protein (μg/ml), B-Carbohydrate (nm/ml), C-Chlorophyll (mg/g of sample).
### Table 6: Horsegram: Biochemical properties.

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A- Protein (μg/ml), B-Carbohydrate (nm/ml), C-Chlorophyll (mg/g of sample)

Results show (Table 1-6) that, in comparison to the control, all plants exposed to morning raga at specified morning time and evening raga at specified evening time showed similar or more amounts of protein and carbohydrates than the other other set of plants whose music was interchanged to morning raga at evening time and evening raga at morning time. In Bengal gram Evening raga in evening time showed high amounts of carbohydrate and chlorophyll Table.\[^1\]

In Green gram, Fenugreek and Coriander, protein, carbohydrate and chlorophyll content were much higher in the evening rag exposed to evening time [Table 2, 3,4 ]

In wheat germ, Protein, carbohydrate and chlorophyll content was markedly high in morning rag at morning time [Table 5]. According to the vedic calendar, wheat is one of the navadhanyas associated to the sun which shows an excellent response to morning raga at morning time.\[^20\]

In horse gram, Morning rag at morning time and evening raga at evening time brought out the best results [Table 6].

![Figure A: Average increase in all the parameters of various plants.](image-url)
Evening raga played at evening time triggered maximum chlorophyll production (Table 1,2,3,4,6) in almost all herbs except wheat germ and significant increase in growth of Coriander. The main note (vadi swara) in the morning raga Bhupalam is Shuddha Dha and Shuddha Re, corresponding western notes being (D, A). In evening raga Bhageshri it is Shuddha Madhyam (F).

**DISCUSSIONS**

**Evening Raga at Evening time**

It is evident from the graph that Evening rag at Evening time shows greater concentrations of biochemical parameters. This indicates enhanced nutrient absorption from roots and oxygen absorption from leaves.

**Interchanged Timing**

Morning rag at Evening time and Evening rag at Morning time showed good physical growth in plants than control and the morning exposure to morning time. Although the protein production was more than the control, chlorophyll amounts were similar and carbohydrates were much lower.

**Morning Raga at Morning Timing**

Plants exposed to Morning raga at morning time showed lesser height compared to the control and other plants. However the number of leaves and and percentage of healthy plants...
were more than the control. Interestingly, the concentrations of protein, carbohydrates and chlorophyll are very high in morning raga at morning time and evening raga at evening time indicating that the effect of the raga at the specified time is more than what it would have at any random time. Better concentrations of Protein, Carbohydrates and Chlorophyll content in plants may imply that the frequency of waves is shown to directly affect the stomatal opening and the CO2 intake so rate of photosynthesis process occurs quickly. Thus the efficiency of the morning raga was best at morning time to trigger increased biochemical parameters and vice versa. The moisture content (wetness) of the soil was more plants exposed to music in the evening time.

CONCLUSION
The experimental data indicates that there is a correlation between the Indian ragas played at the specified time which was evident with the data of protein and carbohydrate content. Each plant responded by liking a certain raga at a certain time of the day. This “Raga and Time” concept can be used for therapeutic time based healing of Raga therapy to maximize the effect and benefits of the Raga at the specified time. Agriculturists could employ this knowledge of the right raga to be played at the appropriate time as favoured by both medicinal and ornamental plants to help speed up growth, quality, quantity and disease resistance. The nutritive value, vitamin and mineral content of plants can be increased to sustain large demands of our growing population. Chemical pesticides and fertilizers could be avoided to ensure good health of society at large. There is a great scope for time specific raga approach in autistic patients. Autistic patients, especially children can be made to listen to the appropriate music at a specific times in the morning for increasing their neuromotor capacities, gross motor skills, language and communication. In the night time appropriate music can be used for relaxation in patients with sleep disorders and ADHD. The therapeutic effects of Music Therapy is more pronounced in psychological disorders. Hence the results are expected to be highly promising. There is a great scope for time specific raga approach in autistic patients. Autistic patients, especially children can be made to listen to the appropriate music at a specific times in the morning for increasing their neuromotor capacities, gross motor skills, language and communication. In the night time appropriate music can be used for relaxation in patients with sleep disorders and ADHD. The therapeutic effects of Music Therapy is more pronounced in psychological disorders. Hence the results are expected to be highly promising.
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