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BIOACTIVE COMPONENT STUDY OF GARCINIA CAMBOGIA RIND EXTRACTS AND HARNESSING IT'S POTENTIAL FOR FOOD PRODUCT DEVELOPMENT: A NOVEL TREATMENT FOR PCOS

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Abstract:

Garcinia Cambogia (kudam poli) traditionally known as kokum butter is used as a medicinal plant; claimes to possess antioxidant properties and significant antimicrobial activity. Garcinia Cambogia is found commonly in the evergreen forests of Western Ghats, Konkan south, Travancore, and in the Shola forests of Nilgiri. The fruit's active ingredient-HCA extracts is effectively used to relieve the symptoms of PCOS due to its potent antihyperglycemic & lipolytic effects. It is a low glycemic index product, the HCA content is said to reduce the conversion of carbohydrates into fats.The present study aimed at evaluating the bioactive efficacy of medicinal plant under study by preparation of alcoholic and aqueous extracts of Garcinia Cambogia rind and comparing the bioactive content in each of the extracts. The study also determines the potential bioactive component for treatment of PCOS. The aqueous and alcoholic(methanolic) extracts were prepared for the comparative assessment of the presence of total phenol, total flavonoid and total tannin content.Total phenol and tannin content was determined using Folin-Ciocalteu's method and content was expressed as mg GAE(gallic acid equivalent)/g of the plant tissue while total flavonoid content was assessed by Aluminium calorimetric assay and it's amount was expressed as mg QE(quercetin equivalent)/g of the plant tissue . The major bioactives found in Garcinia extracts were HCA(Hydroxy citric acid), total phenols(0.048 mg GAE/ g), total tannins(0.041 mg GAE/ g) and total flavonoids(0.31 mg QE/ g). The

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developed food products from garcinia extracts were subjected to sensory evaluation by sensory panelists(confirmed PCOS candidates) which were well accepted by them i.e with a mean of 6.77±0.72. Further a microbial analysis was also conducted to determine the shelflife of the extracts and it's best accepted product variation.Extensive literature review states that higher consumption of total dietary flavonoids have insulin sensitizing potential along with reversing of the steroidogenesis condition both of which play an important role in the pathophysiology of PCOS. Intake of total dietary flavonoids have also been found to be beneficial in the treatment of metabolic syndrome and PCOS.

Keywords: Garcinia cambogia,Polycystic ovarian disease, bioactives, steroidogenesis, flavonoids.

INTRODUCTION

Polycystic ovary syndrome(PCOS) is considered a multifacet disease characterized by metabolic and reproductive disorders associated with hyperandrogenism and insulin resistance[1].

It is one of the common conditions affecting women of reproductive age and characterized by menstrual irregularities ,signs of androgen excess, and in many cases obesity. Increased insulin resistance and compensatory hyperinsulinemia play a key role in the pathogenesis of PCOS. Therefore, insulin sensitizing agents have been studied in the management of PCOS[2,3]

PCOS is also an endocrine disorder with the above discussed symptoms which affects 5-15 % in women of childbirth age[4,5].

The clinical manifestations of PCOS are heterogenous and considerable diversity can be seen in hyperandrogenemia, hirsutism, acne and alopecia.PCOS patients also have a high risk for the development of severe metabolic disorders, such as metabolic syndrome, type 2 diabetes , cardiovascular disease and endometrial cancer[6].

Since the pathogenesis of PCOS is unclear and heterogenous, the treatment of PCOS is also not unified and multifacet in nature. Weight reduction and lifestyle modification are highly recommended therapies that improves metabolic status, symptoms of ovulation irregularity and protects PCOS women from cardiovascular diseases and type 2 diabetes[7].

Plant part extracts are used to isolate specific chemical compounds that are major targets to be used for mankind.The basis for reporting bioactive compounds in plants is investigated from the fact that these plants have been used in traditional and herbal medication.

One such novel medicinal plant that potentially helps in treating PCOS & is low glycemic index food is Garcinia cambogia.The fruit's active ingredient-HCA(Hydroxy Citric Acid) is effectively used to relieve the symptoms of PCOS due to its potent antihyperglycemic & lipolytic effects.

It is a low glycemic index product, the HCA content is said to reduce the conversion of carbohydrates into fats. This is done by blocking the enzyme citrate lyase which converts citrate to cholesterol & fat respectively. It suppresses appetite thereby reducing weight gain while increasing the production & storage of glycogen-insulin sensitivity [8].The fruit rind is also rich in healthy antioxidants like phenols, flavonoids and tannins ,each of them having significant biological activity on human health. In an ayurvedic science research, Garcinia Cambogia is termed as 'Vrikshamla';effective drug for the management of Medoroga (obesity disorders) and cardiac problems. Further it exerts anti-hyperglycemic, insulin-sensitization, islet protection, anti-dyslipidemic, anti-obesity and antioxidant activities in vitro and in vivo.The study concludes that it has metabolic correction and disease reversible effects[9].

According to a study analyzing the relationship between flavonoid intake and metabolic syndrome(MetS) in Korean women with PCOS stated the significance of flavonoids in the treatment of MetS. The study selected 27 PCOS women with MetS and estimated their dietary intake using MDA(Mini Dietary Assesment) score and intake of six flavonoid classes using a flavonoid data base. The results stated that there was a significant inverse relationship between flavonol intake and risk of MetS ,further concluding that higher the intake of flavonoids lower is the risk of having PCOS related complications[10].

Research method

The dried rind of Garcinia Cambogia was procured locally from the market or it can be obtained from organic store as well.

Preparation of alcoholic extracts:

The solvent used for preparation of the plant extracts involves methanol.

The plant tissue; i.e garcinia rind was cleansed with distilled water, shade dried at room temperature separately and stored in air tight container for further use. Then they were oven dried separately and coarsely powdered.

Extraction was carried out using simple solvent maceration technique.The solvent used for the study is methanol as it is most stable and helpful in extraction of the phytochemicals.

Exactly 30g of the coarsely powdered plant tissue was macerated with motor and pestle and homogenised in 300 ml of methanol(1:10) into a 500ml conical flask and shaken well.The garcinia samples were stored in the dark soaked for 48 hours with tight cotton plugging .

The samples were then centrifuged at 4000rpm for 10-15 min.The procedure was repeated twice and the supernatants were collected and filtered through Whatman No.1 filter paper.The extracts were stored at 4°C and used further for phytochemical analysis[11,12].

Preparation of aqueous extracts:

One gram each of garcinia peel powder was mixed in 100 ml of warm water separately and converted into aqueous extracts which mainly included:

a) Decontamination of the plant

b) Comminuting the plant.

c) Suspension of the mixture obtained in warm water.

d) Maceration of the suspension obtained in the previous step.

e) Separation of the resulting liquid.

Preparation & standardisation of health drink recipes:

Health drink recipe :

Apple seedy delight:(Pomegranate drink)

Method:

1. Peel a fresh pomegranate and separate the fruity seeds from the peel.
2. Add in the fruit, jaggery, cardamom powder, cinnamon powder, tulsi water, salt into the blender.
3. Pulse it for few times in a blender until the outer layer of seeds break and release the juice.

4. Don't blend it, just pulse it because if the seeds break completely, the juice turns bitter.
5. Serve chilled.

Formulation of health drink with garcinia extracts:

The aqueous extracts of garcinia that were prepared earlier were divided into three different variants to be incorporated into the standardized health drinks.

The three aliquots chosen for the preparation of variants were:

30 ml

60ml

90 ml

Note: As mentioned before 1 g of each of the plant tissue was dissolved in 100 ml of warm water.This was done twice to obtain the first two portions of extract from first 100 ml aqueous solution and the third portion from the second 100 ml aqueous solution.

Assessment of the presence and concentration of bioactive components in the prepared extracts:

Qualitative analysis:

The four groups of major bioactives that were analysed in the Garcinia cambogia were: Phenols ,Flavonoids ,Tannins & Terpenoids.

The extract of medicinal plants is analyzed for the presence of flavonoids, phenols, tannins and terpenoids according to standard methods (Edeoga *et al*., 2005;Yadav *et al*., 2012, Harborne *et al.*, 1973,Kokate *et al*., 2000)

Both aqueous and methanolic extracts were used for screening the phytochemicals.

Screening for phenols :

1ml of the extract was treated with 3% ferric chloride. If there is an appearance of deep blue color, then it shows the presence of phenol **[13,14].**

Screening for flavonoids:

1mi of the extract was added with 1ml of sulphuric acid. Orange color formation confirmed the presence of flavonoids [13,14].

Screening for Tannins (Braymers Test):

1ml of the extract was added mixed with 2ml of water. To these 2 drops of 5% ferric chloride solution was added. Appearance of dirty green precipitate indicated the presence of tannins [14,15].

Screening for terpenoids :

2ml of the extract was added with 2ml acetic acid. Then concentrated sulphuric acid was added. Deep red color development showed the presence of terpenoids[16].

Quantitative analysis:

Quantitative estimation of bioactives namely total phenols, total flavonoids and total tannins in the plant extracts were performed using spectrophotometry. Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength .The equipment used for spectrophotometry is spectrophotometer -an apparatus for measuring the intensity of light in a part of the spectrum, especially as transmitted or emitted by particular substances.

Total phenolic content were determined using Folin-Ciocalteu's method. The procedure was performed referring to Singleton *et al.*,1999[17]. with minor modifications. Here gallic acid was used as the standard and the absorbance of the standard and test solutions are measured against the blank at 750nm. Calibration curve was plotted using standard gallic acid concentrations .The total phenolic content was expressed as mg of gallic acid equivalent weight(GAE)/g of extract.

Total tannin content was determined by Folin-Ciocalteu's method. The assessment was performed by referring to Rajeev *et al.*, 2005[18] with slight modifications. Here gallic acid was used as the standard. Absorbance of the test and standard solutions were measured against the

blank at 725nm with UV/Visible spectrophotometer.The total tannin content was expressed in terms of mg of GAE/g of extract.

Total flavonoid content was measured with the aluminium chloride colorimetric/ spectrophotometric assay by referring to Lee & Ismail., 2012 [19]. Here absorbance of the standard and test solutions at measured at 510nm spectrophotometer.The calibration curve was plotted using standard quercetin. The total flavonoid content were expressed as mg of quercetin equivalents/g of extract.

Microbial analysis of developed health drink and aqueous extracts of garcinia cambogia rind:

Microbial analysis is defined as analysis techniques to help profile and identify the bacterial and fungal diversity in a variety of clinical and environmental samples.

The above analysis are usually done by following a 2-step process:
Serial dilution(pour/spread plate)

Coliform forming units CFU) determination

The extracts were sealed and stored in plastic bottles and used during the testing.Serial dilution of each extract was done and then plating was performed using pour plate and spread plate method.

Nutrient Agar(NA) was used for pour plate method while Potato Dextrose Agar(PDA) was used for spread plate method. The dilutions were used in triplicates and the Colony Forming units(CFU) were counted.

Pour plate method was done using 1ml of the dilutions 10^{-7},10^{-8}, 10^{-9} and incubated at 35°C to 37°C for 24 hours.This is used to estimate the CFU for bacteria. Spread plate method was done using 0.1 ml of the dilutions 10^{-4}, 10^{-5}, 10^{-6} and incubated at room temperature (25 to 28°C) for 24-28 hours. This estimates the fungal count of the food.

Sensory evaluation of the developed health drink:

Sensory evaluation of the products was done using a 9-point hedonic scale. The 9-point hedonic scale is also called as the degree of liking scale and is commonly used to check the acceptability of the product among the consumers.

The 9-point hedonic scale was first developed by David Peryam at the Quartermaster Food and Container Institute of the U.S. Armed Forces; for the purpose of measuring the food preferences of soldiers[20].The scale was widely adopted in food industry, for measuring the acceptability of foods and beverages, but also of personal care products, household products, and cosmetics.

The scale has equal intervals as given below ranging from "like extremely" to "dislike extremely" and a central point-"neither like or dislike". The minimum acceptability belongs to 1(dislike extremely) and the maximum acceptability belongs to 9(like extremely).

9-Point Hedonic Scale

Like Extremely
Like Very Much
Like Moderately
Like Slightly
Neither Like nor Dislike
Dislike Slightly
Dislike Moderately
Dislike Very Much
Dislike Extremely

The sensory booth constituted a 30 semi trained panelists who were diagnosed with PCOS few years back and some of them were newly diagnosed with PCOS selected according to the inclusion criteria.They were chosen to evaluate the developed health drink using the 9-point hedonic scale on the basis of appearance, taste -flavour,after taste and overall acceptability. The panelists were briefed about the method of sensory evaluation and were requested to avoid bias during evaluation.

The sensory evaluation was conducted in food analysis lab as it had a sterile environment, odour free atmosphere and adequate light.The drink was served in clean white cups and did not influence the color of the drink.

A resting duration of 30 seconds is usually given between each drink evaluation as it does not influence or mix the tastes of different drinks. This also ensures clarity in the result. The drinks were given a 3 digit code to obscure the identity of the product and presented in a serial order.

Results and Analysis

Calculated nutritive value of the best accepted health drink variation:

Nutritive value of garnate shots:

Table 1. Nutritive value of garnate shots per 200 ml (V2)

NUTRIENT	VALUE
Energy(kcal)	25
Protein(g)	0.9
CHO(g)	38
Fat(g)	-
Fibre(g)	-

Sensory evaluation:

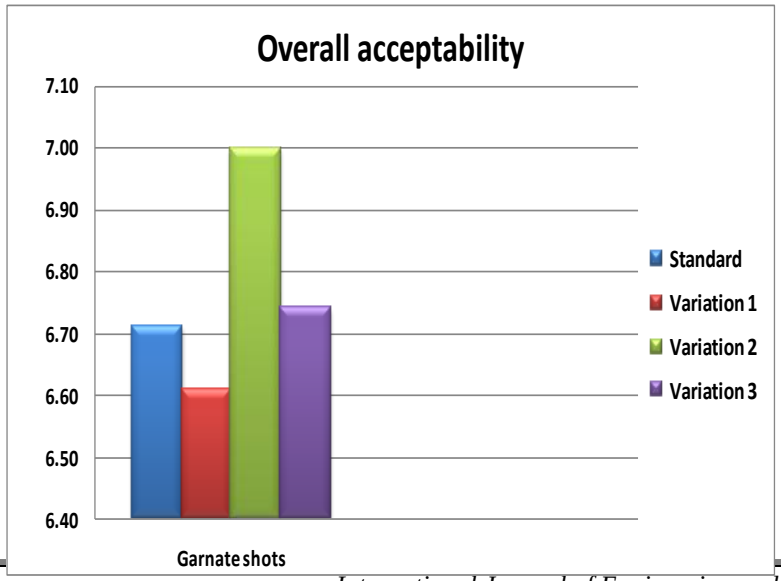
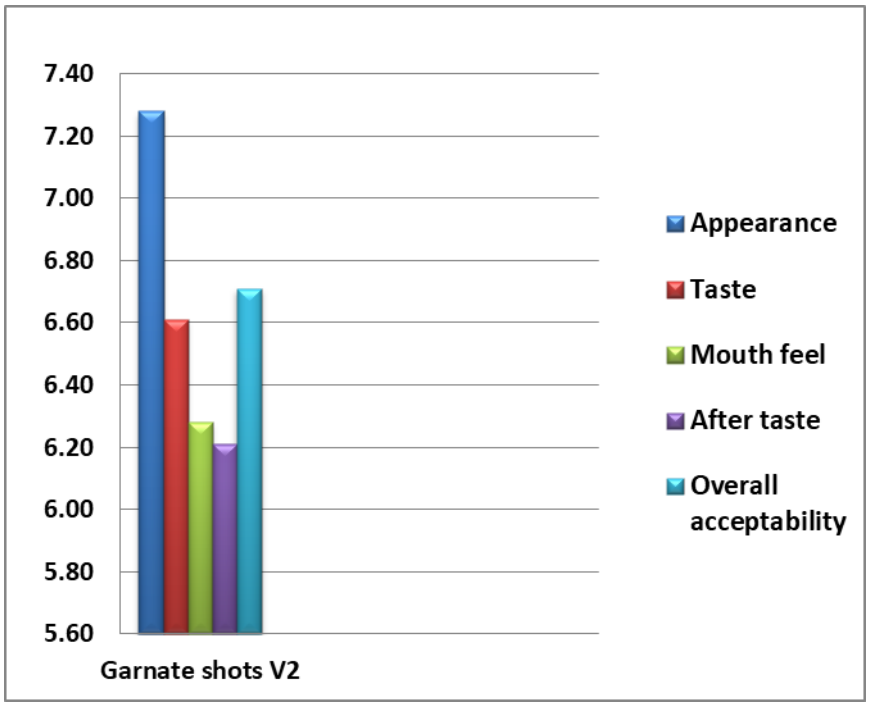


Fig 1.Comparison of overall acceptability between the variations of the health drink

The above figure depicts the comparison of overall acceptability of the health drinks. In garnate shots, variation 2 had the best overall acceptability with a mean score of 7.0±0.72 followed by variation 3, standard and variation 1.



Note: V 2- Variation 2

Fig 2. Comparison between the attributes of best accepted variation

The above figure depicts the comparison between all the attributes of best selected variation in the health drink. In garnate variation 2, appearance had the highest mean score(7.28±0.93) followed by overall acceptability, taste, mouth feel and after taste.

Table 2. Correlation between all the attributes of garnate shots

ATTRIBUTE	R	p	SIGNIFICANCE
Appearance v/s taste	**0.551	0.000	99%
Appearance v/s mouth feel	**0.290	0.000	99%
Appearance v/s after taste	**0.556	0.000	99%
Appearance v/s overall Acceptability	**0.488	0.000	99%
Taste v/s mouth feel	**80.635	0.000	99%
Taste v/s after taste	**0.638	0.000	99%
Taste v/s overall acceptability	**0.822	0.000	99%
Mouth feel v/s after taste	**0.617	0.000	99%
Mouth feel v/s overall acceptability	**0.817	0.000	99%
After taste v/s overall acceptability	**0.686	0.000	99%

NOTE 1: ** Correlations are significant at 0.01 level (2-tailed).

NOTE 2: r = correlation

p= degree of significance

The above table states the correlation scores between all the attributes tested during the sensory evaluation.There existed a higher positive correlation between all the attributes.This meant that there was a direct relationship between any two attributes of garnate shots. For example, in appearance v/s taste or after taste, the r = 0.0551 and 0.056 respectively with p = 0.000 ,which was concluded as the number of positive responses for appearance increased it also simultaneously increased the positive responses for taste and after taste. Appearance had a direct correlation with taste and after taste.

Similarly, taste and overall acceptability also showed a higher positive correlation with r = 0.822 and p =0.000.The other attribute combinations also showed a correlation of similar fashion.

Qualitative phytochemical analysis:

Table 3. Preliminary phytochemical screening of garcinia extracts

EXPERIMENT	RESULT	
	AQUEOUS EXTRACT	METHANOLIC EXTACT
Test for phenols- 1 ml of extact is treated with 3% ferric chloride(few drops) Appearance of blue color shows the presence of phenols.	++	+++
Test for tannins- I ml of extract is treated with 2ml of distilled water.Then treated with 2 drops of 5% ferric chloride. Appearance of dirty green precipitate indicates the presence of tannins.	+	++
Test for flavonoids- 1 ml of extract is treated with 1ml sulphuric acid Orange color formation confirms the presence of flavonoids.	+	+++

Test for terpenoids- 2 ml of the extract is added to 2 ml acetic acid.Then concentrated sulphuric acid is added. Deep red color development showed the presence of terpenoids.	–	+

Note: + slightly present

++ moderately present

+++ significantly present

– absent

The above table indicates the presence or absence of bioactive compounds in garcinia cambogia rind extract. The determination was based merely on the desired color changes expected according to the standard procedure. It can be observed that methanolic extracts showed intense color changes as compared to aqueous extracts. Among the aqueous extracts; only the test for phenols showed moderate positive response and a negative response for terpenoid's test. While the methanolic extracts showed higher presence of phenols, flavonoids and moderate presence for tannins. Terpenoid content was the least in methanolic extracts of garcinia.

According to a study conducted on the preliminary phytochemical screening of fruit rinds of Garcinia cambogia ,the alcoholic extracts were found to contain significant amounts of total phenols and total flavonoids as compared to total tannins.While the aqueous extracts of the fruit rinds showed low amounts of all the phytochemicals tested.The study further concluded that high the phenolic compounds in form of flavonoids and tannins present in the rind, greater is the free radical scavenging property in the cells[12].

Quantitave phytochemical analysis:

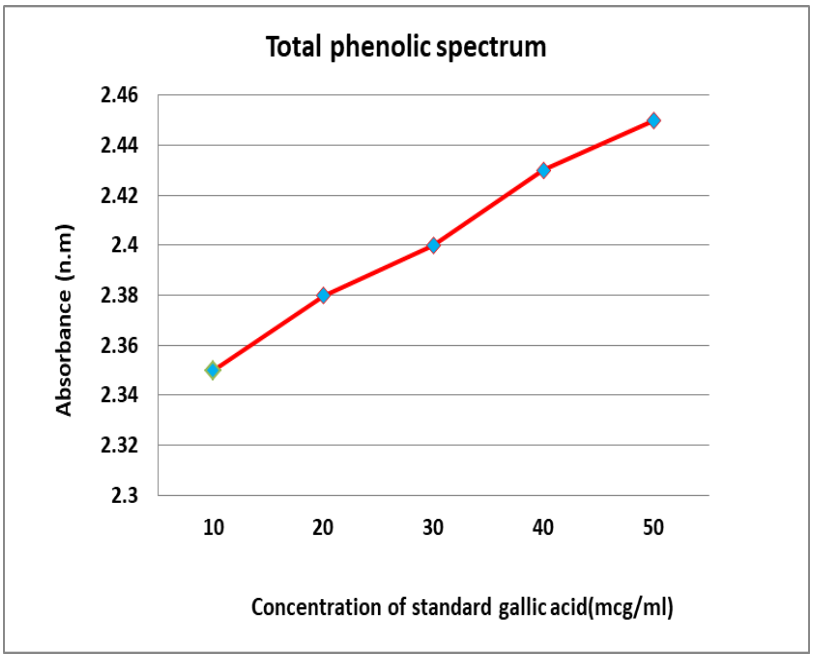


Fig 3. Standard calibration curve for gallic acid

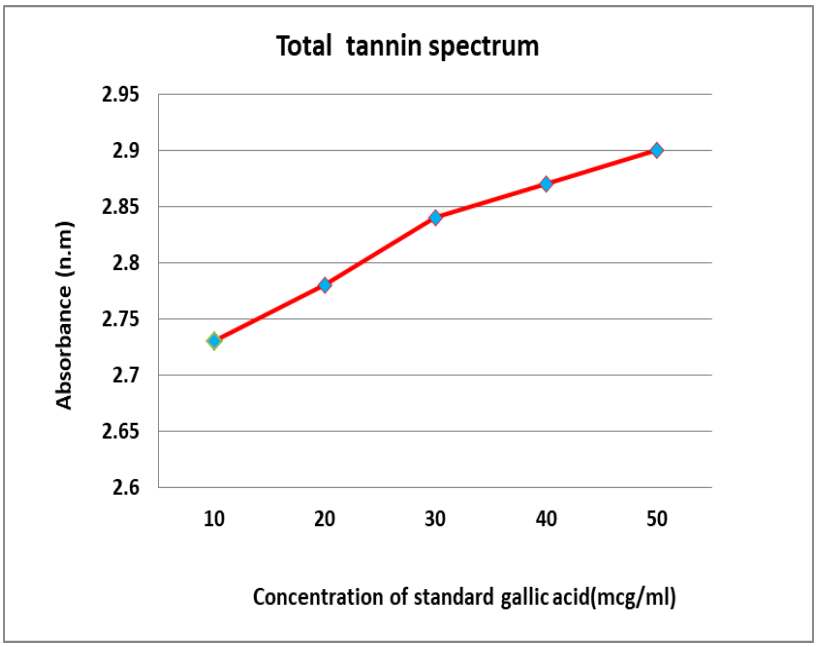


Fig 4. Standard calibration curve for gallic acid

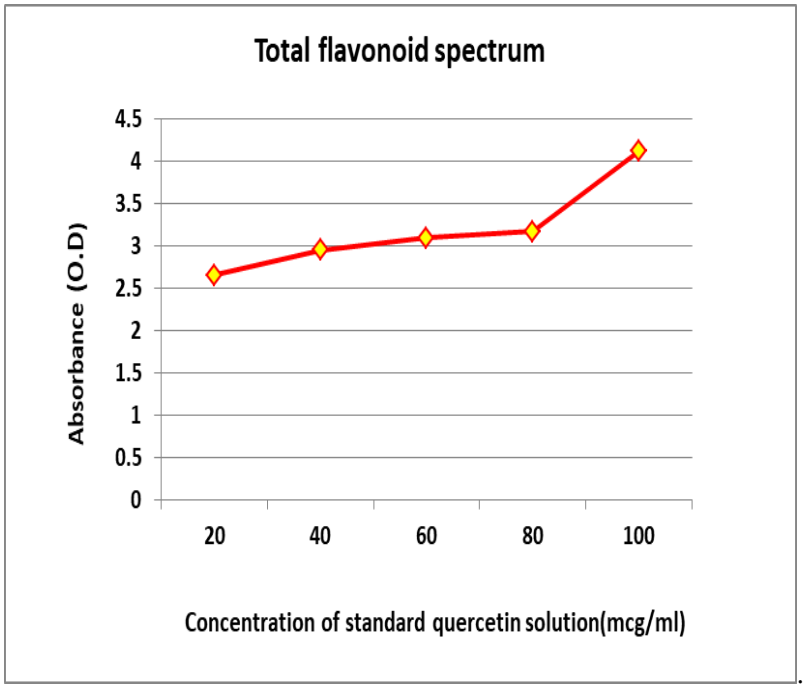


Fig 5. Standard calibration curve for quercetin solution

The major bioactives found in Garcinia extracts were HCA(Hydroxy citric acid), total phenols(0.048 mg GAE/ g), total tannins(0.041 mg GAE/ g) and total flavonoids(0.31 mg QE/ g).Quantification of hydroxyl citric acid was performed using HPLC method in Bangalore Test House(BTH) Centre, Bangalore; which confirmed the presence of 25.67 % of HCA in the dried sample of garcinia cambogia rind.

Table 4. Results of correlation

PHYTOCHEMICALS	R	p	SIGNIFICANCE
Phenol v/s tannin	0.301	0.105	Not significant
Phenol v/s flavonoid	0.369*	0.045	95%
Tanin v/s flavonoid	0.763**	0.000	99%

NOTE : ** Correlations are significant at 0.01 level

*** Correlations are significant at 0.05 level**

r = correlation

p= degree of significance

The above table states the correlation trend existing between total phenol, total tannin and total flavonoid content observed simultaneously in the test plant.The total phenol and total tannin did not exhibit a significant positive correlation with p = 0.105 and r = 0.301.This means as the total phenol content increased ;the total tannin content did not increase in the plant tissue.

However, a significant positive correlation was observed between total phenol and total flavonoid content. Therefore, it was concluded that as the total phenol content increased, there was a simultaneous increase in the total flavonoid content in the medicinal plant extracts with p =0.045 and r = 0.369.

While on the other hand; a higher positive correlation was observed between total tannin and total flavonoid content. This explained that as the total flavonoid content got elevated it led to a simultaneous rapid elevation in the total tannin content in both the plant extracts. These phytochemical contents had 99% significant correlation at p = 0.000 and r = 0.763.

Microbial analysis:

Table 5. Microbial analysis of the garcnia extracts and best accepted variation of garnate shots

Product	**Day**	**Appearance of bacterial and fungal colonies**
Garcinia aqueous extract	1	None
	7	None
	14	None
	21	None
	28	TLTC(Too low to count)
Garnate shots	1	None
	7	None
	14	None
	21	TLTC(too low to count)
	28	Present

The above table depicted the appearance of bacterial and fungal colonies in garcinia extracts and garnate shots. It can be comprehended that garcinia extracts showed better antimicrobial properties as compared to garnate shots which was decided based upon the time period tested for colony growth. Further, it can be concluded that garcinia extract showed no colony formation till the 21st day of microbial estimation while on the 28th day there were growth of TLTC colonies.On the other hand,garnate shots showed the presence of TLTC colonies on the 21st day and countable colonies on 28th day of microbial analysis.

Evaluation of antioxidant properties and antimicrobial activity of G.cambogia fruit extracts against Streptococcus feacalis, Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa and Bacillus subtilis were performed in numerous studies. These studies proved that G.cambogia significantly inhibited the growth of Escherichia coli, Streptococcus feacalis and Pseudomonas aeruginosa as compared to Bacillus subtilis.They showed higher inhibition activity towards Staphylococcus aureus(14.33±0.29mm) as compared to Bacillus subtilis(14.17±0.24 mm) which is a gram-positive bacteria[21].

Table 6. Total coliform forming units(cfu) :DAY 21 ,28 (Garnate shots)

Day	**Test**	**Result**	***Standard cfu values**
21	Total Bacterial Count Total Fungal Count	250 cfu/100 ml 300 cfu/100 ml	Not more than 50cfu/ml
28	Total Bacterial Count Total Fungal Count	450 cfu/100 ml 900 cfu/100 ml	Not more than 50cfu/ml

The above table depicts the total bacterial and fungal count estimated on day 21 and day 28 for garnate shots.

Note:* Microbiological criterion and value of sampling of Fruit beverages (2005), Dr. V. Sudershan Rao, National Institute of Nutrition (ICMR), Hyderabad.

Gram staning:

As a result of the higher outgrowth of bacterial colonies on day 28 in garnate shots, gram staining was performed in order to identify the type of bacteria growing in the food product.The colonies grown in the health drink was found to be gram positive cocci bacteria.

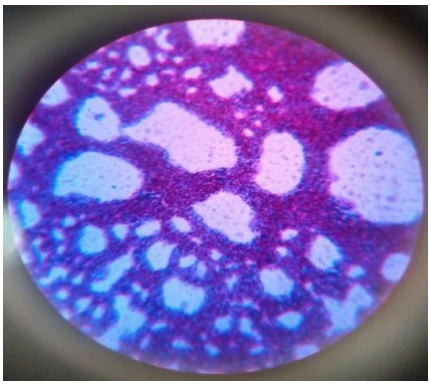


Plate 1. Gram postiive cocci

CONCLUSION:

The methanolic extracts of garcinia cambogia rind showed the higher presence of total dietary flavonoids that have direct relationship with reversing the scenario of metabolic syndrome and insulin resistance. Further the presence of HCA(Hydroxy citric acid) make it a potential drug or a treatment modality for PCOS by aiding in weight loss, suppressing hunger and alevating depression; which are prime significant targets in the treatment of PCOS. Garcinia cambogia serves to be a novel drug or medicine in the treatment of PCOS due to it's higher antioxidant capacity(bioactive content) and HCA content. In-depth investigation of the fruit extracts with human clinical trials needs to be performed to comprehend it's bioavailability and efficacy.

RECMMENDATIONS OF THE STUDY:

Garcinia cambogia can be a potential cure for PCOS and plant extracts could be used for isolating an active compound and further be used for discovery of new drug in the future.Garcinia Cambogia is a good source of dietary total flavonoids which are found to act as antagonists for PCOS.A randomized control trial(clinical intervention) could have been performed on PCOS women for 3 months to find out the efficacy of the garcinia extracts which was not done due to time constraint.

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