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Comparative Study of Bioactive Components from Medicinal Plants for their Efficacy in Food Product Development for PCOS Women

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Abstract: ***Polycystic ovarian disease is one of the commonly observed endocrine disorder; mostly affecting women of child bearing age. PCOS women have a high risk for the development of severe metabolic disorders, such as cardiovascular disease, type 2 diabetes, metabolic syndrome and sometimes endometrial cancer. Alternative complementary medication is the need of the hour to help PCOS women from the side effects of drug therapy.. Therefore this study aims at evaluating the bioactive efficacy of two medicinal plants– Garcinia Cambogia and Berberis Aristata and incorporating their extracts in the development of two food products developed exclusively for PCOS women. 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). While the key bioactives found in Berberis extracts were Berberine (protective alkaloid), total phenols (0.043 mg GAE/g), total tannins (0.056 mg GAE/ g) and total flavonoids (0.43 mg QE/ g). These bioactives positively treat the symptoms of PCOS, protect the body cells from harmful oxidative stress (free radical formation), aid in weight loss and regulate body metabolism. The developed food products from garcinia extracts and berberine 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 and 5.66+1.13 respectively. Further shelf life study of the extracts and best accepted variation of developed food products were performed which indicated that products can be safely consumed upto 3 weeks while their respective extracts can be stored and consumed safely until 28 days.***

Keywords: Polycystic ovary syndrome, Berberis Aristata, Garcinia Cambogia, Hydroxy citric acid, Berberine, phytochemicals

1. Introduction

Polycystic ovary syndrome (PCOS) is one of the commonest endocrine disorder seen in young women of child bearing age and is mainly characterized by anovulation, hyperandrogenism and polycystic ovaries in ultrasonic scanning.

The clinical manifestations of PCOS are heterogeneous and diversity in androgen excess (hyperandrogenemia, hirsutism, acne and alopecia), irregular menstrual cycle, infertility, insulin resistance and obesity. PCOS patients also have a high risk for the development of severe metabolic disorders, such as cardiovascular disease, type 2 diabetes, metabolic syndrome and endometrial cancer.

According to an international consensus definition of PCOS established, requires the fulfillment of atleast two of the following criteria: oligo and /or anovulation; clinical and/or biochemical signs of hyperandrogenism; and/or polycystic ovaries [1]

It represents a condition in which an estimate of 10 small cysts of a diameter ranging between 2 and 9mm develop in one or both of the ovaries and/or ovarian volume in atleast one of the ovaries exceeds 10 ml[2]

It is the most common endocrinopathy in women and most common cause of anovulatory infertility, affecting 5-10% of population of reproductive age. These women have increased risk of reproductive abnormalities[3]

Several risk factors have been investigated in relation to PCOS, which includes obesity, glucose intolerance and dyslipidemia. Insulin resistance is known to play a critical role in the pathophysiology of PCOS. Many synthetic drugs exist for the effective treatment and management, however their numerous side effects and high cost have led a way to seek plant based remedies for the treatment of PCOS[4]

Therefore, this experimental study is conducted to formulate a health drink incorporating two such novel medicinal plants Garcinia cambogia & Berberis Aristata to target regularization of menstrual irregularities, insulin sensitivity & weight loss as these medicinal plant can be "paths or leads" to formulate newer synthetic compounds with greater therapeutic usefulness[5]. These herbal plants are also significant sources of polyphenols and flavonoids that contribute to many of their antioxidant and free radical scavenging activities.

2. Literature Survey

Several studies report that PCOS women have increased risk of developing severe metabolic disorder including Type II Diabetes mellitus, cardiovascular diseases, obesity and insulin resistance[6]. According to a longitudinal study -Polycystic ovary syndrome (PCOS) and Type 2 diabetes mellitus (T2D) are both obesity-related conditions that share similar epidemiological and pathophysiological factors. Insulin resistance is a key factor, whereas obesity has an effect on the expression of each condition. However, the mechanisms by which insulin resistance contributes towards the manifestation of PCOS and T2D differ in important ways:

In PCOS, compensatory hyperinsulinemia results in multiple effects including co-gonadotrophic stimulation of ovarian and adrenal steroidogenesis; in T2D, insulin

resistance contributes towards β-cell exhaustion and ultimately creates hyposecretion of insulin resulting in dysglycemia. The link between PCOS and Type 2 diabetes mellitus is further seen to elevate supraphysiological concentrations of insulin within the systemic circulation. Further there is progression of the obesity that ensures even greater significance of obesity-related conditions such as PCOS and T2D. This suggests that weight loss and improving insulin resistance plays an important role in treating both PCOS and type 2 DM [7].

Garcinia cambogia'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].

Berberis Aristata's key bioactive-Berberine is able to activate an enzyme called Adenosine Monophosphate-Activated Protein Kinase (AMPK) and inhibits the Protein-Tyrosine Phosphatase 1B (PTP1B). With the subsequent AMPK activation, glucose uptake into cells is doubled with improved insulin sensitivity, promoting regeneration and functional recovery of B-cells and reduction in the glucose production in the liver[9]. The effect of AMPK activation is stimulation of hepatic fatty acid oxidation and ketogenesis, inhibition of cholesterol synthesis, lipogenesis, triglyceride synthesis, inhibition of adipocyte lipolysis, stimulation of skeletal muscle fatty acid oxidation, muscle glucose uptake and modulation of insulin secretion by pancreatic beta cells[10-12].

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[13].

3. Materials & Methods

Selection & procurement of raw materials

The raw materials for the study were selected on the basis of their ease of availability, therapeutic effects and economic feasibility.

The major ingredients used for the study include:

- Dried rind of garcinia fruit
- Root extract of berberis aristata (Daruharidra)

The dried rind of the Garcinia cambogia fruit was procured from local supermarket, while the daruharaidra root powder was procured from Indian Herbs Extraction, Nainital, India.

Materials for the preparation of health drink:

The current study aimed at selection of the following ingredients for preparation of standard drink: lemons, pineapple, orange, pomegranate, mint, tulsi, aloevera, jaggery and traditional Indian spices like cinnamon, star anise powder and cardamom.

Preparation of aqueous extracts:

One gram each of garcinia peel powder and berberis root powder were 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 of alcoholic extracts:

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

Each of the plant tissues; i. e garcinia rind and berberis root were cleansed with distilled water, shade dried at room temperature separately and stored in air tight container for further use. Then each of them 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 each of the coarsely powdered plant tissues were 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 while berberis extracts were soaked for 48 hours with tight cotton plugging [14].

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[15, 16].

Preparation & standardisation of health drink recipes

Health drink recipe 1:

Apple-seedy delight (Pomegranate drink)

Method:

- Peel a fresh pomegranate and separate the fruity seeds from the peel.
- Add in the fruit, jaggery, cardamom powder, cinnamon powder, tulsi water, salt into the blender.
- Pulse it for few times in a blender until the outer layer of seeds break and release the juice.
- Don't blend it, just pulse it because if the seeds break completely, the juice turns bitter.
- Serve chilled.

Health drink recipe 2:

Orange -pineapple shots:

Method:

- Wash the pineapple fruit and cut into small cubes.
- Add tulsi leaves into medium boiling water, as the color of the water changes, strain the liquid and discard the leaves.
- Now add in the pineapple cubes, jaggery, mint, tulsi water, aloe vera gel, ginger and star anise powder in a blender and blend well.
- Strain the contents and obtain the clear liquid.
- Garnish with mint leaves and serve chilled.

Formulation of health drink with garcinia and berberis extracts:

The aqueous extracts of garcinia and berberis 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.

Health drink selected for garcinia extract is apple seedy delight and that for berberis extract is orange-pineapple shots.

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 and Berberis extracts 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[17, 18]

Screening for flavonoids:

1mi of the extract was added with 1ml of sulphuric acid. Orange color formation confirmed the presence of flavonoids [17, 18].

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 [18, 19]

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 [20]

Quantitative analysis:

Quantitative estimation of bioactives namely total phenols, total flavonoids and total tannins in each of 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[21] 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[22] 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[23]. Here absorbance of the standard and test solutions were measured at 510nm spectrophotometer. The calibration curve was plotted using standard quercetin. The total flavonoid content was expressed as mg of quercetin equivalents (QE)/g of extract.

Comparative shelf life study of the extracts and the developed health drinks:
The aqueous extracts and the health drinks prepared were tested and analyzed in order to obtain the shell life, longevity and duration of efficacy of the bioactives present in the food component extracts. The analysis performed constitutes:

Serial dilution (pour/spread plate)

Coliform forming units CFU) determination

The analysis was done in order to study the shell life of the extracts and their bioactivity in-vitro. The Institute of Food Science and Technology (IFST, 1993) defined shelf life as the period of time during which, any formulation. extract or a food product will, Remain safe

Certainly sustain its biological activity

To retains it's desired sensory, chemical, physical, microbiological characteristics

To comply with any label declaration of the nutritional data, this can be stored under the nutritional conditions.

The extracts and health drinks 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 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. Ideally, clinical practionists suggest garcinia drinks should be consumed 30-45 minutes prior to meals, while berberis drink can be consumed atleast 30 minutes after meals[24]

4. Results & Discussion

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)	-

Nutritive value of berberine shots:

Table 2: Nutritive value of berbeirne shots per 200 ml (V2)

Nutrient	*Value*
Energy (kcal)	37
Protein (g)	0.56
CHO (g)	30
Fat (g)	-
Fibre (g)	-

Sensory evaluation:

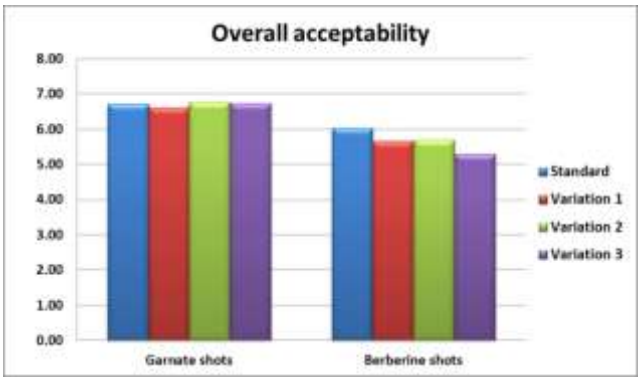
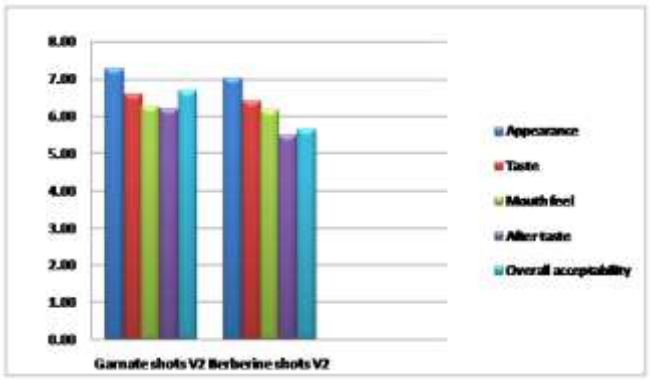


Figure 1: Comparison of overall acceptability between the 2 health drinks

The above figure depicts the comparison of overall acceptability of the health drinks. It was observed that garnate shots were better accepted than berberine shots. 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.While in berberine shots, standard had the highest overall acceptability with a mean score of 6.04+1.14, followed by 2nd, 1st and the least accepted one was the variation 3.



Note: V 2- Variation 2

Figure 2: Comparison between the attributes of best accepted products

The above figure depicts the comparison between all the attributes of best selected variation in each of the health drinks. In garnate variation 2, appearance had the highest mean score (7.28+0.93) followed by overall acceptability, taste, mouth feel and after taste. Similarly in berberine variation 2, appearance had the highest preference (7.04+0.78) followed by taste, mouth feel, overall acceptability and after taste. However, the mean scores of all the attributes of garnate V2 were higher compared to those of berberine V2.

Table 3: 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	0.635**	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.

Table 4: Correlation between all the attributes of berberine shots

Attribute	r	p	Significance
Appearance v/s taste	0.274	0.012	Not significant
Appearance v/s mouthfeel	0.165	0.135	Not significant
Appearance v/s after taste	0.009	0.368	Not significant
Appearance v/s overall Acceptability	**0.368	0.001	99%
Taste v/s mouth feel	**0.738	0.000	99%
Taste v/s after taste	**0.669	0.000	99%
Taste v/s overall acceptability	**0.70	0.000	99%
Mouth feel v/s after taste	**0.790	0.000	99%
Mouth feel v/s overall acceptability	**0.673	0.000	99%
After taste v/s overall acceptability	**0.718	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 reports the correlation between all the attributes tested in sensory evaluation of berberine shots. It was clear that except for the first three attribute combinations, there existed a significant correlation between other attribute combinations.

In appearance v/s overall acceptability, r = 0.368 and p = 0.001; similarly in taste v/s mouth feel and taste v/s after taste, r = 0.738 & p = 0.000 and r = 0.669 & p = 0.000 respectively. This showed that as the semi-trained panelist expressed their liking towards one attribute there was a

simultaneous increase in the liking of other attribute in the combination.

In this case, there was a direct correlation existing between taste, after taste, mouth feel, overall acceptability and appearance of berberine shots.

Qualitative analysis:

Table 5: Preliminary phytochemical screening of garcinia extracts

Experiment	Result	
	Aqueous Extract	Methanolic Extact
Test for phenols- 1 ml of extract 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.

Similar study performed on preliminary phytochemical screening of G. cambogia fruit extracts also revealed the presence of phenolics, flavonoids and tannins in higher amounts. It was also stated that G. cambogia exhibited a wide range of antioxidant capacities, thus making them a valuable source of natural antioxidants[25]

Table 6: Preliminary phytochemical screening of berberis extracts

EXPERIMENT	RESULT	
	AQUEOUS EXTRACT	METHANOLIC EXTACT
Test for phenols- 1 ml of extract 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 berberis root 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 immediate color changes as compared to aqueous extracts. Among the aqueous extracts; only the test for flavonoids showed moderate positive response while a negative response for terpenoid's test. While the methanolic extracts showed higher presence of flavonoids, tannins and moderate presence of phenols. Terpenoid content was the least in methanolic extract of berberis.

Similar study performed on the detection of phytochemical presence in B. aristata root extracts also reported that presence of total flavonoids were significantly higher compared to almost negligible amounts of total phenols[26]

Another phytochemical study confirmed that the B. aristata leaf extracts (methanolic) showed higher presence of total polyphenols and total flavonoids while they were nil in total terpenoid content. The same study showed low amounts of all the phytonutrients in their respective aqueous extracts of the leaf of B. aristata[14]

Quantitative phytochemical analysis:

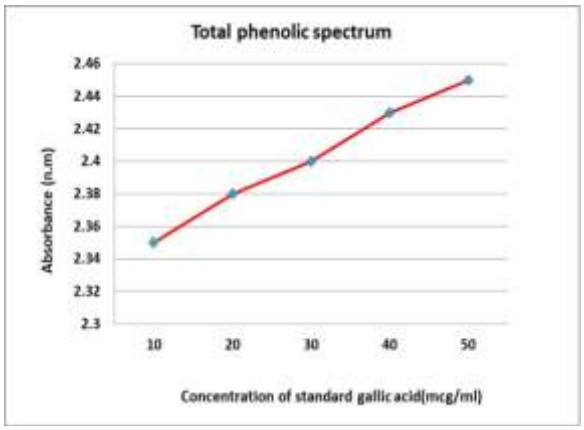


Figure 3: Standard calibration curve for gallic acid

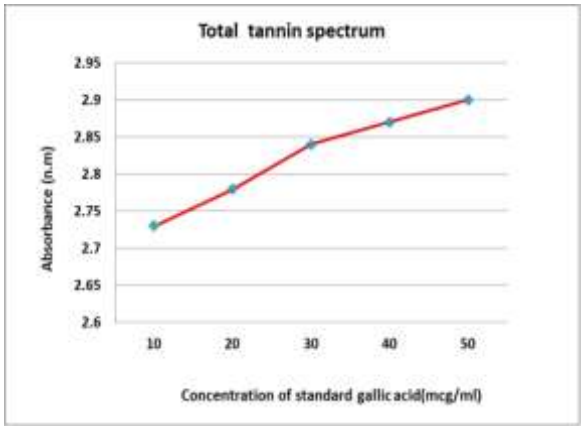


Figure 4: Standard calibration curve for gallic acid

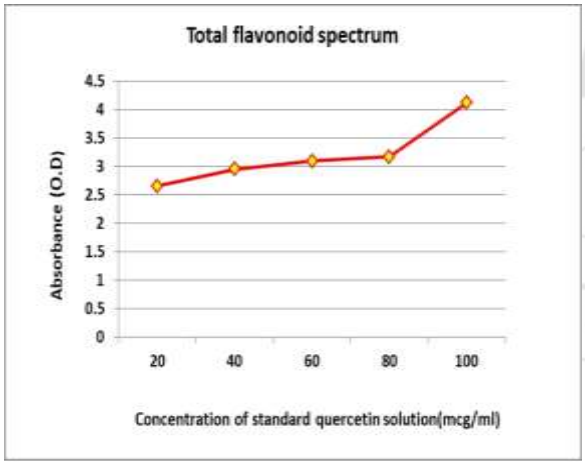


Figure 5: Standard calibration curve for quercetin solution

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). While the key bioactives found in Berberis extracts were Berberine (protective alkaloid), total phenols (0.043 mg GAE/g), total tannins (0.056 mg GAE/ g) and total flavonoids (0.43 mg QE/ g).

Table 7: 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 both the test plants. 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 both the plants.

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 both 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.

Table 8: ANOVA test performed on all the phytochemicals

		Sum of Squares	**df**	**Mean Square**	**F**	**Significance**
Phenol	Between Groups	1306.536	1	1306.536	**32.845****	. 000
	Within Groups	1113.816	28	39.779		
	Total	2420.352	29			
Tannin	Between Groups	1038.291	1	1038.291	**28.294****	. 000
	Within Groups	1027.503	28	36.697		
	Total	2065.794	29			
Flavonoid	Between Groups	1285.503	1	1285.503	1.211	. 280
	Within Groups	29717.520	28	1061.340		
	Total	31003.024	29			

Note: ** Correlations are significant at 0.01 level

The above table depicts the ANOVA values of each of the phytochemicals that states the difference between total phenol, total tannin and total flavonoid content observed together in garcinia and berberis extract.

With reference to the total phenolic content, there was a significant difference observed in both the plants, i. e at f = 32.845; mean of garcinia extract and berberis extract were 16.15 and 2.95 respectively.

Similarly, there was significant difference in total tannin content; with f = 28.294 where mean scores of garcinia and berberis extract were 3.67 and 15.44 respectively.

However, there was no significant difference found in total flavonoid content in both the extracts with f = 1.21 ;where the mean scores were 59.29 and 72.39 of garcinia and berberis extracts respectively.

Shelflife analysis:

Table 9: Microbial analysis of the berberine extracts and best accepted variation of berberine shots

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

The above table reported the appearance of bacterial and fungal colonies in berberine extracts and berberine shots. The data revealed that bereberine extracts showed better antimicrobial properties as compared to berberine shots which was estimated on the total time period tested for colony growth. It was concluded that berberine 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 contrary, berberine shots showed the presence of TLTC colonies on the 21st day and LTC colonies on the 28th day of microbial analysis.

According to ethnobotanical studies on Berberis aristata DC. root extracts (methanolic and aqueous)showed wide antibacterial activity against Gram-positive bacteria at a concentration of 50 µg/disc.

Among the gram-negative bacteria tested, the antibacterial activity was limited against E. coli, Dysenteriae type 1 and the best activity being against V. cholerae The antibacterial activity of the extracts against clinical isolates was comparable to those of standard strains. It was also stated the methanolic and aqueous extracts of B. aristata showed best antimicrobial activity towards V. cholerae at 32.0±1.9 mcg/disc and 14.0±1.7mcg/disc[27]

Table 10: Total coliform forming units (cfu): Day 28 (Berberine shots)

Day	Test	Result	*Standard cfu values
28	Total Bacterial Count Total Fungal Count	190 cfu/100 ml 256 cfu/ 100 ml	Not more than 50cfu/ml

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

Table 11: 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 7 14 21 28	None None None None TLTC (Too low to count)
Garnate shots	1 7 14 21 28	None None None TLTC (too low to count) 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[25].

Hence, shelf life study of the extracts and best accepted food products indicated that products can be safely consumed up to 3 weeks safely while their respective extracts can be stored and consumed safely until 28 days.

Table 12: 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

Note:* 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.

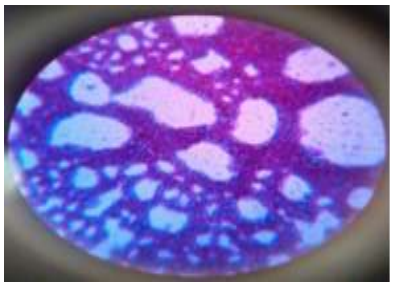


Plate 1: Gram positive cocci

5. Conclusion

The developed health drinks rich in bioactives can positively treat the symptoms of PCOS, protect the body cells from harmful oxidative stress (free radical formation), aid in weight loss and regulate body metabolism. From the microbial analysis it was confirmed that the plant extracts are GRAS (generally regarded as safe) products which can be safely consumed on an average for a month. Both the extracts were rich in dietary flavonoids which are known to have a significant inverse relation with PCOS related complications.

6. Further Scope

1. A randomized control trial (clinical intervention) for 3 months could have performed on PCOS women to find out the efficacy of the health drinks which were not done due to time constraint.
2. The microbial analysis of the extracts and developed health drinks could have been conducted simultaneously for determining their efficacy.
3. The bioactive extracts can be incorporated into a neutraceutical medicine or a capsule to determine its bioactive efficacy.

References

[1] The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group (2004) Revised 2003 consensus on diagnostic criteria and long term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 19: 41–47.
[2] Agarwal, A., Gupta, S., & Sharma, R. K. (2005). Role of oxidative stress in female reproduction. Reproductive biology and endocrinology, 3(1), 28.
[3] Fauser, B. C. J. M., Tarlatzis, B. C., Rebar, R. W., Legro, R. S., Balen, A. H., Lobo, R., & Boivin, J. (2011). Consensus on women's health aspects of polycystic ovary syndrome (PCOS). Human Reproduction, 27(1), 14-24.
[4] Bency Baby T, Smitha Rani, Remya K, Shebina P Rasheed and Azeem AK, (2016). Polycystic ovarian syndrome: Therapeutic potential of herbal remedies-A review, International Journal of herbal Medicine (5):91-96.
[5] Rani Smitha, Bency Baby T, Remya K and Azeem, (2016). Polycystic ovarian syndrome: Therapeutic potential of herbal remedies-A review, International Journal of herbal Medicine (5):91-96.
[6] Rocca, M. L., Venturella, R., Mocciaro, R., Di Cello, A., Sacchinelli, A., Russo, V., & Morelli, M. (2015). Polycystic ovary syndrome: chemical pharmacotherapy. Expert opinion on pharmacotherapy, 16(9), 1369-1393.
[7] Barber, T. M., Bennett, A. J., Groves, C. J., Sovio, U., Ruokonen, A., Martikainen, H., ... & Järvelin, M. R. (2007). Disparate genetic influences on polycystic ovary syndrome (PCOS) and type 2 diabetes revealed by a lack of association between common variants within the TCF7L2 gene and PCOS. Diabetologia, 50(11), 2318-2322.
[8] Patil, M. M., & Appaiah, K. A. (2015). Garcinia: Bioactive compounds and health benefits. Introduction to Functional Food Science, 110-125.
[9] Singh A, Duggal S, Kaur N, Singh J. (2010).Berberine: Alkaloid with wide spectrum of Pharmacological activities. J Natural Produ;3:64-75.
[10] Sabnis M. Chemistry and pharmacology of Ayurvedic medicinal plants. Varansi:Chaukhambha Surabharati Prakashan (2006):23-35. 64. Singhal GD, Sharma KR. Opthalmic and otorhinolaryngological considerations in ancient Indian surgery. Singhal Publications 1976:13- 23
[11] Ni Yanxia.(1995)Therapeutic effect of berberine on 60 patients with noninsulin dependent diabetes mellitus and experimental research. Chinese J Integrated Trad and Western Medic;1:91-95
[12] Winder WW, Hardie DG. (1999).MP activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. Am J Physiol;277(1 Pt 1):E110
[13] Oh J. S., Ahn, M. J., Han, C. J., Kim, H., Kwon, O., Chung, H. W., & Chang, N. (2014). Relationship between flavonoids intake and metabolic syndrome in Korean women with polycystic ovary syndrome. Journal of Nutrition and Health, 47(3), 176-185.
[14] Sharma, K. R., Kalauni, S., Awale, S., & Pokharel, Y. R. (2015). In Vitro Free Radical Scavenging Activity of Methanol Extracts of Some Selected Medicinal Plants of Nepal. Austin J. Biotechnol. Bioeng, 2, 1-5.
[15] Murugan, S. B., Deepika, R., Reshma, A., & Sathishkumar, R. (2013). Antioxidant perspective of selected medicinal herbs in India: A probable source for natural antioxidants. Journal of pharmacy research, 7(4), 271-274.
[16] Krishnamoorthy, V., Nagappan, P., Sereen, A. K., & Rajendran, R. (2014). Preliminary phytochemical screening of fruit rind of Garcinia cambogia and leaves of Bauhinia variegate—a comparative study. Int J Curr Microbiol Appl Sci, 3(5), 479-486.
[17] Kokate, C. K.(2000). Practical Pharmacognosy, Vallabh Prakashan, Delhi.107-111.
[18] Harborne, J.B.(1973).Phytochemical methods, Chapman & Hall Ltd, London.188.
[19] Edeoga, H.O., Okwn, D.E and Mbaebie, B.O(2005). Phytochemical constituents of some Nigerian

medicinal plants, African Journal of Biotechnology.4:685-688
[20] Yadav, M., Chatterji.S., Gupta., SK and Watal, G.(2012). Preliminary phytochemical screening of medicinal plant of Ziziphus Mauritiana Lam. Fruits, International Journal Of Current Pharmaceutical Research 4(3):0975 7066.
[21] Singleton VL, Orthofer R, Lamuela-Raventos RM(1999).Analysis of total phenolics and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent methods Enzymol.299, 152-178.
[22] Rajeev S., Pawan KV., Gangadeep S.(2012).Total phenolic, flavonoids and tannin contents in different extracts of Artemisia absinthium. J Intercult Ethnopharmol.1(2):101-104.
[23] Lee WH., Safinar I.(2012).Antioxidant activity total phenolics and total flavonoids of Syzygiumpolyanthum (Wight) Walp leaves.Int J Med Arom Plants.2(2):219-228.
[24] Rajesh KR.., Yadav., Amit KR.., Singh & A.K Pandey(2016) Vrikshamla(Garcinia Cambogia):A novel natural gift for metabolic syndrome, Int.J. of General Med and Phar;ISSN(P):2319-3999.
[25] Irulandi, K., Geetha, S., Mehalingam, P., & Nirmalkumar, N. (2016). Evaluation of Antioxidant Properties and Antimicrobial Activity of Garcinia cambogia Hort. Ex Boerl (Clusiaceae) fruit extracts. JOURNAL OF ADVANCED APPLIED SCIENTIFIC RESEARCH, 1(3), 74-83.
[26] Mittal, M., Juyal, V., & Singh, A. (2012). Phytochemical, antidiabetic, and cytoprotective properties of Berberis aristata DC. Root Extracts. Pharm Crop, 3, 64-68.
[27] Shahid, M., Rahim, T., Shahzad, A., Latif, T. A., Fatma, T., Rashid, M., & Mustafa, S. (2009). Ethnobotanical studies on Berberis aristata DC. root extracts. African Journal of Biotechnology, 8(4)

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