

Development of Giloy (*Tinospora Cordifolia*) based Herbal Squash incorporated with Pineapple (*Ananas Comosus*)

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ABSTRACT

The research project was undertaken with an objective to develop giloy based herbal squash incorporated with pineapple and to determine its physical and biochemical properties. Squash prepared with giloy and pineapple, together this enhances the medicinal, nutritional and other properties of squash. As both pineapple and giloy have immense health benefit. The treatment T2 which is (50% giloy & 50% pineapple) accepted maximum for all sensory quality attributes. New developed squash observed to be safe up to 2 months in room temperature. Moreover, this research work also provides insight on physical, biochemical, functional, textural, microbial properties of fresh prepared squash. In the case of commercialization and market survey this product is acceptable in food market.

This research work was an effort in the direction of producing value added instant beverage. Due to functional and nutritional goodness of both the plants, it can be further exploited in the development of healthy beverage product.

The demand for immunity booster food products is increasing owing to the increasing numbers of health issues. Giloy (*Tinospora Cardifolia*) among the most important ayurvedic plant which act as, immunoenhancer, anti-inflammatory, anti-oxidant, anti-carcinogens, anti-diabetic, anti-hypertensive, anti-cancer, anti-allergic and what not, also pineapple (*Ananas comosus*) is rich in vitamin-C concentration. Consumer's attitude towards immune boosting foods was positive; in this pandemic we all realize the value of health and healthy food to sustain our lives.

Keywords: Giloy, Pineapple, Squash, Ayurvedic, immunity, Medicinal

I. INTRODUCTION

Medicinal potential of various plant species has remained untapped. There is necessity of putting more efforts to make their utilization in diet as well as medicinal purpose. To promote the utilization of the medicinally important plant species, processing and value addition would be the better avenue.

The therapeutic use of herbal medicine is gaining considerable momentum in the world since the past decade. Due to toxicity and side effects of allopathic medicines, there is an increased rely on use of herbal medicines. Large number of the population of our country uses traditional medicines for their primary health care. So there is a great need for the recognition of the medicinal and economic benefits of flora and fauna (Sangeeta et al. 2015).

1.1 GILOY

Tinosporacordifolia (Menispermaceae) commonly known as Giloy, a Hindu mythological term refers to the heavenly Elixir. Giloy is used in the traditional medicinal system since ages. Its stem and roots are used as herbal remedies. The plant is a large, glabrous, deciduous climbing shrub and distributed throughout subtropical and tropical Indian sub-continent, extending from the Himalayas down to the southern part of Peninsular India. The stem is bitter in taste and stimulates bile secretion, stomachic, diuretic and cures jaundice. It is a best remedy for children suffering from upper respiratory tract infections (Vedavathy and Rao 1991). The crude extract of dry stem of *Tinosporacordifolia* enhanced immune responses (Manjrekaret al. 2000). The extract of T.

cordifolia has an anti-hyperglycaemic property (Rajalakshmi et al. 2009). It might increase the leucocytes and phagocytic cells (Dikshit et al. 2000).

Giloy / Guluchiar considered as a most divine herb because of its various Ayurvedic uses. It is called as nectar/Amruta for its divine healing nature. Guluchi increases the immunity of the body. It fights against infecting organisms. The leaves of the plant in juice cure psychological disorder. The leaves are also used for swine flu treatment. It is also used for the treatment of fever due to infection. Guduchi is a remedy for diabetes. The traditional use of the leaves is to cure urine infection and stomach ulcer. Mixed with cow milk, Guduchi leaves are used to cure leucorrhoea. It is also used as antiaging juice. The plant oil is used to cure skin diseases and promote healthy skin. It brings down the high blood sugar. Guduchi purifies blood and levels the blood pressure. Traditionally, Guduchi is used to cure bowel problem. The starch prepared from it called 'palo' is popular in India. The leaf juice mixed with cumin seeds is used to cure certain internal inflammation. It is also used to cure rheumatic pain.

The leaf extract also used to cure jaundice and anaemia. This also can be used for the treatment of malarial fever and cough. It removes the toxins from the brain. It cures asthma and clears the respiratory tract. The juice of the leaf, root and stem helps in digestion. It also helps to bring down bleeding during menstruation.

The juice is also used in the treatment of the cardiac disorder. Guduchi increases memory power. It enhances natural immunity in the human body. Guduchi increases appetite. It purifies milk in mothers. Guduchi adds strength and vigour to the body and removes the weakness. Because of the antioxidant nature, Guduchi is also used in the treatment of cancer. (Bina Rani et al. 2017)

Traditionally people consume it in the crude form as a remedial measure in certain ailments. No work has been reported on the estimation of quality attributes and product development of Giloy. So, keeping in view its therapeutic as well as nutritional values, the present study was envisaged with the objective of the development and evaluation of value added product by using its stem.

Giloy is a bit bitter, so we incorporated it with pineapple, because pineapple is rich in vitamin C and other minerals due to its high immunity and therapeutic properties. Pineapple has a fruity flavour and sweet taste with this our squash tastes promising and it doubles its acceptability.

1.2 SQUASH

Squash is non-alcoholic concentrated syrup that is usually fruit-flavoured and usually made from fruit juice, water, and sugar or a sugar substitute. Some traditional squashes contain herbal extracts, most notably elderflower and ginger. Squash must be mixed with a certain amount of water or carbonated water before drinking (Jenny Joseph et al. 2015).

This beverage may be served as fresh juice drink as soon as it is expressed from the fruit. It could also be processed then packaged for future use as a processed juice such as squash (Gatchalian and De Leon, 1992). Squash is a diluted fruit juice. Among all beverages squash is quite popular all over the world as nutritious soft drinks.

Citrus fruits (particularly orange, lime and lemon) or a blend of fruits and berries are commonly used as the base of squash. Popular blends are apple with blackcurrant, raspberry with pomegranate, and orange or peach with mango. Less popular single-fruit squashes are also produced, such as pineapple, pomegranate, raspberry, and strawberry. Blending means to combine (varieties or grades of the same substance) to obtain a mixture of a particular character, quality, or consistency (Jenny Joseph et al. 2015).

Fruit squashes are becoming popular in comparison with synthetic beverages evidently because of their taste, flavour, nutritive value and their storage stability. The beverage product squash has a good demand in this subcontinent as well as many other foreign countries. A number of research workers in different countries investigated the formulations of different fruit based soft drinks, squash, non-carbonated fruit juice drink etc. containing sugar, water, citric acid, KMS, artificial colour and flavour (Ahmad, et al. 1988). A fruit juice beverage is a clear or nearly clear unfermented liquid which is developed from the removal of the sweet watery sap from live fruits.

1.3 PINEAPPLE

Pineapple is a tropical fruit crop with several nutritional benefits. It is a good source of vitamin B1, B6, and vitamin C (Remizeet al. 2018), (Sun, et al. 2016). There are many cultivars of pineapple (*Ananas comosus*) in the world, but pineapple 'Queen Victoria' is particularly appreciated for its sweet flavour. Sweetness relies on the ratio between acidity and concentration of sugars (Sun, et al. 2016) but depends also on climatic conditions and cultural practices (Dorey et al. 2015). Several studies show the impact of climatic conditions on physicochemical parameters

of the fruit before harvest, especially in Reunion Island (Dorey et al. 2015, Sanewaskiet al. 2015).

Pineapple is mainly consumed fresh and canned. The commercial 'Queen' cultivar class is not suitable for canning as it results in a large amount of waste due to its morphology. However, it can be processed into juice. Pineapple juice is the third most consumed fruit juice worldwide after orange and apple (Upadhyayet al. 2013). Quality and shelf life of untreated juice depend on the raw material and the applied processes (Montero-Calderón et al. 2020). Sensory defects during pineapple juice shelf life result from browning and carotenoid destruction. Similarly, a trained sensory panel described minimally processed pineapple as "sugared", "pineapple", and "fresh" when juice was tasted the day of preparation and "fermented", "alcoholic and "chemical" after three to seven days of storage at 40C, together with being browner and shinier and losing firmness (Leneveu-Jenvrin et al. 2020).

Since the 1820s, pineapple has been commercially grown in greenhouses and many tropical plantations. Further, it is the third most important tropical fruit in world production. In the 20th century, Hawaii was a dominant producer of pineapples, especially for the US. (UN Food and Agriculture Organization, Corporate Statistical Database (FAOSTAT 2017).

II. 2. MATERIAL AND METHODS

2.1 Treatment Details:

The treatment combination for the Giloy juice and Pineapple juice were made as below.

T0: 100% Giloy squash,

T1: 75% Giloy and 25% pineapple

T2: 50% Giloy and 50% pineapple

T3: 25% Giloy and 75% pineapple and

T4: 100% pineapple squash

2.2 Raw Materials

2.2.1 Giloy

Tinosporacordifolia(Thunb.) Miers, (Guduchi) is one of the important dioecious plants belongs to the family Menispermaceae. The stems and leaf of plant is full of medicinal and ayurvedic benefits. (Choudhary, 2014).

2.2.2 Pineapple

Pineapple (*Ananuscomosus*, Bromeliaceae) is a wonderful tropical fruit having exceptional juiciness, vibrant tropical flavour and immense health benefits. Pineapple contains considerable calcium, potassium, fibre, and vitamin C. It is low in fat and cholesterol. (Joy, 2010).

2.2.3 Sugar

Sugars occur naturally in a wide variety of fruits, vegetables, milk and dairy foods. In addition, they are produced commercially and added to foods both for their sweetness as well as other functions such as the texture of foods. When metabolized, sugars have approximately 4 calories per gram, the same as both protein and other carbohydrates. The most common sugar used in addition is sucrose, Sucrose often called table sugar, sucrose is a disaccharide that is composed of one glucose unit and one fructose unit joined together by a chemical bond that is readily broken in the small

Intestine. Sucrose is found naturally in fruits and vegetables, but in the highest quantities in sugar beets and sugar cane. When sucrose is digested or placed in an acidic environment (such as in many ready-to-drink beverages), it 'inverts' and yields 50% glucose and 50% fructose.

2.2.4 Water

Potable water is used for the preparation of squash, good quality fresh water is used for squash. Water is used from soaking to mixing to grinding. Water also maintain the consistency of squash if used excessive water it can be control later by boiling for a short period of time.

2.3 Squash Preparation

2.3.1 Giloy Juice

Take 10g of Giloy stem, cut into desirable amount, then crush it onto rolling board and pin, remove the outer skin and soak in 500ml of potable water for overnight.

2.3.2 Pineapple Juice

Take 1kg Pineapple, Grade and Cut the pineapple, Blend with approx. 250ml of water, Mix both juice as per decided ratios. Add powder sugar half the squash quantity (up to 40 to 45°Bx), Add Potassium Meta bisulphate (KMS) 0.06g/100ml. boil at 120°C, Bottling and Capping at ambient temperature.

Fig. 1- Freshly prepared Giloy – Pineapple squash (From treatments T0 to T4)



2.4 Determination of Biochemical Analysis

2.4.1 PH Analysis

The zero of the dial is set mechanically. The knob for temperature compensation is fixed for the temperature of the solution. The electrodes are dipped into a standard buffer solution of known pH. Adjustment is made so the dial reads the value of buffer solution. The electrodes are removed and slightly rinsed with distilled water. The electrodes are dipped into the sample solution. The dial shows the pH value of sample solution. (AOAC method)

2.4.2 Determination of Titrable acidity

Titration acidity can be expressed conveniently in grams acid per 100gm or 100ml as appropriate, by using the factor appropriate to the acid.

Slightly coloured solution

Take 10ml well mixed juice, dilute 250ml with neutralized or recently boiled water. Titrate with 0.1N Noah using 0.3 phenolphthalein for each 100ml of the solution to pink end point persisting for 30seconds. Report acidity as ml 0.1N Noah per 100gm or 100ml as required. (Ref., A.O.A.C 17th edn, 2000, official method 942.15 Acidity (Titrable) of fruit products read with A.O.A.C official method 920. 149 preparation of test sample.)

Calculation

$$\text{Acidity} = 9aN/W$$

Where, a = burette reading, N = normality of Noah, W = weight of sample

2.4.3 Total Soluble Solid

Measurement of the refractive index of the test solution at 20°C, using a refractometer, and use of tables correlating refractive index with soluble solids content (expressed as sucrose), or direct reading of soluble solids content on the refractometer.

Procedure

Put a small quantity of the test solution (2-3 drops are sufficient) on the fixed prism of the refractometer and immediately adjust the movable prism. Suitably illuminate the field of view. Bring the line dividing the light and dark parts of the surface in the field of view to the crossing of the threads and read the value of refractive index. Determine the percent sugar from the table. (Ref: IS 13815: 1993/ISO 2173: 1978 Fruit and Vegetable Products Determination of Soluble Solid Content – Refractometer method)

2.4.4 Ascorbic acid method

Accurate 20 ml of each of the freshly prepared sample solution was transferred in 250ml conical flask. Then add 150ml of D/W. To this flask 5.0 ml of KI solution (0.6 M), 5 ml of hydrochloric acid HCl (1.0 M) and few drops of starch solution were added. Each of the five solutions was then titrated against KIO₃ (0.002 M) from the burette until the appearance of blue-black colour which indicate the end point of the reaction. The titration was repeated three time for each of the squash samples. The results were recorded, tabulated and calculated for ascorbic acid determination for each samples.

Calculations

1. Number of moles of KIO₃

$$N = C \cdot V$$

Where,

N = number of moles of KIO₃

C = molar concentration of KIO₃

V = average burette reading

2. Number of moles of ascorbic acid

$$N_{aa} = N \cdot 3/1$$

Where,

N_{aa} = number of moles of ascorbic acid

N = number of moles of KIO₃

3. Convert moles into mass of ascorbic acid

$$M = N_{aa} \cdot 176.12g$$

Where,

M = mass of Ascorbic acid

Calculations

Reducing sugar (%) = $\frac{\text{mg of invert sugar} \times \text{volume made up} \times 100}{\text{Titration Reading} \times \text{Wt. of sample} \times 1000}$

$$\text{Titration Reading} \times \text{Wt. of sample} \times 1000$$

Total Reducing Sugar (%) = $\frac{\text{mg of invert sugar} \times \text{Final vol. made up} \times 100}{\text{Titration Reading} \times \text{Wt. of sample} \times \text{Aliquot taken for inversion} \times 1000}$

$$\text{Titration Reading} \times \text{Wt. of sample} \times \text{Aliquot taken for inversion} \times 1000$$

Total sugar (as sucrose) (%) = (Total Reducing Sugar – Reducing Sugar)X 0.95 + Reducing Sugar
Added sugar = Total sugar – Reducing sugar
(Ref :- AOAC 17th edn, 2000 Official Method 925.35 Sucrose in Fruits and Fruit Products read with AOAC Official method 923.09 Lane and Eynon general volumetric method)
*HPLC method can also be used for sugar profiling.

N_{aa} = number of moles of Ascorbic acid

176.12g = Molar mass of Ascorbic acid

2.4.5 Reducing, Non- Reducing and Total Sugar Standardization of Sucrose Solution

10% dilute invert sugar standard solution was prepared and the pH was adjusted to 8.0. It was titrated with 10.0 mL of boiling Fehling's A and Fehling's B solution end point was determined by mixing 1% methylene blue until the blue colour of the indicator disappeared to a brick red end point titration was completed within 3 minutes and blank titration was performed without hydrolysing sucrose sample titration was performed in triplicates and the average value was taken.

Determination of Initial Reducing Sugar Content

Accurately 5 mL of fruit juice sample was measured and 50 times diluted solution was prepared and the pH was adjusted to 8.0. is solution was transferred into a burette. Titration was performed as the above procedure.

Hydrolysis of the Fruit Juice Sample and Analysis of Total Sugar Content

About 5 mL of fruit juice sample was mixed with 3 mL of conc. HCl and it was kept at 68°C for 30 minutes. The pH of the mixture was adjusted to 8.0, and 50 times diluted solution was prepared using this hydrolysed sample. This solution was transferred into a burette. Titration was performed as the above procedure.

III. RESULT AND DISCUSSION

The various treatment combination like T0 is 100% Giloy squash, T1 is 75% Giloy and 25% pineapple, T2 is 50% Giloy and 50% pineapple, T3 is 25% Giloy and 75% pineapple and T4 is 100% pineapple squash were analysed. The biochemical and sensory analysis of Giloy and pineapple squash revealed following findings.

Table 1- Nutritional composition of freshly prepared Giloy - Pineapple squash

Parameters	Treatment				
	T0	T1	T2	T3	T4
TSS (°Brix)	41	43	44	41	41
Ph.	3.45	2.07	1.78	1.76	1.72
Acidity (%)	0.058	0.19	0.22	0.36	0.45
Ascorbic Acid (mg/100ml)	10.95	16.34	21.62	25.28	30.78
Total Sugar	41.58	41.39	40.08	39.36	39.00
Reducing Sugar	21.11	21.01	20.95	19.05	18.90
Non-Reducing Sugar	20.47	20.38	19.13	20.31	20.10

Nutritional composition of freshly prepared Giloy – Pineapple squash was analysed in the laboratory, the analysis revealed that –

Total Soluble Solids (TSS)

Nutritional composition of freshly prepared giloy squash was analysed in the laboratory. The analysis revealed that TSS of treatment T0, T1, T2, T3, T4 are 41, 43, 44, 41, 41 degree Brix respectively. The TSS of treatment T2 (44 Brix) was found highest as compared to other treatment combinations. The TSS of given sample is same as FSSAI specification of Squash which is between 40 to 45°Brix.

pH

The pH of different treatments are 3.45, 2.04, 1.78, 1.72, 1.94 respectively and all the treatments are acidic in nature. The highest pH recorded in the treatment T0 (3.45) which is 100% giloy and the lowest pH is of treatment T4 (1.72) which is 100% pineapple. 100% giloy have high pH because it is not acidic in nature and all the recorded pH are low due to high amount of sugar used in the preparation of squash. On the other side pineapples are highly acidic and this results in low pH of treatment T4. This is the reason why squash should be diluted in a certain level before consumption.

Acidity

The lowest acidity in treatment T0 (0.058) and highest in T4 (0.45) were recorded. The acidity of treatments shown above is supported by the findings of Sangeeta and Shilpa (2015) where they have highest acidity in T4 which is 100% kinnow (Citrus fruit) and lowest in T0 which is 100% giloy.

Ascorbic acid

Ascorbic acid content or Vitamin c concentration is much higher in pineapple than other fruits even it contain more than that of citrus fruits. Treatment T4 (30.78) contain highest amount of vitamin C concentration because it is 100% pineapple squash, pineapples are rich in manganese a good source of vitamin c, and lowest concentration in T0 (10.95) 100% giloy.

Total Sugar

Total sugar concentration was reported highest in T0 (41.58) in 100% giloy squash as compared to other treatments combinations. The highest content may be due to higher content of sugar added in squash because giloy self not have any sweet taste. Lowest Total sugar content is in T4 (39.00) 100% pineapple, we didn't add much sugar in this concentration due to natural sugar present in pineapple.

Reducing sugar and Non-reducing sugar

The reducing sugar and non - reducing sugar content is also high in treatment T0 (100% giloy) 21.11 and 20.47 respectively. The lowest concentration is slightly different, in reducing sugar the lowest concentration in treatment T4 (100% Pineapple), which is 18.90, but the non – reducing sugar content is lower in treatment T2 (50% giloy and 50% pineapple) which is 19.13. Similar findings were also found or observed by Sangeeta and Shilpa (2015). Slight difference is seen and this is due to the different combination of fruit in both squashes, their temperatures and climatic conditions.

Table 2- Sensory analysis of freshly prepared Giloy – Pineapple squash

Parameters	Treatment				
	T0	T1	T2	T3	T4
Colour	7.34	7.4	7.65	7.62	7.13
Flavour	6.2	6.98	8.27	7.31	8.0
Taste	5.1	6.12	7.61	7.82	7.91
Consistency	7.2	7.41	8.83	7.9	8.1
Overall Acceptability	6.46	6.97	8.09	7.66	7.83

The sensory analysis was recorded as per hedonic scale. It is taken as an average of the points given by 10 people, some of them are specialist and few are common customers. The overall results of sensory analysis revealed that the treatment combination of T2 (50% Pineapple and 50% Giloy) is said to be best as compared to all other combinations.

IV. CONCLUSION

The thesis entitled “Development of Giloy (*TinosporaCardifolia*) based herbal squash incorporated with Pineapple (*Ananascomosus*)” is an effort towards making a healthy drink for the people of all age group. The giloy and pineapple both have immense health benefit which leads me to the new product. A squash is the type of beverage which can be consume by diluting it with any carbonated beverages or simply with water. The results are very promising for further research works.

First we start with the soaking giloy branches for overnight, so the whole giloy branch will become soft and easy to grind with, we prepare separate juice first of giloy and pineapple than mix them with according to our treatments. Than we add powdered sugar in each concentration till the TSS reach 40 to 45°Bx. And then add preservative potassium Meta bi sulphate, than we boil each sample at 70°c for few seconds. Ultimately store the squash in PET bottles. I took reference of several proximate analysis of giloy and pineapple to know more about their composition like moisture, ash, crude fibre, calcium, manganese, NDF, ADF etc., some biochemical analysis to know about the composition of product we conduct pH, reducing sugar, non - reducing sugar, total sugar, acidity, ascorbic acid and TSS analysis, then I conduct sensory analysis with panellist and ask them to rate my products in several categories like

taste, flavour, colour, aroma, consistency and over all acceptability. Here my all lab work is finished and now I go for my best treatment among others.

After concluding all the results and discussion, the treatment T2 which is 50% giloy and 50% pineapple is likely to most accepted squash because it appear to be good in sensory analysis as well as biochemical analysis. In sensory the overall acceptability of this Treatment is 8.09 the highest among others. Which concluded that the given treatment is best in taste, consistency, aroma, flavour and in colour. On the other side the most important is biochemical analysis where the TSS of treatment T2 is 44°Bx. Acceptable as per the FSSAI specification and also the other parameters are pretty much convincible. As per the future the further research work could be carried out on the aspects like different fruit combination with giloy which can increase the value of squash even more and also if want to commercialize the product one should study the shelf life and storage condition as well.

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