Formulation of Herbal Tea using Selected parts of Passiflora Incarnata and Plumeria Rubra

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Abstract

Plumeria Rubra & Passiflora Incarnata high amount of flavonoid and alkaloids with this they also contain high potential antioxidant properties that scavenge the free radical. Herbal Tea is considered to be the true tea which is only prepared by adding Herbs, spices, flowers and other plants(Bihani, 2021). They are the infusion or decoction of plant parts (flower, stem & leaves). The flower contains the properties of Anti-inflammatory activity, antioxidant, anti-microbial, Antipyretic and antinociceptive activity & Antidiabetic and hypoglycaemic activity(Khan & Nabavi, 2018). A sensory evaluation was conducted for proper formulation and they were tasted by three members and came to the conclusion to use Sample HT 2. There was also proximate analysis which has also provided the data for sample HT 2. The toxicity analysis was conducted and there has been a mild toxin of 21 % in the sample(Maluf et al., 1991).

Keywords: P.Incarnata, P.Rubra, Herbal Tea, proximate analysis &toxicity analysis.

1. Introduction

Herbal Tea is the true tea that is only prepared by adding Herbs, spices, flowers and other parts of plants. They are the infusion or decoction of plant parts (flower, stem & leaf's). Unlike coffee and herbal tea (where this is also available decaffeinated), most herbal teas do not naturally contain caffeine. Herbal Tea can be made from Fresh or dried flowers, leaves or roots. The history of Herbal tea takes place before 2100 years from now and had been originated back in China and Southeast Asia(Dhawan et al., 2004).

The other name of Passiflora Incarnata is purple passionflower and they are fast-growing & wildly grown vine. P. Incarnata are widely grown in the middle east of the USA, some parts of Europe and selected-part of south & southeast Asia. The plant grown has an annual flowering with one-day self-life of one day. The fruit of the plant is used for jam, jelly and desserts. P.Incarnata have a high amount of flavonoid and alkaloids (chrysin, apigenin, luteolin, quercetin, kaempferol, and isovitexin), with this, they also contain high potential antioxidant properties that scavenge the free radical(Rudnicki et al., 2007)(Doyama et al., 2005).

Fig 1: Purple Passiflora flowers

Plumeria Rubra is also known as Frangipani and the flower & leaves tend to fall off when they mature which are usually seasonal leaves shed. P.Rubra are native to Mexico, Central America, Colombia, and china(Bacar et al., 2017). The appearance of a flower occurs at the start of summer and said to have a shelf-life of

one day after blossom. P.Rubra have a high amount of the carbohydrate(galactose & rhamnose), Polyphenol (Pyrogallol, Catechin Epicatechin) and protein. They also contain a high amount of antioxidant that scavenge the free radicals(Bihani, 2021).

Fig 2: Frangipani flowers

2. Objectives of the study:

2.1 To analyze the nutritional value of selected parts of Passiflora & Plumeria Rubra

2.2 To identify the antioxidant activities of selected parts of Passiflora & Plumeria Rubra

2.3 Toxicology analysis of selected sources

3. Review of the Literature

3.1 In this study, the leaves are dried and they are mixed with three different solvents which are water, methyl alcohol and chloroform. They used a rota vapour to dry out the methyl alcohol concentration and a uniform suspended methyl alcohol concentration was dried in three different concentrations which is 75,100 &150mg/kg. (Kamaldeep Dhawan, Suresh Kumar, Anupam Sharma, 2003)

3.2 In this paper, For quantitative and qualitative analysis, a high-performance liquid chromatography (HPLC) was used with diode-array detection (DAD) to extract and fractions, using the validating species-specific method. A stock solution was prepared with the sample (1 mg/mL) at10% aq. As for the working solution we had to prepare a sample aliquot up to 900µL and adding only 10% of ascorbic acid. Syringe filters were used of size 0.45µm and its diameter is 33mm before it is sent to HPLC analysis. (Lara Alexander, Dalene de Beer, Magdalena Muller, Marieta van der Rijst, Elizabeth Joubert, 2018).

3.3 In this study, FRAP methodology was optimized for Monoterpenes study. Earlier results were unreliable due to ferric reduction (FRAP) in cancer-preventing reagents and they do not change regarding any of the aromatic compounds in plants. There were changes in the FRAP method so replacing methyl alcohol with various solvents like n-heptane, butyl alcohol, and ethyl acetate and adding polysorbate 20. Moreover, a thin layer chromatography was conducted (TLC) FRAP test was taken. The result of the TLC had shown vital changes in the slow movement in the selected aromatic compounds (linalyl alcohol, alpha-phellandrene, & alpha-terpinene) in testing with the polysorbate 20 in stretch replacing with methyl alcohol with other solvent didn't show any changes. (Karolina A. Wojtunik-Kulesza, 2020)

3.4 This study focussed on cytotoxicity test with the living cell tissue and they are the biological method used to test the toxicity which affects the cells. The toxins are those which affect the living cells, body and organs accordingly. There are many ways in which the toxicity test can be conducted and one of the methods is the Brine Shrimp Lethality Test (BSLT) which are quick and economical also. This toxicity analysis uses shrimp larvae and artemia salina leach and by monitoring the mortality rate of the shrimp larvae and artemia salina leach. (Nurul Hidajati, Qodriyah, 2018)

3.5 The determination of total phenolic content (TPC), total flavonoids content (TFC), and antioxidant capacities of different kinds of edible flowers in China and comparison were studied in this paper. DPPH is a chemical used to evaluate the Characteristic of plant-free radical scavenging activity of the edible flower sample as reported by the calorimetric and ABTS method. In Xu and Chang's method,

Trolox is used as a standard for calorimetric and the ABTS method. The results were identical to both of the dry weight bases in micro-mole TE per gram of sample. A Colorimeter experiment was conducted with FRAP assay are using an external standard as Ferrous ion iron and the output was showed in form of a micromole of Fe2+E per 100gram. (Jingyun Zhenga,b, Xiaoming Yua, Meenu Manindera, and Baojun Xua, 2018)

4. MATERIALS AND METHODS

4.1 Formulation of Herbal Tea

The chosen portion of the plant's Plumeria Rubra and Passiflora-like flowers that are harvested and placed in the container after being washed. Flowers that have been cleaned have had any dust or other impurities removed. The flowers are dried for three hours at 60°C in a fluidized bed dryer and by air as well. The flower is then thoroughly dried, ground into a fine powder, and sieved at 85grid. They were combined with tea powder together with P. Incarnata and P.Rubra. They are scaled to 1 gramme per unit.

Table1: Formulation Herbal Tea

S.NO	Ingredients	Formulation		
		HT Sample 1	HT Sample 2	HT Sample 3
1	Tea Powder	0.3	0.2	0.3
2	*P.Rubra*	0.35	0.2	0.2
3	*P.Incarnata*	0.35	0.6	0.5
Total		1.0 Gram	1.0 Gram	1.0 Gram

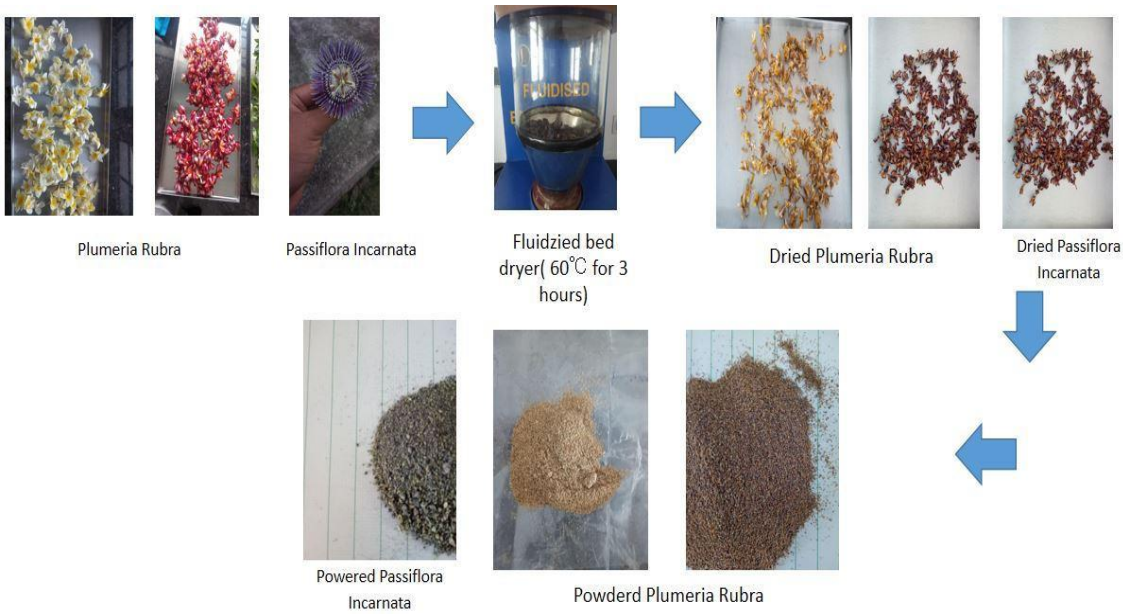
4.2 Herbal Tea concoction

4.2.1 A gramme of dry herbal tea powder is added to 120ml of boiling water after it has been preheated.

4.2.2 After 3 minutes of heating, the mixture is filtered and presented to the customer at the preferred temperature.

4.3 Methodology

Fig 3. Methodology for making Herbal Tea



4.4 PROXIMATE ANALYSIS

4.4.1 PROTEIN - Kjeldahl Method

4.4.1.1 Digestion

The sample is weighed into a digestion flask and then it is kept on heating for digestion in the presence of sulfuric acid, anhydrous sulfate and a catalyst is added such as copper(Of & Page, 2012). Digestion happens by any nitrogen present in food is converted to ammonia and other organic particles like CO2 and H2O. The ammonia in this state is liquid so there will not be any liberation in an acid solution.

$$N(food) \rightarrow (NH_4)_2SO_4$$

4.4.1.2 Neutralization

At the next step after the digestion process, they are sent through a tube to a flask which receives the basic solution where the NaOH is added to the solution which changes (NH4)2SO4 (Ammonium Sulphate) into NH3 (ammonium)(Rudnicki et al., 2007).

$$(NH_4)_2SO_4 + 2\ NaOH \rightarrow 2NH_3 + 2H_2O + Na_2SO_4$$

The NH3 (ammonium) which is formed in the digestion flask is then sent through a condenser which is then collected in a flask that contains boric acid in high quantity. As the ammonium and boric acid are combined the solution turn to a lower pH thus forming ions of ammonia and borate.

$$NH_3 + H_3BO_3 \text{ (boric acid)} \rightarrow NH_4^+ + H_2BO_3^- \text{ (borate ion)}$$

4.4.1.3 Titration

As ammonium borate is formed in the flask then analysis was conducted to determine the nitrogen content in the digested food.

$$H_2BO_3^- + H^+ \rightarrow H_3BO_3$$

The Titration is done by adding a standard Hydrochloric Acid or Sulfuric acid and to know the endpoint a preferable indicator is added. They are titrated till the colour change appears.

$$\%\,N = \frac{x\ \text{moles}}{1000\ \text{cm}^3} \times \frac{(v_s - v_b)\ \text{cm}^3}{m\ \text{g}} \times \frac{14\ \text{g}}{\text{moles}} \times 100$$

The concentration of nitrogen and the concentration of hydrogen should be equal to that of the initial food.

Convert the nitrogen content to protein content after determining the nitrogen content and using a proper formula to convert:

%Protein = F %N.

Fig 4: Kjeldahl method (Protein Determination)

4.4.2 FAT -Soxhlet Method of Extraction

We should get 1 gramme of fat from 30 grammes of sample heated to 102 degrees Celsius for two hours in a thimble (Bihani, 2021). By adding solvent and heat for 16 hours, the Soxhlet device can be used to extract the fat from the thimble. Every hour for the next 16 hours, the sample in the Soxhlet device is weighed, and the weight of the empty container is also recorded. At 90 to 100°C, the Soxhlet apparatus is maintained (Khan & Nabavi, 2018).The thimble is cooled and weighed until it reaches below 2 milligrams. Additionally, they are weighed once every half-hour gap and the lowest weight taken off is recorded.

Calculation

Fig 5: Fat (Soxhelt apparatus)

$$\%\,Fat = \frac{(m_{Total} - m_{Beaker})}{m_{Sample}} \bullet 100\% \qquad (1)$$

% Fat: Percentage of fat in the sample
m_{Total}: Beaker + extract [g]
m_{Beaker}: Empty beaker weight [g]
m_{Sample}: Sample weight [g]

4.4.3 CRUDE FIBER

Procedure

The Dry process is done before the solvent is added by taking 5grams of sample and drying it at 107℃. The sample from the hot air oven is taken out and it is weighed around 2.5grams. The sample is taken in a Soxhlet flask in which pet ether and sample is mixed to a certain concentration and kept in the Soxhlet apparatus for one hour(Alexander et al., 2019).After the processing of the sample in the Soxhlet apparatus, it is then put into a beaker and diluted H_2SO_4 added in the beaker which is bought to a boiling state.

The beaker is set aside with the sample present inside with it and they are connected to a knockback condenser then it is heated to a certain point where it starts to boil. The beaker is occasionally mixed so that the sample does not stay on the sides and exposed to acid(Indian Standard PROCESSED-CEREAL BASED COMPLEMENTARY FOODS-SPECIFICATION (Second Revision), 2006).Boil the beaker for half an hour continuously and they are filtered with 18 layers of linen cloth. This process is continued until the sample is no longer acidic by washing in boiling water again and again.

As the solution which was filtered are kept aside and a NaOH solution was prepared with a knockback condenser connected with the flask at boiling temperature. The sample is washed with the NaOH solution with the knockback condenser set up.The setup was set with a knockback condenser at boiling temperature for half an hour and the vapours are send through a series of sieve to filter them. the sample which is the flask is filtered and washed with boiling water. The sample is scooped out and then they are mixed with 15ml of ethyl alcohol.The sample is dried and placed in a ceramic container with the help of a hot air oven it is heated at 107℃. The sample matter is fully burned to ash and it is cooled in a desiccator and it is weighed.

$$\text{Crude Fibre (dry basis)} = \frac{M_1 - M_2}{M} \text{ x } 100$$

Fig 6: Crude fiber

Where,

M1- Mass of crucible and contents before ashing,

M2- Mass for crucible containing asbestos and ash

M- Mass for dried material taken for test

4.4.4 CALORIFIC VALUE

Gauge your two food things on a weighing scale to 0.01 grams. Gauge the void can, at that point fill it around 1/4 to 1/3 full with water, and gauge it once more. Record the underlying temperature of the water in the can. Set the can on the remain with the thermometer in it Cautiously skewer the food thing on the test, touch off it with the lighter, and afterwards hold it straightforwardly under the can until it consumes itself out and will not re-light. Record the most noteworthy temperature came to by the water. Rehash the strategy with the second food thing. You can re-utilize the water in the can, yet need to record the new beginning and finishing temperature (Dhawan et al., 2004). When completed, tidy up and dissect your information.

4.4.5 PHENOL

At first, the sample is weighed 1 gram and it well grinds with the pestle. The mixture of the volume is ten times the volume of 80% ethyl alcohol. A laboratory centrifuge is used at the speed of 10,000 RPM for 20 minutes which forms a supernatant solution. The extraction process is repeated five times the volume of 80% ethyl alcohol. The same process of centrifuge is done and the 5 times supernatant solution is taken. The supernatant solution is dried using an evaporator and the sample which remained back should be mixed with 5 to 10ml of water. The sample & water mixture is pipette within 2ml into a test tube and it makes up to 5ml. Add 0.5ml Folin's phenol Reagent and after few minutes mix it with 20% Sodium Carbonate solution in both the solution. Before placing the test tube into the water they are well shaken and cooled after it is heated for a minute(Alexander et al., 2019).

Calculation

Sample test shows the concentration of phenols and they are expressed in terms of mg per 100grams of the sample.

4.4.6 VITAMIN A - HPLC Liquid Chromatography

Sample preparation

To 5gm of margarine is weighed and added to a 250ml flask.300mg of ascorbic acid was added to it. Add 50ml of ethyl alcohol and 15% of 50% KOH.A process name Saponification goes by knockback condenser. The disappearance of fatty drop indicates successful Saponification. The sample was taken and it is washed with 50ml ethanol or ethyl alcohol into a 250ml funnel. The 120ml of water was added to the sample and 3 times 50 ml hexane was mixed with the sample during the extraction. The hexane was washed with water a few time and it is rota evaporated at 50℃ and 260mbar. The remaining was dissolved in 10ml of methanol(Garai, 2017).

Fig 7: HPLC Liquid Chromatography

4.4.7 TOTAL SUGARS

Requirements

- 5% Phenol: In 1 liter of water 50 gram of Re distilled phenol is added and another solution was prepared by adding 94% H2SO4 and 6% water.
- Standard glucose: 100ml concentrated mixture(stock) is nixed with 100ml of water.
- Working Standard: 10ml of a concentrated mixture (stock) is mixed with 100ml of distilled water.

Procedure

The sample is mixed with a working sample to form a solution in which they are added to 5 different test tube in a proportion of 0.2 gram of variation till 1ml.The sample solution is prepared are set out to two different test tubes with the proposition of 0.1ml & 0.2ml and making it up to 1ml with water respectively. In test tubes, 5% Phenol and 96% H2SO4 is added and shaken well for ten minutes. After shaking the test tubes are kept for an ambient temperature of 20 minutes in a water bath. The total amount of carbohydrate present in the sample is shown in the graph by which the calculation is done(Khan & Nabavi, 2018).

Calculation

Absorbance corresponds to 0.1ml of the test

$= x$ mg of glucose

100mL of the sample solution contains

$= \frac{x}{0.1} \times 100$mg of glucose

= % of total carbohydrate present

4.4.8 VITAMIN C

Requirements

Standard solution: In a Flask 100ml of 4% oxalic acid with 100mg of ascorbic acid is mixed to create a standard solution (1ml/1mg).

Working Solution: a 100ml solution (100µg/mL) was prepared 10ml of standard solution is diluted and 4% oxalic acid with it.

Procedure

Take a clean and dry 100ml conical flask and place it in the platform where the working sample is added with a help of a measuring cylinder 5ml is taken. The working sample is added to the conical flask and 10 ml 4% oxalic acid dissolved solution is added. The conical flask is placed under a burette that contains dye is titrated against the solution prepared before. As we titrate the colour changes to pink and it continues for few minutes by the time the consumed dye represents the amount of ascorbic acid consumed is equal. The sample is taken around 0.5 to 5 gram from the conical flask and it is added to centrifuge tubes with 4% oxalic acid. The remaining is made up of a known value. After centrifuge, the supernatant solution is taken around 5 ml with that 10ml of 4% oxalic acid added to a conical flask for Titration. As we titrate the colour changes to pink and it continues for few minutes by the time the consumed dye represents the amount of ascorbic acid consumed is equal(Yaman, 2020).

Calculation

Amount of ascorbic acid mg/100g sample

$$= \frac{0.5\text{ mg}}{V_1\text{ mL}} \times \frac{V_2}{5\text{ mL}} \times \frac{100\text{ mL}}{\text{Wt. of the sample}} \times 100$$

4.5 TOXICITY ANALYSIS - Invitro cytotoxicity - Test on extract

Table 2: Toxicity table

Evaluation Criteria		
S.No.	**Grade(%)**	**Reactivity**
1	**0**	**None**
2	**1-20**	**Slight**
3	**21-50**	**Mild**
4	**51-70**	**Moderate**
5	**>71**	**Severe**

Test Details:

Source of cell line: NCCS, Pune

Justification: L929 is an established and well-characterized cell line that has demonstrated reproducible results

Culture media: MEM medium supplemented with fetal bovine serum

Assay Method: MTT Assay

Reagent: MTT Solution (mg/ml)

Incubation: 37oC with 5% CO2

Absorbance: 570 nm

Imaging: Inverted Phase Contrast Microscope

Test sample preparation: MEM

Analyst ID: SM

4.6 SENSORY EVALUATION

A composite scoring test was used for the sensory evaluation. The example scorecard is given below(Oduro et al., 2013).

Sensory Evaluation

Code: 001

Product: Herbal Tea

Description: Taste the product and give a score to the specific characteristic of the product.

Sensory attributes	Score	HT Sample 1	HT Sample 2	HT Sample 3
Color	10			
Aroma	15			
Flavor	15			
Aftertaste	30			
Acceptability	30			
Total Score	100			

5. RESULT AND DISCUSSIONS

The sensory evaluation of the sample was done and they three sample HT 1, 2, & 3. The sensory evaluation indicates that sample HT 2 is good with aroma, flavour, after taste and acceptability. The sample HT 2 was sent to the proximate analysis which concluded within the result of having 65.12% of carbohydrate by anthrone method and by performing Kjeldahl method(Of & Page, 2012) using Soxhlet apparatus 16.12% of protein is shown.

Determination of Fat test(Bihani, 2021) is performed, and the fat was 3.60% present in the sample. Determination of Crude fibre was done, and the result was 11.33% in the sample. The DGHS method calculated to know the Calorific Value of sample 360.64Kcal/100g which would show how many calories does 100g of the sample will provide. The antioxidant is the herbal tea, so the test was performed using the FRAP method which has provided 20.94mg/g in the sample.

The phenol, vitamin A, Total sugar & vitamin C are present in the sample and they go through a biochemical method which the results are provided for Phenol it is 1603.80mg/100g, vitamin A has 77.58 IU, total sugar has 10.55% is present in the sample and vitamin C has 284.07mg/100g.

Toxicity Analysis

Fig 9: Control and sample cells

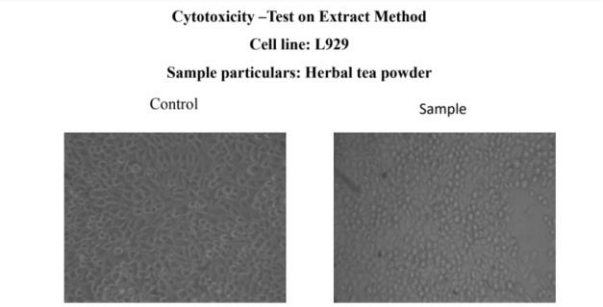


Table 3: Toxicity Analysis Result Sensory Evaluation

Result :

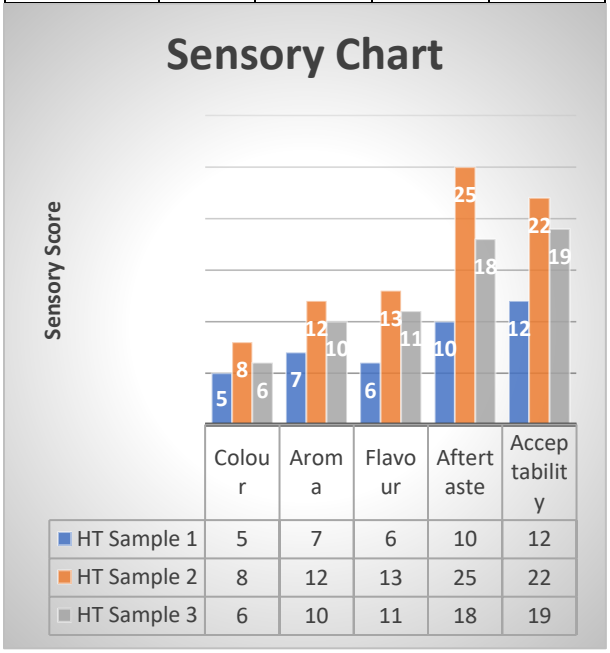
Cotton Seed Oil Extract	B2100010-1 Powder: *Herbal tea powder*		
	Cytotoxicity (%)	Cell Viability (%)	Cytotoxic Reactivity
Sample	26.1	73.9	Mild
Image(s)	-NA-		
Inference : : As per ISO 10993:5 the test sample Herbal tea powder showed Mild cytotoxic reactivity to L929 cells after 24 h contact. Control gave none cytotoxic reactivity as expected.			

Code: 002

Product: Herbal Tea

Description: Taste the product and give a score to the specific characteristic of the product.

Sensory attributes	Score	HT Sample 1	HT Sample 2	HT Sample 3
Color	10	5	8	6
Aroma	15	7	12	10
Flavor	15	6	13	11
Aftertaste	30	10	25	18
30		12	22	19
Total Score	100	40	80	64

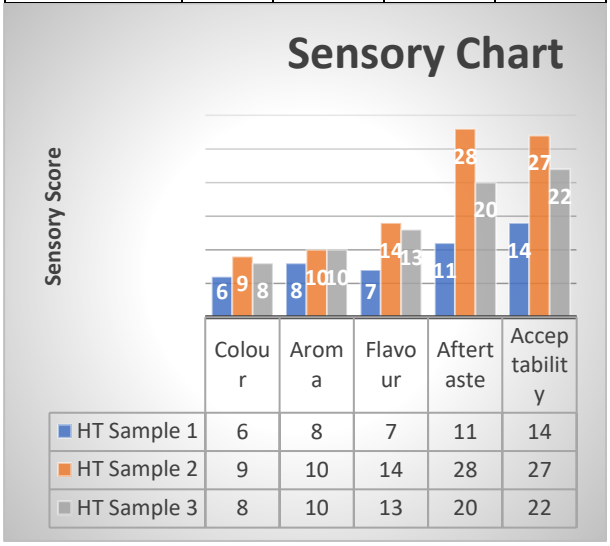


Code: 003

Product: Herbal Tea

Description: Taste the product and give a score to the specific characteristic of the product.

Sensory attributes	Score	HT Sample 1	HT Sample 2	HT Sample 3
Color	10	6	9	8
Aroma	15	8	10	10
Flavor	15	7	14	13
Aftertaste	30	11	28	20
Acceptability	30	14	27	22
Total Score	100	46	88	73



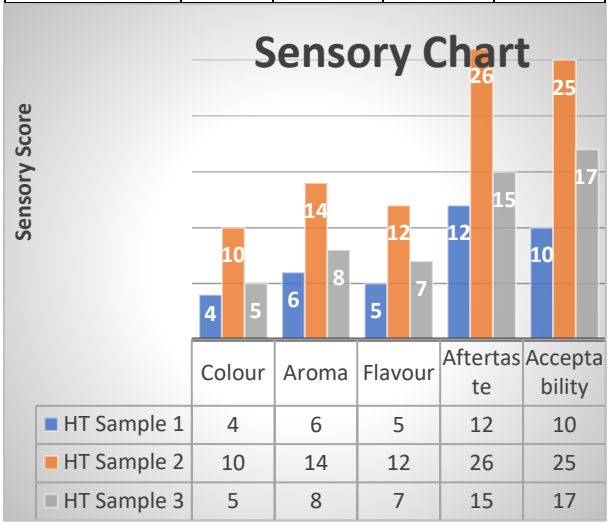
Code: 004

Product: Herbal Tea

Description: Taste the product and give a score to the specific characteristic of the product.

Sensory attributes	Score	HT Sample 1	HT Sample 2	HT Sample 3
Color	10	4	10	5
Aroma	15	6	14	8
Flavor	15	5	12	7
Aftertaste	30	12	26	15

Acceptability	30	10	25	17
Total Score	100	37	87	52



6. CONCLUSION

Plumeria Rubra & Passiflora Incarnata are good sources of micronutrient which provides a high amount of vitamins, polyphenols, and dietary minerals. There have been three types of herbal tea formulation which also show how they differ according to different compositions(Fisher et al., 2000)(Malongane et al., 2020).Sample HT 1, 2, &3 are sensory evaluated in which the sample HT 2 was highly recommended, and it has been taken for proximate analysis. The result was showed a high amount of antioxidants, phenols & vitamin C. A toxicity analysis was also conducted for Sample HT 2 which showed a mild toxin of 21% which is present in the sample(Bihani, 2021)(Oduro et al., 2013). For further studies to study the rate of diabetic control in the formulated product and to find the suitable Primary and secondary packaging material. The of study the shelf life and nutritional degradation rate concerning various temperature (Hot beverage temp)(Fisher et al., 2000).

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