

## Research on 'Preparation and Evaluation of Polyherbal Chocolate

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### ABSTRACT

Polyherbal syrup prepared from multi component which gives synergistic effect to boost body's natural immunity. The polyherbal immunity booster syrup formulation consist of Dragon fruit (*Hylocereus undatus*), Jamun (*Syzygium cumini* L), Sweet Lemon (*Citrus limetta*), Tulsi (*Ocimum sanctum*). The extract of dragon fruit is added into sweet lemon it gives flavour to syrup and Tulsi leaves extract is added as antibacterial agent and jamun vinegar is added as a quantity sufficient and Sodium Benzoate used as preservative and Sodium Bicarbonate for pH adjustment (Buffer). This formulation acts as immunity booster with antioxidant property. The formulation was prepared and evaluated for the parameter, such as pH, viscosity, density, specific gravity and organoleptic properties. The prepared syrup was evaluated immediately after preparation and all tested parameter along with turbidity or homogeneity were compared with changes in accelerated stability testing. final syrup was found to be pH 6.5, specific gravity 1.0097 kg/m<sup>3</sup>, and density found to be 2.589 g/cm<sup>3</sup> and viscosity found to be 0.474 cp. The formulated syrup was also evaluated by organoleptic properties such as colour, odour, taste and appearance. Colour of the formulated syrup was found to be yellowish brown, odour found to be aromatic, taste found to be slightly sour and appearance found to be clear. stability testing shows the formulation was stable at various environmental conditions. The main purpose of adding Jamun Vinegar to make syrup sugar-free. It can be conveniently used by diabetic patients.

**Keywords:** Polyherbal syrup, Dragon fruit, Antioxidant, Immunity booster, Decoction, Sugar free, Antidiabetic.

### I. INTRODUCTION

In the wake of the Covid 19 outbreak, entire mankind across the globe is suffering. Enhancing the body's natural defence system (immunity) plays an important role in maintaining optimum health. We all know that prevention is better than cure. While there is no medicine for COVID-19 as of now, it will be good to take preventive measures which boost our immunity in these times. Ayurveda, being the science of life, propagates the gifts of nature in maintaining healthy and happy living. Ayurveda's extensive knowledge base on preventive care, derives from the concepts of "Dinacharya" - daily regimes and "Ritucharya" - seasonal regimes to maintain healthy life. It is a plant-based science. The simplicity of awareness about oneself and the harmony each individual can achieve by uplifting and maintaining his or her immunity is emphasized across Ayurveda's classical scriptures. Ministry of AYUSH recommends the following self-care guidelines for preventive health measures and boosting immunity with special reference to respiratory health. These are supported by Ayurvedic literature and scientific publications.

Various epidemic respiratory diseases emerges now a days, common cold, influenza, flu, pneumonia, whooping cough etc. Very booming now a day pandemic disease is COVID-19 worldwide caused more than 1.21 million deaths and may expecting more in coming days. COVID-19 caused by Novel CoV (Corona Virus)-2019 and mainly affects respiratory system (Ahad HA., et al. 2020)1 . Mostly in various epidemic respiratory diseases it is suggested that enhance the immunity by regular exercise, food and supportive lifestyle. Making healthy lifestyle choices by consuming nutritious foods and getting enough sleep and exercise are the most important ways to boost our

immune system. In addition, research has shown that supplementing with certain vitamins, minerals, herbs, and other substances can improve immune response and potentially protect against illness (Chindarkar P., 2020)<sup>2</sup>. The Ministry of AYUSH has recommended the some self-care guidelines as preventive measures and to boost immunity with special reference to respiratory health (Anonymous; 02/06/2020)<sup>3</sup>. The medicinal plants like tulsi, lemongrass, lavang, sunthi, dalchini, kalimiri and haldi have well known for immunity booster/enhancer. In current research work author prepare the immunity boosting rasayankvatha, dragonfruit, sweet lemon, jamun vinegar and evaluated it first time. In present study ingredient selected on basis of their individual proven pharmacological study like Tulsi plant and dragon fruit known for immunity boosting, sweet lime, jamun vinegar, consist terpenoids and they are have a antimicrobial activity. Immunity is ability of individual to resist diseases. Immunity booster works by strengthening your body's immune system against disease causing pathogens<sup>[1]</sup>. The polyherbal immunity booster syrup formulation consist of Dragon fruit (*Hylocereus undatus*), Jamun (*Syzygium cumini* L), Sweet Lemon (*Citrus limetta*), Tulsi (*Ocimum sanctum*)<sup>[2]</sup>.

Polyherbal syrup is prepared by decoction process. Mixing a decoction of herbs with a Jamun Vinegar. It helps for the thickening the formulation. And addition of sodium benzoate help to preserve the formulation and increase its shelf life. Syrup are very efficient delivery vehicle use for the immunity booster. Because it gives the soothing to swallow and ingest as compared to the tablet and capsule. This syrup formulation gets absorbed and reaches into systemic circulation. Most of herbal syrup was originally derived from plant herbal medicine refers to use extract of fruit for medicinal purpose. Along with other dosage from herbal drugs also formulated in form of syrups. Today syrup is used for treatment of many ailments and to overcome symptoms of diseases. The antioxidant syrup is used to treat the cancer because of many stresses condition and other oxidative reaction in body the free radical is generated, by using this syrup the condition is overcome. Ayurvedic syrup can be easily administered by oral route and by mixing with orally administered foods and beverages<sup>[3,4]</sup>. Following ingredients used in polyherbal syrup:

#### Ingredient used in formulation:

##### 1. Dragon fruit (*Hylocereus undatus*):

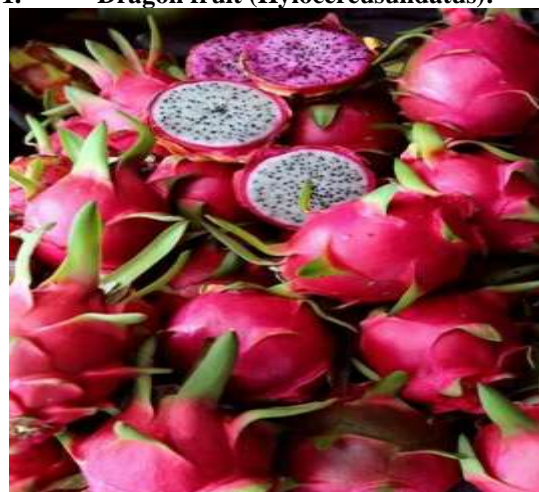


Fig no.1: Dragon Fruit

Dragon fruit belongs to genus *Hylocereus* of the order Caryophyllales belongs to family Cactaceae. Dragon fruit is beneficial for health and thus it prevents various diseases such as improving eye health, as well as improving the function of kidney, brain, making the bones strong, reduces risks of diabetes, cholesterol and colon cancers<sup>[5]</sup>. This fruit is loaded with variety of nutrients and minerals like Vit.C, Vit.B1, Vit.B2, Vit.B3, carotene, calcium, proteins, lipids, zinc, flavonoids<sup>6,7</sup>. Dragon fruit or pitaya is an exotic tropical plant that brings multiple benefits to human health thanks to its high nutritional value and bioactive compounds, including powerful natural antioxidants. Extracts from stems, flowers, peels, pulps of dragon fruit own a range of beneficial biological activities against pathogenic microbes including bacteria, fungi and viruses, and diseases like diabetes, obesity, hyperlipidemia, and cancer<sup>[8]</sup>. Moreover, dragon fruit extracts have cardiovascular- and hepato- protective properties, as well as prebiotic potential. Vietnam is a tropical country with favourable climate conditions for the development of pitaya plantations, which have great adaptability and tolerance to a wide range of environmental conditions (e.g., salinity adaptation, favour light intensity, drought resistance, etc.). The dragon fruit, thanks to its nutritional properties, biological activities, and commercial value has become a cost-effective good for the Vietnamese economy, particularly in the poorest areas of the Mekong delta region, and a driving force in the sustainable development of Vietnam under the challenges posed by the global climate change such

as saline intrusion and drought. Dragon fruit contains the antioxidants vitamin C, beta-carotene, lycopene and betalain. Studies have linked diets high in antioxidants to a reduced risk of chronic disease<sup>[6,9]</sup>

## 2. Jamun (*Syzygiumcumini* L):



Fig no.2: Jamun

Jamun belongs to family myrtaceae. Jamun has been proved to be a most beneficial fruit to human body. It having better antioxidant, antibacterial, antidiabetic, blood purifier, antidiarrheal, hepatoprotective, anti-inflammatory, antifungal, antifungal property. It also used for gastric ulcer. Jamun (*Syzygium cumini* L.) belongs to poly-embryonic species of the family Myrtaceae or Myrtle is known as *Syzygium jambolanum* and *Eugenia cumini* Chase and Reveal, Other common names are Jambul, Black Plum, Java Plum, Indian Blackberry, is an evergreen tropical tree, native to the Indian subcontinent and found growing throughout the Asian subcontinent, Eastern Africa, South America, Madagascar, Australia and have also naturalized to Florida and Hawaii in the United States of America. Jamun fruit has oblong berries, having dark purple or bluish colour and light pinkish pulp (75%) rich in anthocyanins and single dark brown seed (25%). Jamun fruit contains various essential chemical compounds and is a rich source of different nutritional and bioactive compounds. The fruit has carbohydrates, free amino acids, water soluble vitamins, minerals, and essential oil The color and flavor characteristic of the fruit are due to the different phenolic compounds present in the fruit<sup>10</sup>. The fruit have high tannin content so its taste is astringent and the fruit is useful for the treatment of various diseases as an astringent, antiscorbutic, antidiabetic, antidiuretic, chronic diarrhoea, antidiarrheal and

enlargement of spleen. The fruit also gaining more popularity among consumers because of its balanced sugar, acid, and tannin. There are many phytochemicals present in the different plant parts, such as fruit pulp, seed, stem bark, leaves, flowers, and roots.<sup>[11]</sup>

## 3. Sweet Lemon (*Citrus Limetta*):



Fig no.3: sweet lemon

Sweet lime (*Citrus limettarisso*) is also known as 'Mosambi' in the Indian subcontinent region. It has multiple pharmacological effects and its constituents are extensively utilized for many clinical applications. Traditionally it has been widely used in the treatment of the scurvy, indigestion and constipation, diabetes, ulcers, urinary disorder and for improvement of immune system. Advancement in analytical techniques led to identification of multiple constituents among which d-limonene was found to be in abundant. Being chief component of *Citrus limetta* and having better pharmacokinetic and pharmacodynamics properties of d-limonene, it is worth full to explore it in detail. This study is focused on highlighting the phytochemical investigations, traditional uses and clinical applications of *Citrus limetta* as well as its chief constituent-limonene<sup>12</sup>. Sweet lemon belongs to family Rutaceae. It consists of health promoting phytoconstituent and nutritional carotenoids, fatty acids and specially polyethanols. Sweet lemons are the excellent source of Vit.C to strengthen the immune system while reducing inflammation and contains other nutrients including vit. A, fibers, calcium, potassium, folate, and niacin. Sweet lemon having immunity boosting as well as antibacterial property. *Citrus limon* (lemon) is native to South Asia and is an evergreen small tree belongs to family rutaceae and is well familiar nutritional and medicinal property most important specie of the citrus of wastes and by-products

which found major source of pectin, water soluble and insoluble antioxidants and essential oils)<sup>[13]</sup>

#### 4. Tulsi (*Ocimum sanctum*):



**Fig no.4: Tulsi**

Tulsi belongs to family Lamiaceae. It is good for mental health. Because it addresses psychological stress condition such as anxiety, depression. Tulsi is antispasmodic, appetizer, carminative and stomachic. It is used in stomach cramps, vomiting, constipation and useful for diabetes. It is useful for reducing cholesterol levels and heart disease patients, and reduces blood pressure. plant has a great deal of essentialness for humankind, because of the complex restorative advantages it gives. Tulsi leaves are broadly utilized in the readiness of Ayurvedic prescriptions. It is known to advance the life span of life. The extricates acquired from the plant are widely brought to use for relieving different illnesses, for example, the basic cold, irritation, intestinal sickness, coronary illness, migraines, stomach issue, kidney stones, heart issue, and some more<sup>3</sup>. The Indian basil Tulsi additionally helps in the decontamination of environment. Tulsi plant fills in as a marvellous repellent in battling against flies, mosquitoes and creepy crawlies. It is particularly significant in fighting malarial fever. It is a tonic for the sensory system and in this manner helps a lot in honing the memory. This fragrant plant underpins the evacuation of mucus and catarrhal issue from the bronchial cylinder. It additionally does something amazing in forestalling stomach issue. The herb Tulsi is known to fix the respiratory issue. The decoction arranged by blending nectar, ginger and Tulsi leaves is very useful in fighting bronchitis, flu and asthma. The leaves of Tulsi plant are amazingly valuable during the blustery

season, when infections like jungle fever and dengue defraud the nation. Heat up the delicate leaves of Tulsi tea and offer it to the patient. The juice separated from Tulsi leaves fills in as the best solution for cut down fever. Tulsi is a fundamental fixing in the planning of Ayurvedic hack syrups. It is exceptionally valuable in disposing of cold and influenza. Indeed, for sore throat, the leaves of therapeutic plant Tulsi are of extraordinary worth. Simply heat up the leaves of Tulsi in water and request that the patient swish with this decoction. Tulsi can reinforce the kidneys. For those experiencing the issue of renal kidney stones, the decoction arranged by blending the juice of Tulsi leaves with nectar, whenever taken truly for six successive months can remove these stones through the urinary tract. For keeping up solid heart, Tulsi is of most extreme worth. It helps in bringing down the degree of cholesterol in blood. Consequently, Tulsi plant fills in as the best solution for dispose of cardiovascular maladies<sup>[14]</sup>

#### 5. Sodium benzoate:



**Fig no.5: Sodium benzoate**

Sodium benzoate (according to the European nomenclature E211) is a salt of benzoic acid and is well soluble in water, tasteless, and odorless, and due to its antifungal and antibacterial properties, it is a preservative added to food in strictly defined doses. Due to its properties, sodium benzoate is used to preserve food products with an acidic pH, such as fruit pulp and purees, jams, pickles, pickled herring and mackerel, margarine, olives, beer, fruit yogurts, canned vegetables, and salads. It inhibits the growth of bacteria, yeast, and mold. Sodium benzoate was approved as the first of all food preservatives by the Food and Drug Administration (FDA). The permissible limit of its consumption is 0–5 mg/kg of body weight<sup>15</sup>. The drug substance attribute, affects the drug product development, manufacturing, performance and

stability. The following aspects were considered during the product development.<sup>[18]</sup>

Sodium bicarbonate used as a buffer in polyherbal syrup form a intaining PH<sup>[16]</sup>.

**Sodium bicarbonate:**



**Fig no.6: Sodium bicarbonate**

**Role of ingredient in Herbal syrup:**

Table no. 1

Sr.No.	Ingredients	Role
1.	DragonFruit	Antioxidant
2.	Basilleaves	Antibacterial
3.	SweetLemon	Flavoringagent
4.	JamunVinegar	Q.S.
5.	SodiumBenzoate	Preservative
6.	SodiumBicarbonate	Buffer

**Materials and Methods**

**Material:**

**Collection and authentication:**

All the fruits such as Dragon fruit, sweet lemon, Jamun, Tulsi and herbs Plant materials were collected from authenticated herbal suppliers and their genuine was checked and confirmed by comparing with the standard. The raw materials

were primarily identified by the Ayurvedic parameters such as Varna (colour), Gandha (odour), Ruchi (taste), Aakruti (shape) and Parimana (size)<sup>17</sup>. Sodium Benzoate, Sodium Bicarbonate were purchase from Raj chemicals Mumbai.

**Methods and procedure:**

Preparation of polyherbal syrup

**1. Formulation No. 1 (F1)- For 100 ml.**

Table no. 2

Sr.No.	Ingredients	Quantity
1.	Dragonfruit	16 ml
2.	Basilleaves	8 ml
3.	Sweetlemon	11 ml
4.	JamunVinegar	65 ml

**Procedure:**

**1. Decoction of Dragon fruit**

Two Dragon fruit were taken and outer peel was removed. Then fruit cut into small pieces and added in 100 ml water. Then heated slowly to get extract. The extract was filtered and cooled down. From whole extract 18 ml of solution measured.

**2. Decoction of Sweet Lemon**

Two Sweet lemons were cut into small pieces and slowly added in 100 ml of water with constant heating to get extract. Then extract was filtered and cool down. From whole extract 8 ml of solution measured.

**3. Decoction of Tulsi**

Above 20 g of tulsi added into 100 ml of water and heated to get extract. The extract then filtered and cooled. From whole extract 6 ml of solution get measured.

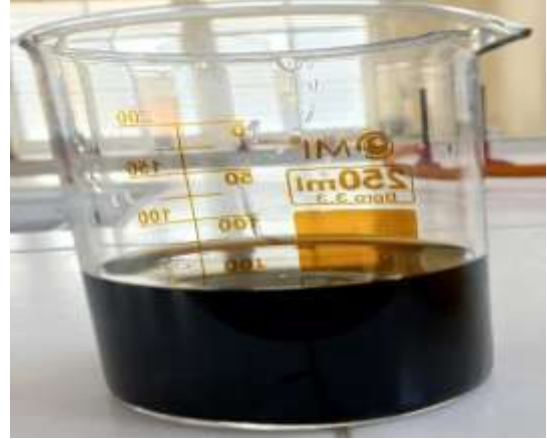
**4. Jamun Vinegar**

Jamun vinegar have collected from authentic ayurvedic shop and sufficient quantity of it have added into polyherbal syrup.

5. We have added sodium benzoate and sodium bicarbonate as an preservative and buffer respectively.

6. All extracts were mixed together to obtain 100 ml of polyherbal syrup.

7. This obtained syrup was transferred to amber color bottle, close it tightly and place it into cool place<sup>[3]</sup>



**Fig no.8: Jamun vinegar**



**Fig no. 9: sweet extract lemon**



**Fig no.7: Dragon fruit extract**



**Fig no. 10: Tulsi extract**

2. Formulation No. 2 (F2)-For 100 ml.

Table no. 3

Sr.No.	Ingredient	Quantity
1.	Dragonfruit	13 ml
2.	Basilleaves	6 ml
3.	Sweetlemon	10 ml
4.	JamunVinegar	63 ml
5.	SodiumBenzoate	8 ml

In above F2 formulation we have added sodium benzoate as a preservative to inhibit microbial growth; because F1 formulation was not stable at environmental condition.

Dissolve 0.40 g of Sodium Benzoate in 40 ml of water and 3.5 ml of dilute Hydrochloric acid. Allow to stand for 5 minute and filter first 5 ml of filtrate. Taken subsequent 20 ml of filtrate and added water to make 50 ml and performed the test.

**Preparation of Sodium Benzoate solution: (50 ml solution)**

3. Formulation No. 3 (F3)- For 100 ml.

Table no. 4

Sr.No.	Ingredient	Quantity	Role
1.	Dragonfruit	18 ml	Antioxidant
2.	Basilleaves	6 ml	Antibacterial
3.	Sweetlemon	8 ml	Flavoring agent and Antioxidant
4.	SodiumBenzoate	8 ml	Preservative
5.	SodiumBicarbonate	2 g	Buffer
6.	JamunVinegar	60 ml	Q.S. and Antioxidant

In above formulation F3 we have added sodium bicarbonate as a buffer to maintain pH; because above two formulation F1 and F2 were shows acidic pH.

- vi. outside the tube using tissue paper.
- vi. Weight bottle with unknown liquid on analytical balance (w<sub>2</sub>).
- vii. Calculate weighting rams of unknown liquid (w<sub>3</sub>).

Formula for density: Density of liquid under test (syrup) = weight of liquid under test / volume of liquid under test = W<sub>3</sub>/V.

**Evaluation Parameter:**

**1. Procedure to determine density:**

- i. Clean thoroughly the specific gravity bottle with chromic acid or nitric acid.
- ii. Rinse the bottle at least two to three times with distilled water.
- iii. If required, rinse the bottle with an organic solvent like acetone and dry.
- iv. Take the weight of empty dry bottle with capillary tube stopper (w<sub>1</sub>).
- v. Fill the bottle with unknown liquid and place the stopper, wipe out excess liquid from



Fig no. 11: Density



Fig no. 12: Specific gravity

**2. Procedure to determine Specific gravity:**

- i. Clean thoroughly the specific gravity bottle with chromic or nitric acid.
- ii. Rinse the bottle at least two to three times with purified water.
- iii. If required, rinse the bottle with an organic solvent like acetone and dry.
- iv. Take weight of empty dry bottle with capillary tube stopper.
- v. Fill the bottle with distilled water and place stopper; wipe out excess liquid from side tube using tissue paper (w2).
- vi. Weight bottle with stopper and water on analytical balance(w2).
- vii. Repeat the procedure for liquid under test by replacing the water after emptying and drying as mentioned in step 4 to 6.
- viii. Weight bottle with stopper and liquid under test on analytical balance (w3).

**Formula for specific gravity:**

Specific gravity of liquid under test (syrup) = weight of liquid under test /weight of water = w5/w4.

**3. Procedure to determine Viscosity:**

- i. Thoroughly clean the Ostwald viscometer with warm chromic acid and if necessary, used an organic solvent such as acetone.
- ii. Mount viscometer in vertical position on a suitable stand.
- iii. Fill water in dry viscometer upto mark G.
- iv. Count time required, in second for water to flow from mark A to mark B.
- v. Repeat step 3 at least 3 times to obtained accurate reading.
- vi. Rinse viscometer with test liquid and then fill it upto mark A, find out the time required for liquid to flow to mark B.
- vii. Determination of densities of liquid as mentioned in density determination experiment

**Formula for viscosity**

Viscosity = Density of test liquid × Time required to flow test liquid/ Density of water × Time required to flow water X viscosity of water [3]





Fig no.13: Viscosity

#### 4. pH determination:

The pH determination of syrup by using two techniques.

##### a. PH Meter

Determination of pH place and accurately measured amount 10 ml of final syrup in a 100 ml volumetric flask and made up the volume upto 100 ml with distilled water. The solution was sonicated for about 10 min. pH was measured with the help of digital pH meter<sup>[3,19]</sup>.

##### b. pH Paper

- i. On a white tile place a clean pH paper strip.
- ii. Drop of the sample on the pH paper using the clean dropper.
- iii. Observe the change in colour of the pH paper.
- iv. Now compare the colour obtain on pH paper with the colour shades on the standard pH chart<sup>[4]</sup>.



Fig no. 14: pH paper



Fig. no. 15: pH meter

#### 5. Stability testing:

Stability testing of the prepared herbal syrup was performed on keeping the samples at accelerated, temperature conditions. The final was taken in culture tubes and were kept at accelerated temperature at 4 degrees Celsius, room temperature and 47 degrees Celsius respectively. The samples were tested for turbidity and homogeneity at the interval of 24 hr., 36 hr. and 72 hr. to observe any change.<sup>[18]</sup>

## II. RESULT AND DISCUSSION

### Result of evaluation parameters:

Table no. 5

Sr.No.	Parameter	F1	F2	F3
1.	Density	2.432 g/cm <sup>3</sup>	2.256 g/cm <sup>3</sup>	2.589 g/cm <sup>3</sup>
2.	Specificgravity	1.0123 kg/m <sup>3</sup>	1.0102 kg/m <sup>3</sup>	1.0097 kg/m <sup>3</sup>
3.	Viscosity	0.42cp.	0.473cp.	0.474cp.
4.	Ph	3.5	3.3	6.5
5.	Organoleptic character:			
	1.Colour	Yellowishbrown	Yellowishbrown	Yellowishbrown
	2.Odour	Aromatic	Aromatic	Aromatic
	3.Taste	Mildelysweet /Slightlysour	Mildelysweet /Slightlysour	Mildlysweet /Slightlysour
	4.Appearance	Turbid	Clear	Clear

### DISCUSSION:

In present age, herbal products are the epitome of safety instead of synthetic drugs which are harmful to human beings and environment. In today's era the herbal products are the symbol of safety in contrast to the synthetic drugs which are regarded as unsafe to human being and environment. Although, herbs had been priced for their medicinal, flavoring and aromatic qualities for centuries. It's time to promote them globally the prepared herbal syrup is having anti-oxidant activity. The final formulation (F3) was obtained is stable than formulations F1, F2. As per the evaluation tests F3 is optimized formulation. The formulation (F3) having antioxidant property as we used herbs and fruits having antioxidant property<sup>[3]</sup>. The formulated syrup was also evaluated by organoleptic properties such as colour, odour, taste and appearance. Colour of the formulated syrup was found to be yellowish brown, odour found to be aromatic, taste found to be slightly sour and appearance found to be clear. The prepared syrup was evaluated immediately after preparation and all tested parameter along with turbidity or homogeneity were compared with changes in accelerated stability testing. final syrup was found to be pH 6.5, specific gravity 1.0097 kg/m<sup>3</sup>, and density found to be 2.589 g/cm<sup>3</sup> and viscosity found to be 0.474 cp<sup>[19]</sup>.

### III. CONCLUSION:

The polyherbal syrup consisting of the four herbal constituents which have antioxidant

property. The polyherbal syrup was evaluated by the physiochemical properties of prepare syrup like colour, odour, taste, appearance, pH, viscosity, density, specific gravity.

The final formulation (F3) was obtained is stable than formulations F1, F2. As per the evaluation tests F3 is optimized formulation. The formulation (F3) having antioxidant property as we used herbs and fruits which having antioxidant property. We used special herbal ingredient that is Jamun Vinegar to make syrup sugar-free. It can be conveniently used by diabetic patients.

The polyherbal syrup having antioxidant property hence it will be very helpful for researchers as well as industries to make the similar formulations on large scale.

### Future prospective:

This polyherbal syrup needs to be further evaluated for its antioxidant potential in animal and human subjects.

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