Research Protocol

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Ayurveda Section

Pharmaceutical Development and Quality Control Assessment of Novel Syrup (Ojolyte) and Evaluation of its Antioxidant Effect: A Research Protocol for an In-vitro Study

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ABSTRACT

Introduction: Ayurveda, an ancient holistic medical system, emphasises achieving balance for overall well-being. The efficacy, safety, stability and palatability of drug dosage forms are crucial in both traditional and modern pharmaceutics. In ayurveda, this is addressed by *Bhaishajya Kalpana*, which focuses on drug formulation and preparation. The concept of polyherbal medicine is highly valued, as combining multiple herbs can enhance therapeutic effects and reduce toxicity. Nutraceuticals, which are made from food or plants, are becoming more and more popular since they provide health advantages beyond simple nourishment. Modern pharmaceutical advancements aim to improve dosage forms for better shelf life, patient compliance and market acceptance. *Mantha* is a traditional ayurvedic cold infusion, offers a liquid form that is easy to ingest and acts quickly due to rapid absorption. Building on this, *Sharkara Kalpana* (syrups) were introduced to ayurveda, offering improved palatability, masked undesirable tastes, and extended shelf life due to their high sugar concentration and osmotic pressure, which inhibits microbial growth.

Need of the study: *Ojolyte* syrup, an emerging formulation, is a mineral-rich, antioxidant, fruity energy drink that replenishes electrolytes and quenches thirst. It is suitable for all age groups, pregnancy, morning sickness, post-chemotherapy care and hangover management, offering a palatable alternative to conventional supplements.

Aim: To assess the pharmaceutical development and quality control Assessment of novel syrup (Ojolyte) and evaluation for anti-oxidant effect

Materials and Methods: The experimental study will be conducted at MGACH&RC, Salod (H), Wardha, Maharashtra, India from January 2025 to December 2025. Nine ingredients including *Phoenix dactylifera*, *Punica granatum*, *Vitis vinifera*, *Garcinia cambogia*, *Tamarindus indica*, *Emblica officinalis*, *Cyperus scariosus*, rock salt and rock sugar are used in specified proportions. Initially, *Mantha* is prepared by soaking and churning the ingredients in cold water, followed by filtration. This filtrate is then processed into syrup by adding 70% sugar and heating to attain one-to-two-thread consistency. Analytical parameters include organoleptic evaluation, physicochemical tests (pH, viscosity, specific gravity, total ash, loss on drying) and microbial load assessment. Advanced analysis involves High-Performance Liquid Chromatography (HPLC) for phytochemical profiling, 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay for antioxidant potential, and nutritional analysis for protein, fat, fibre, carbohydrates, minerals and vitamin C. Shelf-life studies will be conducted under varying temperatures to ensure stability.

Keywords: Antioxidant, Herbal syrup, Nutraceutical, Synergism

INTRODUCTION

The ancient holistic system of medicine known as ayurveda emphasises harmony and balance between the mind, body and spirit in order to support general health and wellbeing. Almost all substances require a certain processing to get a drug that is palatable. In ayurveda such kind of processing is called pharmaceutics or *Bhaishajya Kalpana*. A drug dosage form or drug delivery system is a term given to the form that is eventually used. Safety, efficacy, palatability and stability are the four fundamental needs of an appropriate dosage form. All these four requirements have high value in ayurveda as well. The subdivision known as *Bhaishajya Kalpana* (Pharmaceutical Science) deals with the various drug formulation, preparation and storage, etc., [1]. In order to increase therapeutic efficacy, polyherbal medicine was stressed in the *Sharangdhar Samhita*. As individual plant's active phytoconstituents are insufficient to produce the intended therapeutic outcomes. When several herbs are combined in such a ratio by which the medicinal effect is enhanced and toxicity is decreased [2].

Nutraceuticals, which can be delivered as dietary supplements or functional foods, are bioactive chemicals found in botanical-based sources or common food that provides beneficial effects in addition to the nutritional essential components. Nutraceuticals have been classified based on their use in traditional, non-traditional, recombinant, fortified, herbal, phytochemical, dietary supplements, functional foods, prebiotics and probiotics. Depending upon their nature, various classes of nutraceuticals have a wide range of uses and applications [3].

Dosage form modifications are done for increasing shelf life, palatability, compatibility- patient compliance, adopting to market standards, dosage fixation, easy administration and global acceptance. Modifications can be made to improve form and appearance, increase therapeutic utility or efficacy, make administration and transportation easier, extend shelf life, and improve palatability [4].

Different dosage forms are mentioned in *Bhaishajya kalpana* under which *Mantha* (cold infusion) is one of them which is in liquid form. One part of the drug is churned with four parts cold water to create the cold infusion. After that it is filtered by clean cloth. Eight tola dose should be taken [5]. *Phanta* and *Mantha Kalpana* (cold infusion) are made from medications that are thermo-labile or unstable at high temperatures [6]. Liquid dosage forms are simpler to ingest, more pleasant and more well-liked by all patient strata. Regarding the

efficacy, it is proven that liquids are faster acting than solids due to their fast absorption [7].

By adding sugar to the *Mantha, Sharkara* (syrup) can be prepared. *Sharkara* (syrup) preparations are the concentrated solutions of sucrose obtained in syrup consistency. *Yadavji Trikamaji* introduced these preparations into ayurvedic pharmaceuticals in the 20th century. *Sharkara Kalpana* (syrup) prevents oxidation by being partially hydrolysed into reducing sugars. Syrup's high osmotic pressure stops bacteria, fungi, and mould from growing, which delays decomposition. *Sharkara kalpana* is palatable and it completely masks undesirable taste and odour of the drugs [8].

A thick, viscous mixture of sugar and medicated water is called a syrup. The first step in making syrup is making a decoction, which is made by taking a drug, adding eight times water and boiling it until the volume remains one-fourth of what it was before. After cooling, the decoction is filtered. To prepare the final herbal syrup, the filtrate is taken, sugar is added at a concentration of 66.7%, and the mixture is boiled until it reaches 1-2 thread consistency. The ability to preserve for an extended period of time and the absence of microbial load are two major advancements in syrup. As liquid dosage form can only be stored for 24 hours, this form has a longer shelf life [9].

The major criteria for ayurvedic drugs' acceptance in modern medicine is the quality assessment of their raw materials. These ensure that the drug is pure, safe, potent and efficient [10].

Antioxidants are natural compounds that protect cells from oxidative damage, preventing oxidative stress caused by excessive free radical production, which harms vital biological elements like Deoxyribonucleic Acid (DNA), proteins and lipids. Antioxidants promote overall health by stabilising free radicals, preventing cell damage, and reducing oxidative stress [11].

Electrolytes are essential for life functioning, generating and maintaining electrical neutrality in cells. Imbalanced electrolytes can disrupt bodily functions and cause life-threatening complications [12].

Phoenix dactylifera Linn., *Punica granatum* Linn., *Vitis vinifera* Linn., *Garcinia cambogia* Linn., *Tamarindus indica* Linn., *Emblica officinalis* Gaertn., *Cyperus rotundus* Linn., Rock Salt and rock sugar; all these ingredients are rich in minerals and having antioxidant properties so synergistic effect will be assessed.

Through this study a new formulation will be developed which will be palatable with antioxidant properties and may be rich in minerals.

The aim of the study is to assess the pharmaceutical development and quality control Assessment of novel syrup (Ojolyte) and evaluation for antioxidant effect- In-vitro study

Primary objectives:

1. To prepare *Ojolyte* syrup and to develop its SOP.
2. To evaluate the quality control parameters of herbal formulation.
3. To evaluate antioxidant effect of the *Ojolyte* syrup by in-vitro method.

Secondary objectives:

1. To evaluate minerals concentration and nutritive value of the *Ojolyte* syrup.

REVIEW OF LITERATURE

The nutrition composition and properties [13] of composition of herbal formulation are listed in [Table/Fig-1]. Various studies of Pharmacological activity supporting this study are given in [Table/Fig-2] [14-18].

S. No.	*Dravya* (Drugs)	*Rasa* (Taste)	*Guna* (Properties)	*Virya* (Potency)	*Vipaka* (Bio-transformed rasa)	*Karma* (Therapeutic action)	*Doshaghnata* (pacifying vitiated *doshas*)	Nutrition
1.	*Amalaki (Embilica officinalis* Gaertn.*)*	*Amla* (sour), *Madhura* (sweet), *Kashaya* (astringent), *Tikta* (bitter) *and Katu* (pungent)	*Guru* (heavy), *Ruksha* (dry), *Sheeta* (cold)	*Sheeta* (cold potency)	*Madhura* (sweet)	*Deepana* (metabolism enhancer), *Pachana* (digestion), *Ropana* (healing property), *Trishnaghna* (anti-dypsic), *Grahi* (absorptive), *Mutrala* (diuretic), *Jwaraghna* (anti pyretic), *Balya* (increase strength and immunity), *Hrudya* (palatable) *and Shothaghna* (anti-inflammatory)	*Tridosha shamaka* (pacification of three *doshas*)	Vitamin C, Calcium and Tannin
2.	*Dadima (Punica granatum* Linn.*)*	*Madhura* (sweet), *Amla* (sour), *Kashaya* (astringent)	*Laghu* (light), *Snigdha* (unctuous)	*Anushna* (not too hot)	*Madhura* (sweet) *Amla* (sour)	*Ruchya* (palatable), *Grahi* (absorptive), *Kanthya* (beneficial for throat or voice), *Vishaghna* (reduce toxicity) *and Vatanulomaka* (evacuation of the flatus)	*Tridosha shamaka* (pacification of three *doshas*)	Proteins, Carbohydrates and Minerals Calcium, Iron, Magnesium, Phosphorus and vitamins
3.	*Draksha* (*Vitis vinifera* Linn.*)*	*Madhura* (sweet)	*Snigdha* (unctuous), *guru* (heavy), *mridu* (tender)	*Sheeta* (cold)	*Madhura* (sweet)	*Deepana* (metabolism enhancer), *Pachana* (digestion), *Stambhana* (styptic action), *Dahaprashamana* (relieves burning sensation) *Chardinigraha* (antiemetic)	*Vatapitta shamaka* (pacifies *vata pitta doshas*)	Iron, Calcium, Magnesium, Potassium and Phosphorus
4.	*Kharjura (Phoenix dactylifera* Linn.*)*	*Madhura* (sweet)	*Snigdha* (unctuous), *guru* (heavy)	*Sheeta* (cold)	*Madhura* (sweet)	*Anulomana* (evacuation of the flatus), *Raktashodhaka* (blood purifier), *Mutrala* (diuretic), *Vrushya* (aphrodisiac), *Balya* (increase strength and immunity), *Brumhana* (bulk promotor) *and Dahaprashamana* (relieves burning sensation)	*Vatapitta shamaka* (pacifies *vata pitta doshas*)	Proteins, Vitamins, Carbohydrates and Calcium
5.	*Amlika (Tamarindus indica* Linn.*)*	*Madhura* (sweet), *amla* (sour)	*Guru* (heavy), *ruksha* (dry)	*Ushna* (hot)	*Amla* (sour)	*Deepana* (metabolism enhancer), *Pachana* (digestion), *Rochana* (palatable), *Anulomana* (evacuation of the flatus), *Trishna* (anti-dypsic), *Shramahara* (reduces fatigueness)	*Vatashamaka* (pacifies *vata dosha*)	Copper, iron, calcium, potassium, zinc, magnesium and selenium. Vitamin A, vitamin C, folic acid, riboflavin, niacin and thiamine.
6.	*Vrikshamla (Garcinia cambogia* Linn.*)*	*Madhura* (sweet), *Amla* (sour), *Katu* (pungent)	*Ruksha* (dry), *Laghu* (light)	*Ushna* (hot)	*Amla* (sour)	*Hridya* (palatable), *Yakritottejaka* (liver stimulant), *Jvaraghna* (anti pyretic), *Deepana* (metabolism enhancer), *Pachana* (digestion), *Udararoga* (diseases of abdomen), *Trishna* (anti-dypsic), *Arsharog* (haemorrhoids)	*Kapha-Vatahara and Pittavardhaka* (pacifies kapha *vata dosha* but increases *pitta dosha*)	Fibre, Proteins Sodium, Potassium hydroxycitric acid, and ascorbic acid

7.	*Nagarmotha (Cyperus rotundus* Linn.*)*	*Tikta* (bitter), *katu* (pungent), *Kashaya* (astringent)	*Laghu* (light), *Ruksha* (dry)	*Sheeta* (cold)	*Katu* (pungent)	*Deepana* (metabolism enhancer), *Pachana* (digestion), *Sangrahi* (absorptive), *Lekhana* (scraping), *Jworaghna* (antipyretic)	*Kaphapitta Shamaka* (pacifies *vata pitta dosha*)	Vitamin A, vitamin C, protein, carbohydrate, lipid, iron, phosphorus, sodium, potassium, calcium, magnesium, zinc, copper.
8.	*Saindhava* (Rock salt)	*Lavana* (savoury)	*Laghu* (light), *snigdha* (unctuous), *Sukshma* (minute), *Tikshna* (sharp)	*Sheeta* (cold)	*Madhura* (sweet)	*Rochana* (palatable), *Deepana* (metabolism enhancer), *Avidahi* (reduce burning sensation), *Hikkanashana* (hiccough relieving), *Kapha vilayana* (melting of kapha dosha), *Kapha chedana* (detachment of vitiated dosha), *vibandhaghna* (obstruct eructation)	*Tridoshahara* (pacification of three *doshas*)	Sodium, chloride, iodine, lithium, magnesium, phosphorus, potassium, manganese, iron, zinc
9.	*Sharkara (Saccharum officinarum* Linn.*)*	*Madhura* (sweet)	*Sheeta* (cold)	*Sheeta* (cold)	*Madhura* (sweet)	*Balya* (increase strength and immunity), *Shramahara* (reduces fatigueness), *Shukra shodhaka* (rectifies vitiated dosha of semen)	*Pittavatahara* (pacifies *vata pitta doshas*)	Glucose, sodium, potassium

[Table/Fig-1]: Properties of composition of Ojolyte syrup.

S. No.	Studies	Conclusion
1.	Phenolic contents and antioxidant activity of various date palm (Phoenix dactylifera L.) Fruits from Saudi Arabia [14]	Date fruit is a significant source of hydrophilic antioxidants, as demonstrated by the data presented in this study. This reducing property is generally associated with the presence of polyphenols, specifically flavanols.
2.	*Amalaki*: A review on functional and pharmacological properties [15]	The properties of *Amalaki*, an ayurvedic *Rasayana* drug, are covered in the article. It is a rich source of vitamin C, improves metabolism, digestion, and elimination, and has anti-inflammatory properties. *Amalaki* is a natural antioxidant which rejuvenates tissues and builds *Ojas*, promoting immunity and youthfulness.
3.	Anti photoageing potential of fruits of *Draksha* (*Vitis vinifera L.*) and *Kaashmari* (*Gmelina arborea Roxb.*) [16]	The aim of the current study was to prove the anti-photoaging properties of both *Draksha* and *Kaashmari* fruits applying various parameters. In the DPPH assay, the fruit of *Draksha* exhibited greater activity than the fruit of *Kaashmari*. Thus, both drugs revealed anti-photoaging properties.
4	Role of *amla rasa* in the treatment of *pandu* (Nutritional anemia) [17]	Due to *Rasa kshaya*, *Pandu Rogi* will be having craving for *Amla rasa* but intake of *Amla rasa* due to its similarity in *Yoni* further aggravates *Pitta*. Hence, *Amla rasa* which mitigates *Pitta* at the same time increase *jataragni* should be the choice of drug in the treatment of *Pandu*.
5.	27 Uses of Date Palm in Ayurveda [18]	The effectiveness of ayurvedic preparations using dates as a component has been demonstrated in numerous clinical studies. The mean hangover score, blood levels of alcohol, and acetaldehyde were all significantly reduced by an herbal remedy that included date powder as one of the constituents. This finding demonstrated the potential of this herbal formulation as a novel herbal composition for the treatment of both acute and chronic alcoholic liver diseases and the avoidance of hangover syndrome.

[Table/Fig-2]: Pharmacological activity supporting the study [14-18].

MATERIALS AND METHODS

An experimental study will be conducted in the Department of *Rasashastra* and *Bhaishajya Kalpana*, MGACH&RC, Salod (H), Wardha, Maharashtra, India from January, 2025 to December, 2025. The study will be conducted as per the ethical clearance from Institutional Ethical Committee (IEC no: Ref. No. MGACHRC/IEC/Sep-2023/744). Analytical part of the study will be conducted at the laboratory of Cotex Laxmi Pvt., Ltd., MGACH& RC, Salod (H), Wardha, Maharashtra, India. Analysis of Nutraceutical values and Minerals will be carried out at MGIRI, Wardha. HPLC and Gas Chromatography –Mass Spectrometry (GC–MS) analysis will be carried out at Aakaar biotechnologies Pvt., Ltd., Lucknow, Uttar Pradesh, India. Antioxidant analysis will be carried out at the Central Research Laboratory of JNMC, DMIHER, Sawangi, Wardha, Maharashtra, India.

Drug collection and authentication: The research drug will be procured from Cotex Laxmi Pvt., Ltd., Taxonomist of MGACH&RC will primarily authenticate and verify raw drugs. Raw drugs will be standardised as per API [19].

Composition: List of ingredients used for preparation of *Ojolyte* syrup with their parts to be used and proportion is mentioned in [Table/Fig-3].

S. No.	*Dravya*	Scientific Name	Part to be used	Proportion
1.	*Kharjura*	*Phoenix dactylifera* Linn.	Fruit	4 parts
2.	*Dadima*	*Punica granatum* Linn.	Fruit	1 part
3.	*Draksha*	*Vitis vinifera* Linn.	Fruit	1 part
4.	*Vrikshamla*	*Garcinia cambogia* Linn.	Fruit	1 part
5.	*Amlika*	*Tamarindus indica* Linn.	Fruit	1 part
6.	*Amalaki*	*Emblica officinalis* Gaertn.	Fruit	1 part
7.	*Nagarmotha*	*Cyperus scariosus* Linn.	Rhizomes	1/4 part
8.	*Saindhava*	Rock salt	-	1/8 part
9.	*Sharkara*	*Saccharum officinarum* Linn.	-	70-80%

[Table/Fig-3]: Ingredients with their proportion.
*Note: proportion of drugs may vary according to palatability.

Drug Preparation: Mantha of the mentioned drugs will be prepared. Then, *Ojolyte* syrup will be prepared by adding rock sugar in the polyherbal *Mantha*.

Preparation of Mantha (as per Sharangdhara- Madhyama Khanda-3): All the raw drugs will be washed thoroughly and cut into pieces and seeds will be removed. Then they will be taken together in a clean stainless-steel utensil. Four parts of water will be added. To soak the drug, it will be left undisturbed for four to six hours. Later the mixture will be churned well (manually or mechanically) until the cold water turn warm by rigorous churning and it will be filtered through a clean cloth. The filtrate gained is polyherbal *Mantha.*

Preparation of polyherbal syrup (*Sharkara kalpana*): Polyherbal *Mantha* will be taken in clean vessel. A 70% of sugar will be added into the polyherbal *Mantha* and boil it over mild heat until the liquid will attain syrup consistency (1-2 thread consistency). It will then be filtered to remove any impurities present in sugar. The syrup will be filled in sterile amber colored bottles and will store in dry, cool and dark place.

Analytical Parameters

i) Analytical parameters of polyherbal Mantha:

a) Organoleptic characters: The polyherbal *mantha* will be assessed as per organoleptic parameters. This assessment made use of sensory organs i.e., taste, colour, appearance, odour and touch.

b) Physicochemical parameters:

1. **Viscosity [20]:** The Ostwald viscometer will be thoroughly cleaned with warm chromic acid. Organic solvent such as acetone will be used. A suitable stand will be used to mount the viscometer vertically. The dry viscometer will be filled with water up to mark G. The time it takes for water to move from mark A to mark B will be measured in seconds, this step will be repeated for at least thrice to obtain accurate reading. Determining the liquid densities as specified in the density determination experiment.
2. **pH value [21]:** The drugs' pH will be determined using a 10% aqueous extract. The pH determination procedure will make use of a calibrated digital pH meter, a precision device designed to measure the acidity or alkalinity of a solution. This method provides an accurate and quantitative assessment of the medicinal extract's acidity or basicity, which is critical for understanding its chemical properties and potential effects on biological systems.
3. **Specific gravity [22]:** A liquid's weight per millilitre is the mass in grams of one millilitre of the liquid measured in air at 20°C. The value of this parameter reflects the density of the liquid, which indicates its mass within the specified volume at a given temperature.
4. **Loss on drying at 105°C [23]:** A 10 gm of drug was taken, spread uniformly and thin layered in a shallow petri dish. It was heated at a controlled temperature of 105°C, cooled in a desiccator and weighted. Until two subsequent weights were confirmed to be constant, the procedure was repeated several times. The weight loss % was calculated in relation to the initial weight.

ii) Analytical parameters of Ojolyte syrup:

a) Organoleptic characters:

Organoleptic parameters will be used to assess the *Ojolyte* syrup. The sensory organs-taste, colour, appearance, odour, and touch-were used in this evaluation.

b) Physicochemical parameter:

1. **Loss on drying at 105°C [23]:** A 10 gm of drug was taken, spread uniformly in a shallow petri dish, it was heated at a controlled temperature of 105°C, let it cool in a desiccator and weighted. Until two subsequent weights were confirmed to be constant, the procedure was repeated several times. The weight loss % was calculated in relation to the initial weight.
2. **Total ash [24]:** A 10 gm of each sample of ash will be meticulously measured and placed into crucibles. The samples will be incinerated, allowed to cool and weighted. Weight of the crucible containing the incinerated sample is subtracted from the weight of the empty crucible to determine the total ash value.
3. **pH value [21]:** The drugs' pH will be determined using a 10% aqueous extract. The pH determination procedure will make use of a calibrated digital pH meter, a precision device designed to measure the acidity or alkalinity of a solution. This method provides an accurate and quantitative assessment of the medicinal extract's acidity or basicity, which is critical for understanding its chemical properties and potential effects on biological systems.
4. **Total/reducing sugar [25]:** To start, 100mL of 10% HCl were made for each sample. 10% (100 mL) of HCl was put to a round-bottom flask containing 2 g of PHC. To get rid of the alcohol, this neutralised solution was reduced in volume by half on a heating mantle at 50°c. Through the use of filter paper, this fluid was purified.

   Titration: The flask containing 5 mL of Fehling's solution A and 5 mL of Fehling's solution B was then filled with 30 mL of distilled water. Then, while Fehling's solution was boiling, these filtrate samples were placed in a burette and titrated. Following the addition of two to three drops of methylene blue as an indicator, Fehling's solution was titrated until it turned brick red.

Calculation: Fehling dilution factor × vol. makeup × 100

Burettes reading × g. of sample × 1000

Total sugar: In a flask, 10 mL of PHC extract was put along with 10 mL of HCl. Overnight storage was used for this solution. One millilitre of HCl was used to neutralise the entire sugar content, which produces 100 millilitres in a volumetric flask. Utilise the titrimetric approach to ascertain the overall sugar content.

Non-reducing sugars: Calculating non-reducing sugar content involves subtracting the amount of reducing sugars from the total amount of sugar [26].

5. **Specific gravity [27]:** A liquid's weight per millilitre is the mass in grams of one millilitre of the liquid measured in air at 20°C. The value of this parameter reflects the density of the liquid, which indicates its mass within the specified volume at a given temperature.
6. **Microbial contamination [28]:** Microbial contamination and total viable aerobic count were determined in one-month-old sample using Mac. Conkey and soyabean-casein digest mediums as per method described by World Health Organisation (WHO).
7. **Shelf life [25]:** For the most stable formulations at room temperature, accelerated stability testing of the prepared formulation was carried out. But for the thermal stability, the syrup was kept at 0°C±1°C, 8°C±1°C, 37°C±1°C and 70°C±10C where the sample was placed at freezer, fridge, room temperature and at hot air oven, respectively. For seven days, the sample was kept at various temperatures, and the following parameters were observed to see if any changes occurred.
   1. Loss on drying at 105°C
   2. pH
   3. Total/reducing sugar
   4. Specific gravity
   5. Microbial Contamination

c) Modern sophisticated analysis

1. **HPLC [29]:** 40 mL of deionised water was added to a separate 200 mL beaker containing a 5 gm portion of each sample. After an hour of stirring on a magnetic stirrer, 10 mL of 0.3M copper sulphate were added. A pH meter and 50% sodium hydroxide were used to adjust the pH to 6.4 after stirring. After carefully moving the sample to a 200 mL volumetric flask, deionised water was added to get the volume up to 200 mL. It was properly blended. The sample was filtered into a 5-oz plastic cup with a cap using Whatman 2V filter paper covered with 0.5 g of acid-washed celit. For 2.5 h, it was vortexed on a Sonicator. The sample vials were vortexed every 10 to 15 minutes until there was no residue found on the vial walls. The clear solution was prepared for HPLC analysis against reference standards by filtering it into a 2 mL injection vial using a syringe and 0.2 µm nylon filters.
2. **Antioxidant analysis- DPPH scavenging activity:** The DPPH method will be used to assess the methanolic extracts' capacity to scavenge free radicals. It will be determined by measuring the sample's effect on the absorbance at 516 nm of a coloured DPPH solution in methanol. We'll prepare a stock solution of DPPH (1.3 mg/mL in methanol). The methanolic extract solution will have a concentration of 10 mg/10 mL. A 1 mL of this solution will be transferred to test tubes and diluted with 10 mL

of the same solvent. This is a stock solution. 0.10, 0.15, 0.25, 0.50, and 0.60 mL of the stock solution will be taken and placed in separate test tubes with concentrations of 10, 15, 25, 50, and 60 µg/mL, respectively. To prepare the stock solution, 24 mg of DPPH were dissolved in 100 mL of methanol. Methanol was used to filter the DPPH stock solution, producing a usable mixture with an absorbance of about 0.973 at 517 nm [30]. Five different extract concentrations were mixed with 3 mL of DPPH working solutions in a test tube. As a standard, 3 mL of DPPH in methanol solution is often given. The tubes were then left in total darkness for 30 minutes. Thus, a spectrophotometer (Systronics UV-Visible Spectrophotometer 2201) was used to measure the absorbance at 517 nm. The following formula will be used to calculate the percentage of DPPH free radical scavenging:

Where: Ac = Control reaction absorbance,

$$\% \text{ of antioxidant activity} = \{(Ac-As) \div Ac\} \times 100$$

As = Testing specimen absorbance [31]

3. **Nutritional values:** Nutritional value of Ojolyte syrup. The following analysis shall be conducted using the standard procedure of Rangana 2001 [32] and Association of Official Analytical Chemists (AOAC) 2005 [33]:
    - Protein determination using the Lowry Method.
    - Determination of fat- by Soxhlet method.
    - Fibre is determined using extraction method.
    - Carbohydrate determination: Several methods were used to determine the amount of carbohydrates.
    - Energy value: Calculated by adding the percentages of protein, fat, and carbohydrates, and multiplying the results by 4, 9, and 4, respectively.
    - The colorimetric method is used to determine iron levels.
    - Volumetric determination of calcium.
    - Using the 2,6-Dichlorophenol dye technique to determine vitamin C.

Outcome

Primary outcome:

1. Development and quality control assessment of Ojolyte syrup.

Secondary outcome:

1. Antioxidant effect of Ojolyte syrup;
2. Nutritional value.

Study Guideline: Followed the CRIS guidelines for the study.

STATISTICAL ANALYSIS

Descriptive statistics will be used to analyse the results of the study.

Gnatt chart is explained in [Table/Fig-4]

Steps	Q1 (Jan-Feb)	Q2 (Mar-Apr)	Q3 (May-Jun)	Q4 (Jul-Aug)	Q5 (Sep-Oct)	Q6 (Nov-Dec)
IEC approval						
Drug collection						
Literature review						
Analytical study						
Bioavailability study						
Data analysis						
Writing rest of thesis submission						

[Table/Fig-4]: GNATT chart.

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