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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF THE DIURETIC ACTIVITY OF LEAF EXTRACT OF OCIMUM SANCTUM

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Abstract:

Ocimum sanctum Linn. (Tulsi) is a well-established medicinal herb renowned for its diverse pharmacological activities, including antioxidant, anti-inflammatory, adaptogenic, and neuroprotective effects. However, its therapeutic potential is often limited by poor aqueous solubility and bioavailability of key phytoconstituents. The present study aimed to develop and evaluate self-nanoemulsifying tablet formulations of *Ocimum sanctum* extract to enhance its dissolution rate, stability, and overall pharmacological efficacy. Various tablet formulations were prepared using a combination of surfactants (Tween 80), co-solvents (polyvinylpyrrolidone), and oils (corn oil), and evaluated for physical parameters such as weight variation, hardness, friability, and thickness. The optimized formulation demonstrated acceptable pharmaceutical properties with improved disintegration time and enhanced dissolution profile. Phytochemical screening confirmed the presence of bioactive compounds including flavonoids, alkaloids, glycosides, and saponins. Additionally, behavioral studies such as the open field test suggested potential anxiolytic activity of the formulated tablets. These findings support the feasibility of using SNEDDS technology to improve the therapeutic performance of herbal extracts like *Ocimum sanctum* in a stable and patient-friendly dosage form.

Keywords:

Ocimum sanctum, Tulsi, Self-Nanoemulsifying Drug Delivery System (SNEDDS), Herbal Tablets, Phytochemical Screening.

1. Introduction

Ocimum sanctum Linn., also known as Tulsi or Holy Basil, is a sacred and highly valued herb in Indian traditional medicine systems, especially Ayurveda and Siddha. It belongs to the Lamiaceae family and is native to the Indian subcontinent, where it has been used for centuries for its wide-ranging medicinal properties. Pharmacologically, *O. sanctum* exhibits diverse therapeutic actions including antistress, antioxidant, immunomodulatory, anti-inflammatory, antimicrobial, antidiabetic, and neuroprotective effects, largely attributed to its phytochemical constituents like eugenol, rosmarinic acid, ursolic acid, apigenin, luteolin, and carvacrol (Mondal et al., 2009).

Despite its therapeutic richness, the clinical application of *Ocimum sanctum* is often limited due to challenges in standardization, bioavailability, and stability of its active constituents when administered in crude or decoction forms. Therefore, modern pharmaceutical interventions such as novel drug delivery systems (NDDS) are being explored to overcome these limitations. Among them, Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) have gained significant attention for enhancing the solubility and oral bioavailability of poorly water-soluble herbal drugs (Pouton, 2006). SNEDDS are isotropic mixtures of oils, surfactants, and co-surfactants that spontaneously emulsify in gastrointestinal fluids to form nano-sized emulsions, providing enhanced surface area for absorption.

The integration of *Ocimum sanctum* extracts into a SNEDDS-based tablet formulation offers multiple advantages: improved chemical stability, enhanced absorption of lipophilic constituents, dose uniformity, and patient convenience in oral administration. Tablet formulations are further preferred for their ease of handling, transport, and better patient adherence. The development and evaluation of such formulations involve systematic assessment of physical parameters (e.g., hardness, friability, thickness, weight variation), in vitro drug release, and pharmacological activity such as anxiolytic or antidiabetic potential using validated animal models.

Given the increasing global preference for herbal medicines and the need for scientifically validated delivery systems, this study focuses on formulating *Ocimum sanctum* extracts into self-nanoemulsifying tablets and evaluating their physicochemical and pharmacological properties.

2. Material And Methods

2.1 Collection and Authentication of the Plant Leaves

Fresh leaves of *Ocimum sanctum* were collected during the appropriate season to ensure maximum phytoconstituent content. The plant material was identified based on morphological characteristics such as leaf shape, aroma, and inflorescence structure. Authentication was carried out by a botanist, and a voucher specimen was deposited in a herbarium for future reference, ensuring accuracy and traceability of the plant source.

2.2 Extraction of plant:

The collected leaves were thoroughly washed, shade-dried at room temperature to avoid degradation of thermolabile compounds, and then powdered using a mechanical grinder. The coarse powder was subjected to methanolic extraction using a Soxhlet apparatus for several hours. The extract was then filtered and concentrated under reduced pressure using a rotary evaporator, resulting in a semi-solid mass. This extract was stored in airtight containers under refrigeration for further use in phytochemical or pharmacological studies.

2.3 Determination of Physical Parameters

The physical parameters of *Ocimum sanctum* leaf powder were evaluated to ensure quality, purity, and standardization of the raw material.

- **Moisture content**

The moisture content was determined using the loss on drying method, where a known weight of powdered leaf sample was placed in a hot air oven at 105°C until a constant weight was obtained. High moisture content can lead to microbial contamination and spoilage, while an ideal low value ensures extended shelf life and preservation of active constituents. The result is usually expressed as a percentage of the initial weight of the sample. Acceptable moisture content in medicinal herbs is generally below 10%.

- **Total Ash value**

Total ash value indicates the total amount of inorganic material present in the plant, including both physiological ash (from the plant tissue itself) and non-physiological ash (from external contaminants like dust and sand). A known quantity of the powdered drug was incinerated in a silica crucible in a muffle furnace at about 550–600°C until carbon-free ash was obtained. The residue was cooled in a desiccator and weighed. A higher ash value may suggest the presence of excessive contamination or adulteration.

- **Acid insoluble ash value**

This parameter represents the portion of total ash that is insoluble in dilute hydrochloric acid, mainly indicating the presence of siliceous materials such as sand and earth. After treating the total ash with dilute HCl, the mixture was filtered, and the residue was incinerated and weighed. Acid-insoluble ash is a marker of extraneous matter that cannot be digested, and excessive values indicate poor handling or processing of plant material.

- **Water soluble extractive value**

The water-soluble extractive value determines the amount of water-soluble constituents, such as glycosides, mucilage, tannins, and some alkaloids. This is done by macerating a specific weight of the powdered drug in water for 24 hours with frequent shaking, followed by filtration and evaporation of a portion of the filtrate to dryness in a dish. The residue is weighed and expressed as a percentage of the original sample weight. A higher extractive value typically indicates the presence of abundant water-soluble phytoconstituents, which may correlate with therapeutic efficacy.

2.4 Phytochemical Screening

Phytochemical screening is a crucial step in the preliminary evaluation of medicinal plants to identify the presence of bioactive constituents responsible for therapeutic effects. The methanolic extract of *Ocimum sanctum* leaves was subjected to qualitative phytochemical analysis using standard procedures to detect the major classes of secondary metabolites.

2.5 Detection of Carbohydrate

Subsequent to dissolving 500 mg of the *Ocimum sanctum* extract in five milliliters of distilled water, the solution was filtered to obtain a clear filtrate. This filtrate was used for carbohydrate detection using Molisch's test.

- **Molisch's Test**

Molisch's test is a general test for all classes of carbohydrates, including monosaccharides, disaccharides, and polysaccharides. In this test, 2 mL of the *Ocimum sanctum* extract was mixed with a few drops of Molisch's reagent (a 10% alcoholic solution of α-naphthol). Concentrated sulfuric acid was then gently added down the side of the test tube without shaking. A purple or violet ring at the interface between the two layers confirmed the presence of carbohydrates. This result is due to the dehydration of carbohydrates by sulfuric acid, forming furfural or hydroxymethylfurfural, which condenses with α-naphthol to form a colored complex. The intensity of the ring may provide a semi-quantitative indication of carbohydrate abundance.

- **Fehling's Test**

Fehling's test specifically detects reducing sugars, which possess free aldehyde or ketone groups. The test involves combining 1 mL each of Fehling's A (copper(II) sulfate) and Fehling's B (alkaline tartrate solution) to form a deep blue complex, to which 1 mL of the plant extract is added. After heating in a boiling water bath for several minutes, the formation of a brick-red precipitate (cuprous oxide) indicates the presence of reducing sugars. This suggests that the *Ocimum sanctum* extract contains monosaccharides like glucose or fructose, or disaccharides like maltose or lactose, which may contribute to its nutritive and therapeutic properties.

2.6 Detection of Glycosides

Glycosides are secondary metabolites in medicinal plants composed of a sugar moiety bound to a non-sugar component (aglycone). They play a crucial role in therapeutic actions such as cardiotonic, laxative, anti-inflammatory, and antioxidant effects.

- **Modified Borntrager's**

This test is designed to detect free or combined anthraquinone glycosides. A small amount of the extract was hydrolyzed with dilute hydrochloric acid and heated in a water bath. After cooling, the solution was extracted with benzene. The benzene layer was then separated and treated with dilute ammonia. A pink to red coloration in the ammoniacal layer indicated the presence of anthraquinone glycosides. The modification helps detect glycosides that require hydrolysis to release their aglycones, as commonly found in plant extracts.

- **Killer Killiani**

This test specifically identifies deoxy sugars in cardiac glycosides. A small quantity of the extract was dissolved in glacial acetic acid containing a trace of ferric chloride, followed by the addition of concentrated sulfuric acid along the sides of the test tube. A brown ring at the interface, accompanied by a bluish-green coloration in the acetic acid layer, confirmed the presence of cardiac glycosides. This reaction results from the oxidation of the deoxy sugar portion by ferric ions under acidic conditions, leading to colored complexes.

2.7 Detection of Alkaloids

A total of 0.5 g of the concentrated *Ocimum sanctum* extract was dissolved in 10 mL of dilute hydrochloric acid (0.1 N) and gently warmed for a few minutes to facilitate alkaloid extraction. The mixture was then filtered, and the clear filtrate was used for qualitative tests.

- **Mayer's Test**

Within the sight of alkaloids, filtrates were treated with Mayer's answer, bringing about the advancement of a brilliant cream-hued encourage.

- **Mayer's reagent:**

Mayer's reagent is a solution of potassium mercuric iodide. To perform the test, a few milliliters of the extract were treated with Mayer's reagent. The formation of a cream or pale yellow precipitate indicated the presence of alkaloids. This reaction is due to the complex formation between the alkaloids and the reagent, which results in a visible precipitate.

- **Dragendorff's Test**

Filtrates were treated with Dragendroff's reagent; improvement of red shaded empower shows the presence of alkaloids.

- **Dragendorff's reagent:**

Dragendorff's reagent consists of potassium bismuth iodide. Upon adding this reagent to the plant extract, an orange or reddish-brown precipitate indicates the presence of alkaloids. The color and nature of the precipitate arise from the formation of insoluble bismuth-alkaloid complexes, which are characteristic of many nitrogenous plant compounds.

- **Hager's test**

Hager's reagent is a saturated solution of picric acid. When this reagent is added to the plant extract, the appearance of a yellow crystalline precipitate confirms the presence of alkaloids. The interaction between the alkaloid base and picric acid forms a stable picrate salt, which is visible as a yellow precipitate.

2.8 Detection of Phytosterols and Triterpenoids

Phytosterols and triterpenoids are important non-polar secondary metabolites found in many medicinal plants, including *Ocimum sanctum*. They possess a wide range of biological activities such as anti-inflammatory, hepatoprotective, antioxidant, and immunomodulatory effects. Their presence in plant extracts can be confirmed using the Salkowski Test, a classical color reaction that identifies the sterol and triterpene classes of compounds.

- **Salkowaski Test**

The Salkowski test is a qualitative method used to detect the presence of phytosterols and triterpenoids in plant extracts. In this test, a small quantity of the *Ocimum sanctum* extract is dissolved in chloroform, followed by the careful addition of concentrated sulfuric acid along the side of the test tube without mixing. Upon standing, two distinct layers form. A reddish-

brown coloration at the interface indicates the presence of phytosterols, while a golden-yellow to red or greenish fluorescence in the chloroform layer suggests the presence of triterpenoids. These color changes occur due to the sulfonation and oxidation of the sterol or triterpene nuclei in the acidic environment. The Salkowski test provides a rapid and effective preliminary screening for lipophilic bioactive compounds in herbal extracts.

2.9 Detection of Protein and Amino Acid

Proteins and amino acids are essential biomolecules involved in enzymatic activity, tissue repair, and various physiological functions. Their detection in *Ocimum sanctum* helps in evaluating the nutritional and therapeutic potential of the plant. Two standard qualitative tests—Millon's Test and Ninhydrin Test—are commonly used to confirm their presence.

- **Millon's Test**

Millon's test is specific for detecting phenolic amino acids, especially tyrosine. A small amount of the *Ocimum sanctum* extract is treated with Millon's reagent (a solution of mercuric nitrate in nitric acid). Upon gentle heating, the appearance of a white precipitate that turns red upon further heating confirms the presence of tyrosine-containing proteins. The color reaction is due to the nitration of the phenol group in tyrosine, followed by complex formation with mercury ions.

- **Ninhydrin Test**

The Ninhydrin test is a general test for free amino acids and proteins with free amino groups. A small volume of the extract is mixed with Ninhydrin reagent (1% solution in ethanol or water) and heated in a water bath. The development of a purple or bluish-violet color (Ruhemann's purple) indicates the presence of free amino acids. A yellow color may indicate the presence of proline or hydroxyproline. This reaction occurs as ninhydrin reacts with amino groups to form a colored complex, which is highly sensitive and widely used in amino acid analysis.

2.10 Discovery of Fixed Oils and Fats

- **Oily spot test**

In this test, a small quantity of the plant extract is applied onto a piece of filter paper and allowed to dry. The formation of a translucent greasy spot at the application site, which does not disappear upon drying, indicates the presence of fixed oils or fats. This reaction is based on the ability of lipids to penetrate paper fibers and scatter light differently, creating a semi-

transparent appearance. Unlike volatile oils, fixed oils leave a persistent mark due to their non-evaporative nature.

2.11 Detection of Phenolics and Tannins

Phenolic compounds and tannins are important secondary metabolites in medicinal plants, known for their antioxidant, antimicrobial, astringent, and anti-inflammatory properties.

- **Ferric chloride test**

This test is commonly used for the detection of phenols and tannins. A small quantity of the *Ocimum sanctum* extract is treated with a few drops of 5% neutral ferric chloride solution. The development of a deep blue, green, or black coloration indicates the presence of phenolic compounds or tannins. The color arises due to the formation of colored ferric-phenolate complexes. The specific hue may vary depending on the type and structure of the phenolic or tannin compound present.

- **Lead Acetate Test**

In this test, the plant extract is treated with a few drops of lead acetate solution (10%). The formation of a white or yellowish precipitate indicates the presence of tannins. This occurs as tannins form insoluble complexes with lead ions. This test is particularly effective in detecting hydrolyzable and condensed tannins.

2.12 Detection of Flavonoids

Flavonoids are a diverse group of polyphenolic compounds widely recognized for their antioxidant, anti-inflammatory, cardioprotective, and antimicrobial activities.

- **Alkaline Reagent test**

In this test, a small amount of the *Ocimum sanctum* extract is treated with a few drops of sodium hydroxide solution. The appearance of an intense yellow coloration indicates the presence of flavonoids. This yellow color becomes colorless or pale upon the addition of dilute hydrochloric acid, confirming the reaction. The color change is due to the formation of flavonoid-alkali complexes, which are sensitive to pH.

2.13 Discovery of Saponin

- **Foam Test**

In this test, a small amount of the *Ocimum sanctum* extract is diluted with distilled water and shaken vigorously in a test tube for about 15 minutes. The formation of a stable, persistent froth or foam that lasts for at least 10 minutes indicates the presence of saponins. The foam may rise to a height of 1–2 cm or more, depending on the saponin concentration. This reaction occurs

due to the surfactant nature of saponins, which reduce surface tension and trap air, forming foam.

2.14 Preparation of Drug Loaded Self Nanoemulsifying Tablets

Self-nanoemulsifying tablets (SNETs) are solid dosage forms developed from self-nanoemulsifying drug delivery systems (SNEDDS), which are isotropic mixtures of oils, surfactants, and co-surfactants capable of forming nano-sized emulsions upon dilution in aqueous media. These systems enhance the solubility, dissolution, and bioavailability of poorly water-soluble drugs.

The preparation process begins with the formulation of a liquid SNEDDS. This involves selecting an appropriate oil phase (e.g., medium-chain triglycerides), surfactant (e.g., Tween 80), and co-surfactant (e.g., polyethylene glycol or Transcutol P), which are mixed with the drug under mild heating and stirring to form a homogeneous mixture. The optimal ratios are determined based on phase diagrams and emulsification efficiency.

To convert the liquid SNEDDS into tablets, a solid carrier (e.g., microcrystalline cellulose, colloidal silicon dioxide, or Neusilin) is used to adsorb the liquid formulation, producing a free-flowing powder. This powder is then blended with standard excipients such as binders, disintegrants, lubricants, and glidants to enhance tablet properties. The blend is compressed into tablets using a tablet compression machine under controlled pressure.

The resulting self-nanoemulsifying tablets retain the ability to spontaneously emulsify into nano-sized droplets upon contact with gastrointestinal fluids, ensuring rapid drug release and absorption. This solid dosage form combines the advantages of nanoemulsion-based delivery with the convenience and stability of tablets, making it ideal for enhancing oral bioavailability of lipophilic drugs.

Table 1: Drug Loaded Self Nanoemulsifying Tablets formulation

Ingredient	Composition % w/w (F-1)	Composition % w/w (F-2)	Composition % w/w (F-3)	Composition % w/w (F-4)	Composition % w/w (F-5)
Ocimum sanctum Extract (100 mg/tablet)	70	65	60	55	50
Polyvinylpyrrolidone (Binder)	10	12	15	18	20
Tween 80 (Surfactant)	10	12	12	15	18
Corn oil (Oil phase)	3	3	5	5	5
Magnesium stearate (Lubricant)	2	2	2	2	2
Talc (Glidant)	5	6	6	5	5

2.15 Evaluation of tablets

The tablets were evaluated for in process and finished product quality control tests i.e. appearance, dimensions (diameter and thickness), weight variation, hardness, friability, assay, and drug content.

2.15.1 Physical appearance

The tablets were visually inspected for color, shape, texture, and surface finish. All formulations (F-1 to F-5) exhibited a uniform light green to brownish color due to the natural extract, with a smooth surface, no visible cracks, and no signs of chipping or mottling. The tablets were circular and flat-faced, with acceptable aesthetic properties for oral administration.

2.15.2 Thickness and diameter

- **Measurement of Tablet Diameter:**

To measure the diameter of the tablet, it is first placed horizontally between the jaws of a digital Vernier caliper. Care is taken to align the tablet flat and centered across the jaws, ensuring it rests evenly for an accurate reading. The jaws are then closed gently until they touch both edges of the tablet without applying excessive pressure. The diameter is read and recorded from the caliper display, with the measurement taken at the widest horizontal point of the tablet. This procedure is repeated for ten tablets, and the average diameter along with the standard deviation is calculated to assess uniformity and batch consistency.

- **Measurement of Tablet Thickness:**

To measure the thickness of the tablet, it is stood vertically between the jaws of a digital Vernier caliper. The tablet is positioned perpendicular to the caliper jaws to ensure an accurate reading. The jaws are gently closed until they just touch both flat surfaces of the tablet, avoiding any excess pressure that could distort the measurement. The thickness is then read and recorded from the caliper display, with the measurement taken at the center or thickest point of the tablet. This procedure is repeated for ten tablets, and the average thickness along with the standard deviation is calculated to evaluate batch uniformity.

2.15.3 Hardness

Tablet hardness testing is performed to determine the mechanical strength of tablets and their ability to withstand handling, packaging, and transportation without breaking or chipping. The test is commonly carried out using instruments like the Monsanto, Pfizer, or digital hardness testers. In this procedure, a single tablet is placed between the jaws or plungers of the hardness tester, ensuring that the tablet is properly aligned so that the force is applied diametrically. Pressure is gradually applied until the tablet fractures, and the force required to break the tablet is recorded, typically in kilograms, Newtons, or pounds depending on the instrument used. This process is repeated for at least six tablets, and the average hardness is calculated to assess batch uniformity. Consistent hardness values are crucial for maintaining tablet integrity while ensuring that the formulation still allows for proper disintegration and drug release.

2.15.4 Friability test

The friability test is conducted to evaluate the mechanical strength of tablets and their ability to withstand abrasion and shock during handling, packaging, and transportation. The test is performed using a friabilator, such as the Roche friabilator, which consists of a rotating drum that repeatedly lifts and drops the tablets as it turns. For the test, twenty tablets are randomly

selected, provided their individual weight is at least 650 mg. If the tablet weight is lower, a sufficient number of tablets totaling at least 6.5 grams is used. The selected tablets are first cleaned to remove any dust and then weighed collectively to determine their initial weight (W_1). These tablets are then placed in the friabilator, which is set to rotate at 25 rpm for a total of 100 revolutions over approximately four minutes. During this time, the tablets undergo mechanical stress as they tumble inside the rotating drum. After the test is complete, the tablets are removed, dedusted to eliminate any loose particles, and reweighed to obtain the final weight (W_2). The friability is then calculated using the formula:

$$\text{Friability (\%)} = (W1 - W2 / W1) \times 100$$

A friability value of less than 1% is generally considered acceptable for conventional tablets. In addition to friability, weight variation is also assessed to ensure dosage uniformity. For this, ten tablets are individually weighed, and the average weight is calculated. Each tablet's weight is compared to this average, and the deviation must fall within pharmacopeial limits, typically ±5% to ±10%, depending on the average tablet weight. These evaluations are essential for confirming the physical integrity and consistency of tablet formulations.

2.16 Dissolution Testing

Dissolution testing evaluates how quickly and efficiently a tablet releases its active ingredient in a liquid medium. For *Ocimum sanctum* self-nanoemulsifying tablets, the test is carried out using a USP Type II (paddle) apparatus with 900 mL of dissolution medium (e.g., 0.1 N HCl or phosphate buffer pH 6.8) at 37 ± 0.5°C and 50–75 rpm. One tablet is placed per vessel, and samples are withdrawn at set intervals (e.g., 5–60 minutes), filtered, and analyzed using a UV spectrophotometer. The percentage drug release is calculated and plotted over time. Rapid dissolution indicates good formulation performance and ensures consistent bioavailability.

2.17 Experimental protocol

A total of 30 rats were randomly divided into five groups, with six animals in each group, to evaluate the effects of *Ocimum sanctum* leaf extract over a 30-day experimental period. The analysis was conducted on the 15th and 30th days. Group 1 served as the normal control and received no treatment. Group 2, the disease control, was administered diazepam at a dose of 10 mg/kg to induce a disease-like condition. Group 3 received a low dose of *Ocimum sanctum* leaf extract at 100 mg/kg, while Group 4 was treated with a high dose of the extract at 200 mg/kg. Group 5 received a standard therapeutic drug, such as imipramine at 15 mg/kg, for comparison.

This design aimed to assess the dose-dependent pharmacological effects of *Ocimum sanctum* in relation to both untreated and standard-treated conditions.

2.18 Induction of experimental diabetes in rats:

2.18.1 Animals

Healthy adult Wistar albino rats weighing between 150–200 grams were selected for the study. The animals were housed under standard laboratory conditions with a 12-hour light/dark cycle, room temperature maintained at 22 ± 2°C, and relative humidity of 45–55%. They were provided with a standard pellet diet and water ad libitum throughout the experimental period. Prior to the experiment, the rats were acclimatized to the laboratory environment for at least one week. Only healthy animals with normal fasting blood glucose levels were included. The animals were randomly divided into experimental groups to ensure uniformity and minimize bias. All experimental procedures were conducted in accordance with institutional ethical guidelines and were approved by the Institutional Animal Ethics Committee (IAEC).

2.19 Open field apparatus test

The Open Field Test is a widely used behavioral assay to assess locomotor activity, exploratory behavior, and anxiety levels in rodents. The apparatus consists of a square or circular arena with defined grids marked on the floor, typically enclosed by high walls to prevent escape. The floor is divided into central and peripheral zones to distinguish between exploratory and anxiety-related behavior.

Each rat is individually placed in the center of the open field, and its activity is observed for a fixed period, usually 5 to 10 minutes. Parameters recorded include the number of line crossings (locomotor activity), rearing (vertical activity), grooming (self-care behavior), and time spent in the central vs. peripheral zones (anxiety index). Increased activity and time spent in the center indicate reduced anxiety, whereas preference for the periphery and reduced movement suggest heightened anxiety or sedation.

After each trial, the apparatus is cleaned with 70% ethanol to eliminate olfactory cues. This test helps evaluate the central nervous system effects of *Ocimum sanctum* extract, particularly its potential anxiolytic or sedative properties.

3. Results

3.1 Physico-Chemical Evaluation of Crude extracts

The physico-chemical evaluation of *Ocimum sanctum* crude extract includes parameters such

as moisture content, ash values, and extractive values to assess its quality and purity. Moisture content is measured to ensure stability and prevent microbial growth. Total ash and acid-insoluble ash values indicate the presence of inorganic impurities like silica or sand. Water-soluble and alcohol-soluble extractive values reflect the concentration of bioactive compounds like tannins, glycosides, and flavonoids. These assessments are essential for standardizing the extract and ensuring its consistency and efficacy in herbal formulations.

3.2Physical Test Of Crude extract

Physical testing of *Ocimum sanctum* crude extract involves basic sensory and appearance evaluations that provide initial insight into its identity and quality. These tests include color, odor, taste, and texture. The extract typically appears as a dark brown or greenish-brown semi-solid or powder, depending on the extraction method and drying conditions. It possesses a characteristic aromatic odor, often slightly pungent due to the presence of essential oils, and a bitter, astringent taste. The texture is usually coarse or sticky for semi-solid extracts and fine for powdered forms. These physical characteristics serve as preliminary quality indicators before proceeding to detailed chemical and biological evaluations.

Table 2: Physical Test Of Crude extract

Crude Drug	Nature	Colour	Odour	Taste
Ocimum sanctum	Hygroscopic, semi-solid/powder	Dark brown to greenish-brown	Characteristic aromatic	Bitter and slightly astringent

3.3 Extractive Values

Extractive values help determine the amount of active constituents present in a crude extract using specific solvents. For *Ocimum sanctum*, water-soluble and alcohol-soluble extractive values are commonly measured by macerating the extract with the respective solvent, filtering, and evaporating the solution to dryness. A higher extractive value indicates a greater concentration of bioactive compounds like flavonoids and tannins. These values are essential for assessing the quality, potency, and consistency of the herbal extract.

Table 3: Extractive Values

Crude drugs	Ethanol % w/w	Water % w/w
Ocimum sanctum	18.3	22.6

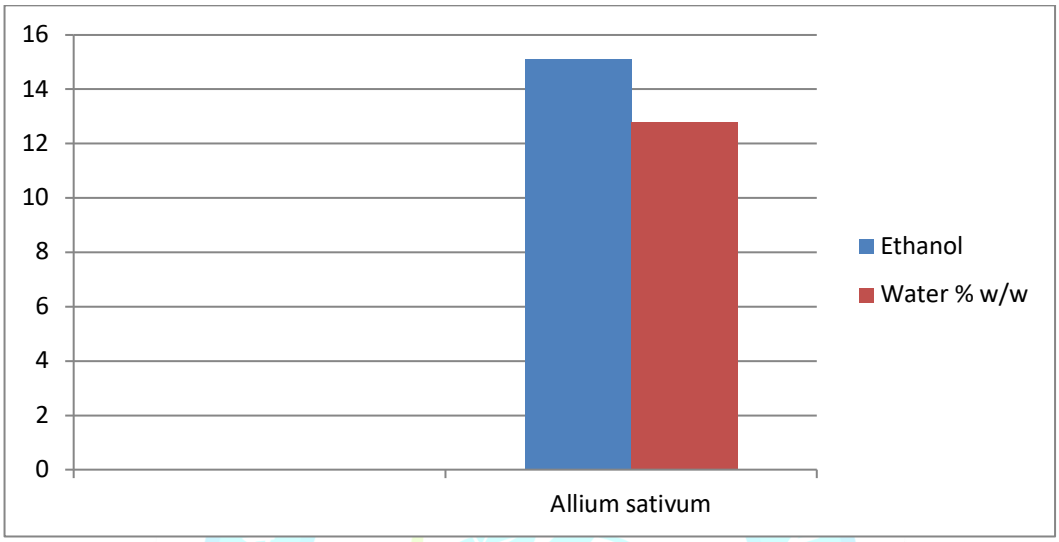

Fig 1: Graph of Extractive Values

3.4 Loss on Drying And Foreign Organic Matter

Loss on drying determines the amount of moisture and volatile matter present in the crude extract. A known quantity of *Ocimum sanctum* extract is weighed and dried at 105°C until a constant weight is achieved. The percentage of weight loss indicates moisture content. Low moisture levels are essential for preventing microbial growth and ensuring extract stability.

Foreign organic matter refers to unwanted materials such as stems, soil, insects, or other plant parts. The extract is visually inspected, and non-target materials are separated, dried, and weighed. The percentage of foreign matter should be within acceptable pharmacopeial limits to ensure purity and quality of the herbal material.

Table 4: Loss on Drying And Foreign Organic Matter

Crude drugs	Loss on drying (% w/w)*	Foreign matter (% w/w)*
Ocimum sanctum	5.2	1.1

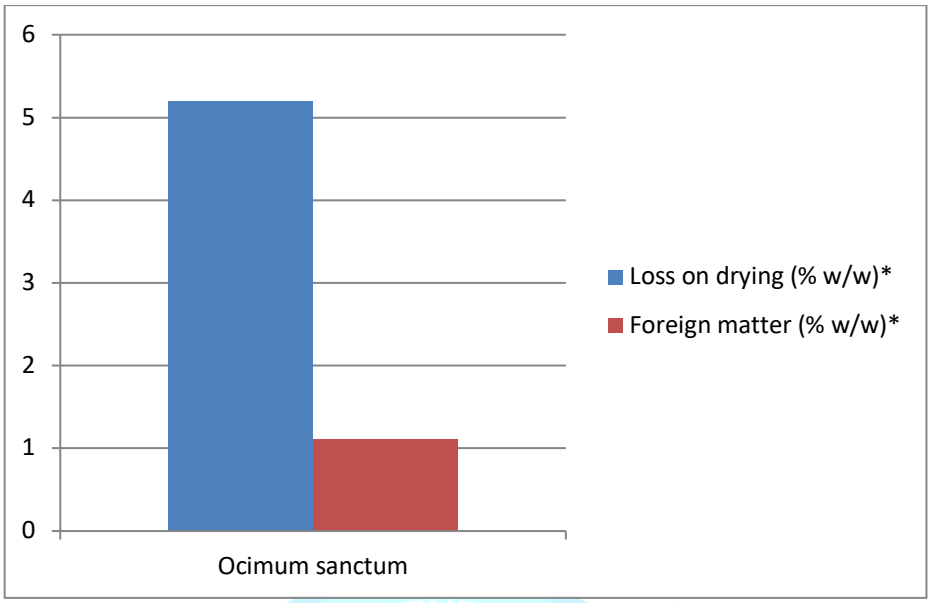

Fig 2: Loss on Drying And Foreign Organic Matter

3.5 Total Ash, Acid Insoluble Ash and Water Soluble Ash Values

Ash values are important for determining the purity and quality of crude plant extracts by measuring the amount of inorganic residue remaining after incineration. Total ash represents the total amount of mineral content, including both physiological and non-physiological ash. Acid-insoluble ash is the portion of total ash that remains undissolved in dilute hydrochloric acid, mainly indicating the presence of silica or sand contaminants. Water-soluble ash is the fraction of ash soluble in water, reflecting the presence of water-soluble minerals. These parameters help assess the cleanliness and authenticity of the *Ocimum sanctum* extract and ensure compliance with quality standards. Regular monitoring of ash values is therefore essential for maintaining consistency, safety, and efficacy in herbal drug development.

Table 5: Total Ash, Acid Insoluble Ash and Water Soluble Ash Values

Crude drugs	Total ash value % w/w	Water soluble ash % w/w	Acid insoluble ash value % w/w
Ocimum sanctum	10.4	4.7	2.3

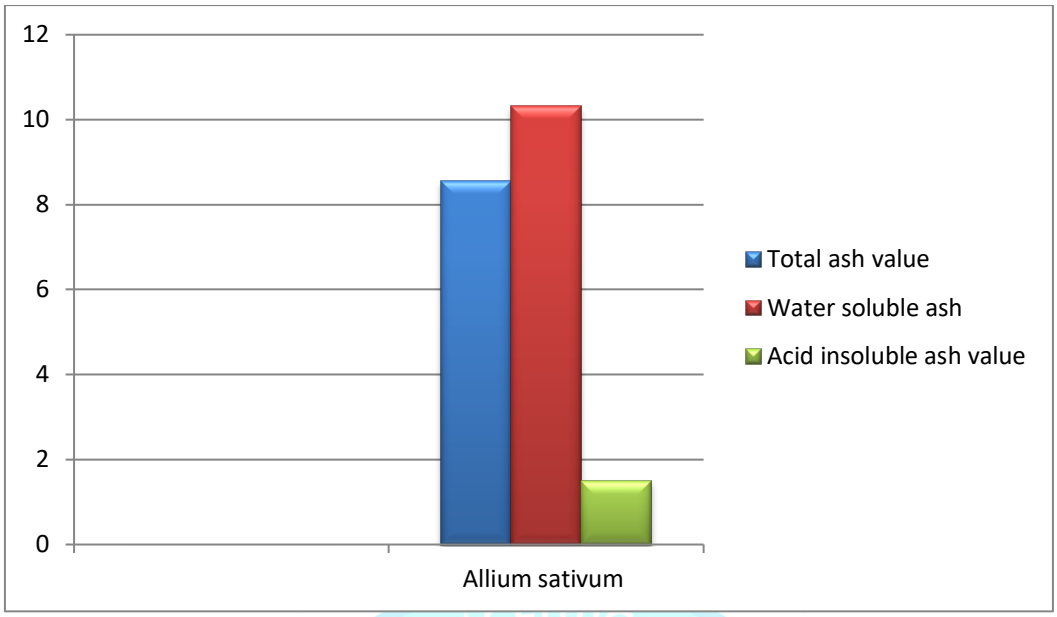

Fig 3: Total Ash, Acid Insoluble Ash and Water Soluble Ash Values

3.6 Phytochemical Screening

Phytochemical screening is a crucial step in the evaluation of medicinal plants, serving as a preliminary analysis to identify biologically active constituents in crude plant extracts. In the case of *Ocimum sanctum* (commonly known as Tulsi), the extract is systematically subjected to a battery of qualitative chemical tests to detect the presence of various phytoconstituents. These include alkaloids (tested using Mayer's, Dragendorff's, and Wagner's reagents), flavonoids (identified by the Shinoda and alkaline reagent tests), tannins (detected using lead acetate and ferric chloride solutions), and saponins (confirmed by the formation of persistent froth in the Foam test).

Glycosides are identified using the Keller-Killiani and Borntrager's tests, while phenolic compounds are revealed through the Ferric chloride and lead acetate reactions. The presence of steroids and terpenoids is determined using the Liebermann-Burchard and Salkowski tests. Proteins and amino acids are evaluated using the Biuret and Ninhydrin tests, whereas carbohydrates are confirmed using Molisch's, Benedict's, and Fehling's tests.

The detection of these diverse phytochemicals in *Ocimum sanctum* reflects its rich pharmacological profile and supports its wide-ranging therapeutic applications, including antioxidant, anti-inflammatory, antidiabetic, and antimicrobial activities. Additionally, the phytochemical profile aids in standardizing plant material for further pharmacological or formulation studies, contributing to quality control in herbal drug development.

Table 6: *Ocimum sanctum* extract were undergone for chemical test and results are shown in Table below:

S. No.	Chemical Tests	Methanol Extract	Water Extract
1.	**Tests for Steroids and Triterpenoids**		
	• Liebermann's Burchard Test	+	–
	• Salkowski Test	+	–
2.	**Test for Saponins**		
	• Foam Test	+	+
3.	**Tests for Alkaloids**		
	• Hager's Test	+	+
	• Mayer's Test	+	+
4.	**Tests for Glycosides**		
	• Borntrager's Test	–	–
	• Keller Killiani Test	+	+
5.	**Tests for Tannins and Phenolic Compounds**		
	• Gelatin Test	+	+
	• Ferric Chloride Test	+	+
	• Lead Acetate Test	+	+
	• Dilute Nitric Acid Test	+	+
6.	**Tests for Flavonoids**		
	• Ferric Chloride Test	+	+
	• Alkaline Reagent Test	+	+
	• Lead Acetate Test	+	+
7.	**Tests for Proteins**		
	• Biuret Test	–	+
	• Xanthoproteic Test	–	+
8.	**Test for Carbohydrates**		
	• Fehling Test	+	+

"+"Found

"-" Not Found

3.7 Evaluation of Tablets

The evaluation of *Ocimum sanctum* self-nanoemulsifying tablets involved the assessment of key physical and mechanical parameters to ensure quality, uniformity, and robustness. All formulations (F1–F5) exhibited acceptable physical appearance with uniform color, shape, and surface finish. Dimensional analysis showed consistent thickness, ranging from 3.2 ± 0.05 mm in F1 to 3.4 ± 0.02 mm in F5, and diameter remained constant across all batches, confirming uniform compression. The weight variation for all formulations was within pharmacopeial limits, with values ranging between 199.3 ± 1.25 mg (F1) and 203.4 ± 1.22 mg (F4), indicating batch uniformity.

Hardness values ranged from 4.4 ± 0.15 kg/cm² (F1) to 5.0 ± 0.13 kg/cm² (F5), reflecting good mechanical integrity, essential for handling and packaging. Friability was below the acceptable threshold of 1% for all formulations, with the lowest friability observed in F5 (0.50 ± 0.01%), indicating excellent resistance to mechanical stress. These results suggest that the tablets possess suitable hardness and durability without compromising friability. Collectively, these evaluations confirm the tablets' suitability for further studies and potential clinical application, ensuring reliable dosing and stability.

Table 7: Evaluation of Tablets

Formulation	Weight Variation (mg)	Hardness (kg/cm²)	Friability (%)	Thickness (mm)
F1	199.3 ± 1.25	4.4 ± 0.15	0.68 ± 0.03	3.2 ± 0.05
F2	200.5 ± 1.18	4.7 ± 0.10	0.63 ± 0.02	3.3 ± 0.04
F3	202.1 ± 1.10	4.7 ± 0.12	0.59 ± 0.02	3.3 ± 0.05
F4	203.4 ± 1.22	4.8 ± 0.14	0.54 ± 0.01	3.4 ± 0.03
F5	201.2 ± 1.15	5.0 ± 0.13	0.50 ± 0.01	3.4 ± 0.02

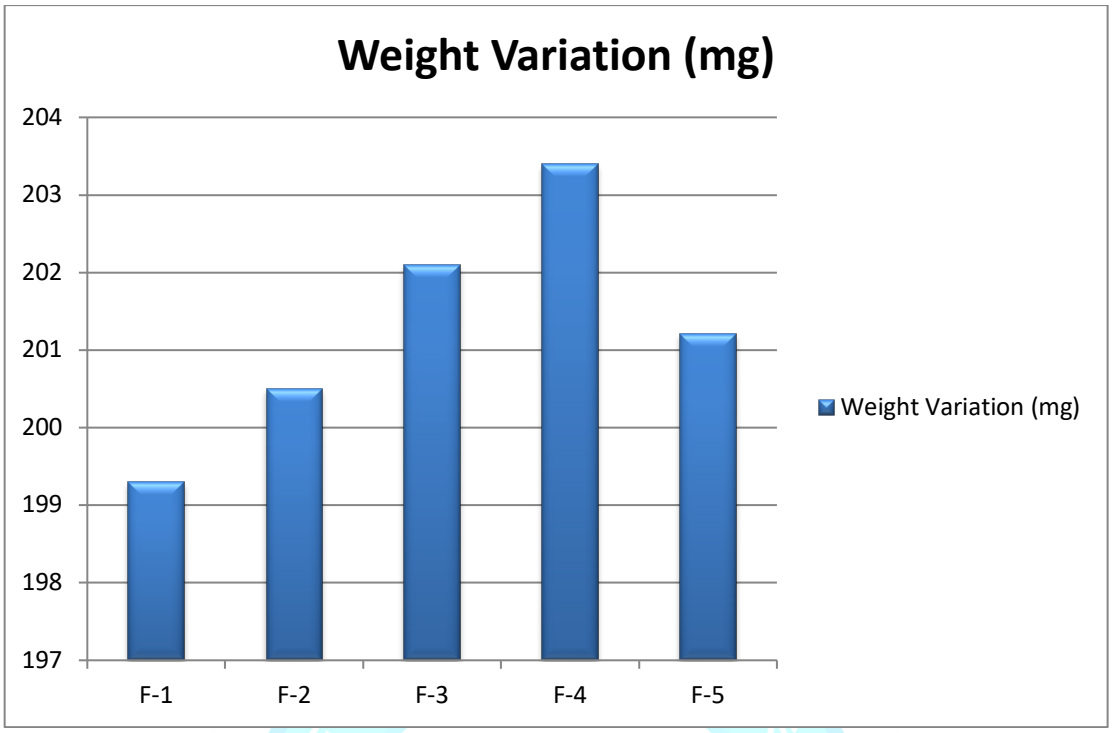

Fig 4: comparison of weight variation

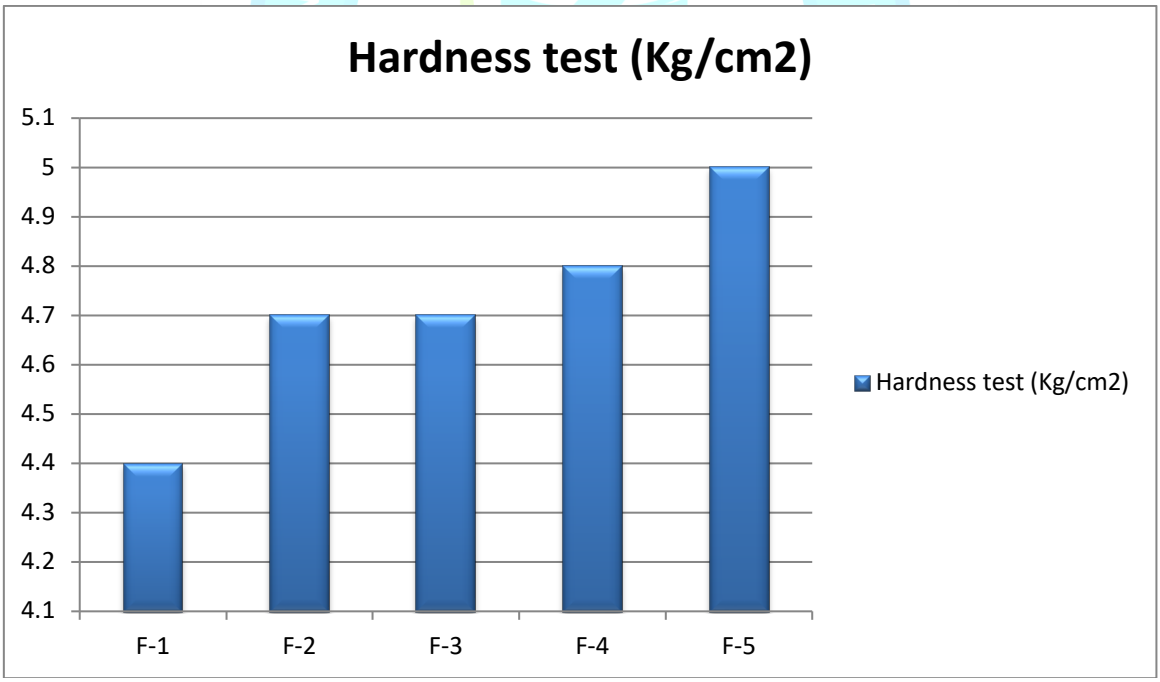

Fig 5: Comparison of hardness

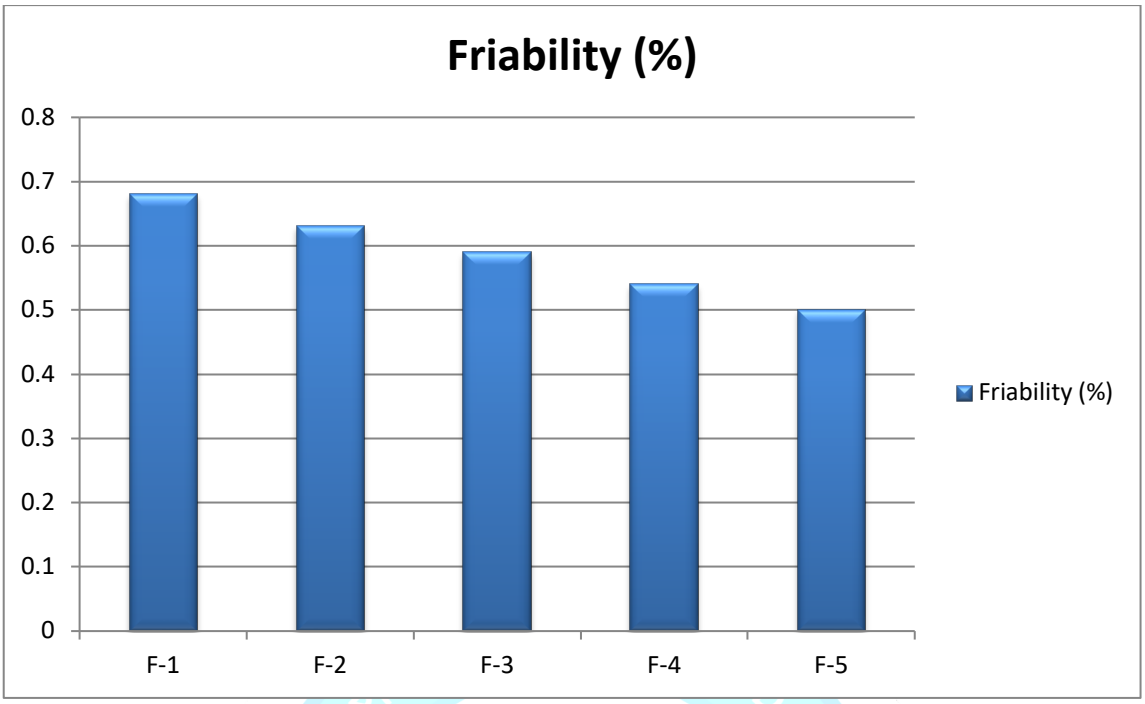

Fig 6: Comparison of friability

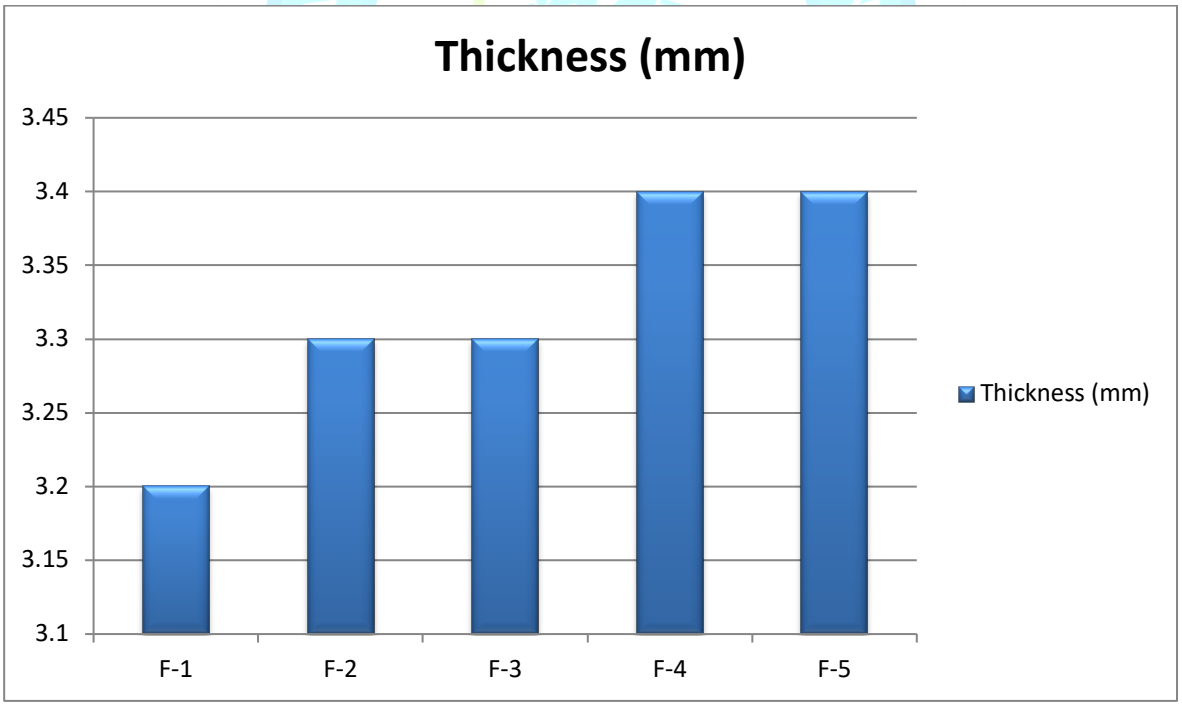

Fig 7: Comparison of Thickness

3.8 Dissolution Testing

Dissolution testing is performed using a USP Type II apparatus to evaluate the rate and extent of drug release. These evaluations collectively ensure that the formulated tablets meet pharmacopeial standards and deliver consistent therapeutic effects.

Table 8: Dissolution Testing

Formulation	Time (2 h)	Time (4 h)	Time (8 h)	Time (16 h)
F1	47.4 ± 1.8	64.9 ± 2.1	80.4 ± 2.5	91.9 ± 2.6
F2	55.0 ± 1.5	71.8 ± 1.9	86.2 ± 2.2	95.6 ± 2.3
F3	60.9 ± 1.3	77.0 ± 1.7	90.9 ± 1.9	98.3 ± 2.0
F4	65.8 ± 1.2	81.9 ± 1.5	94.6 ± 1.8	100.4 ± 1.9
F5	70.6 ± 1.1	87.1 ± 1.4	97.7 ± 1.6	101.1 ± 1.7

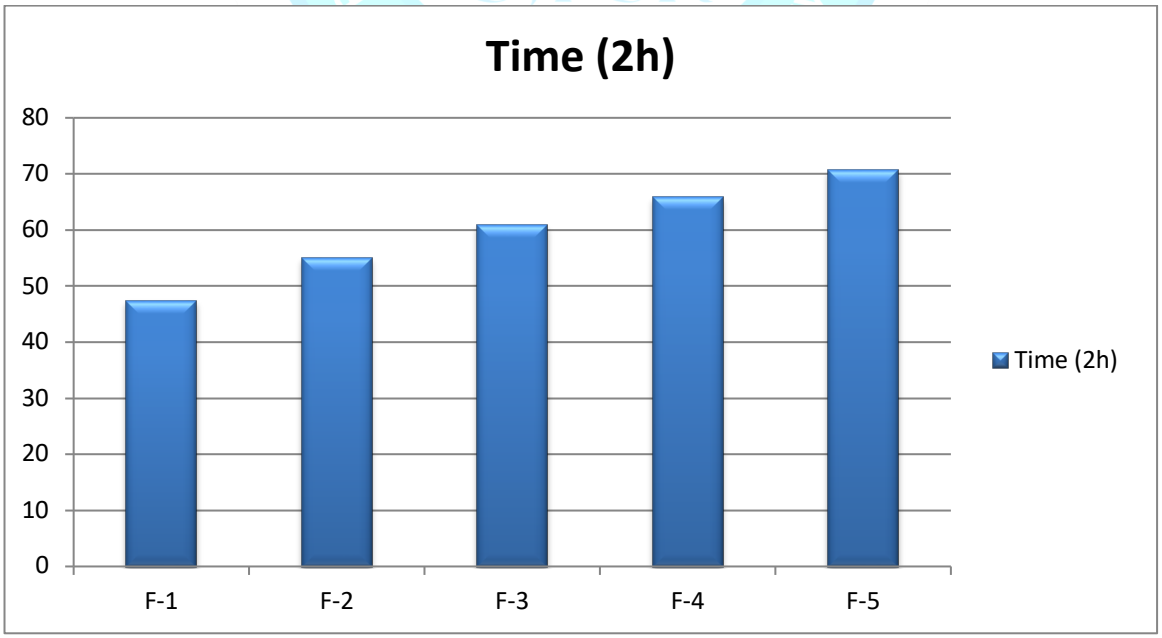

Fig 8: dissolution testing data

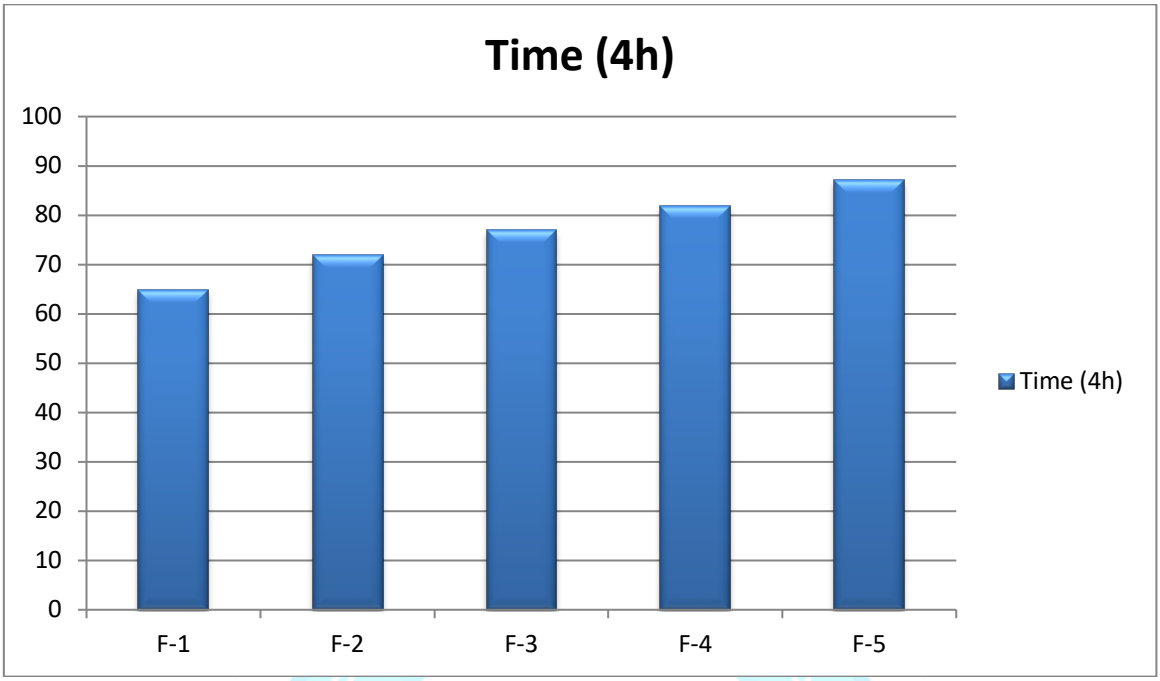

Fig 9: dissolution testing data

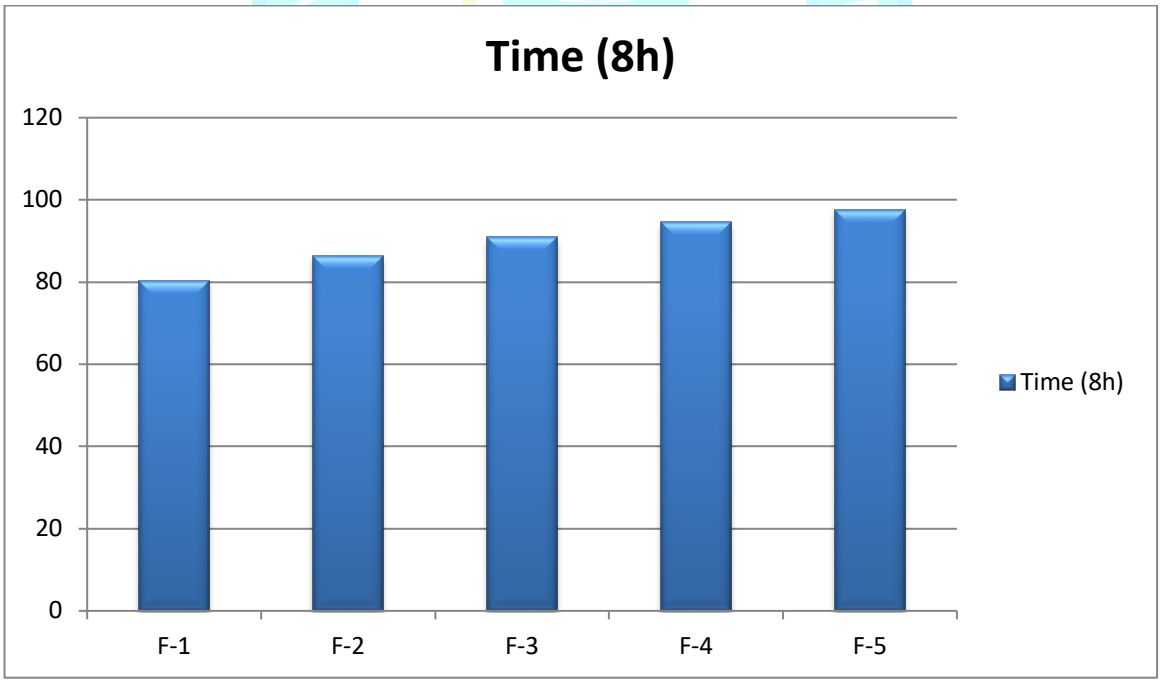

Fig 10: dissolution testing data

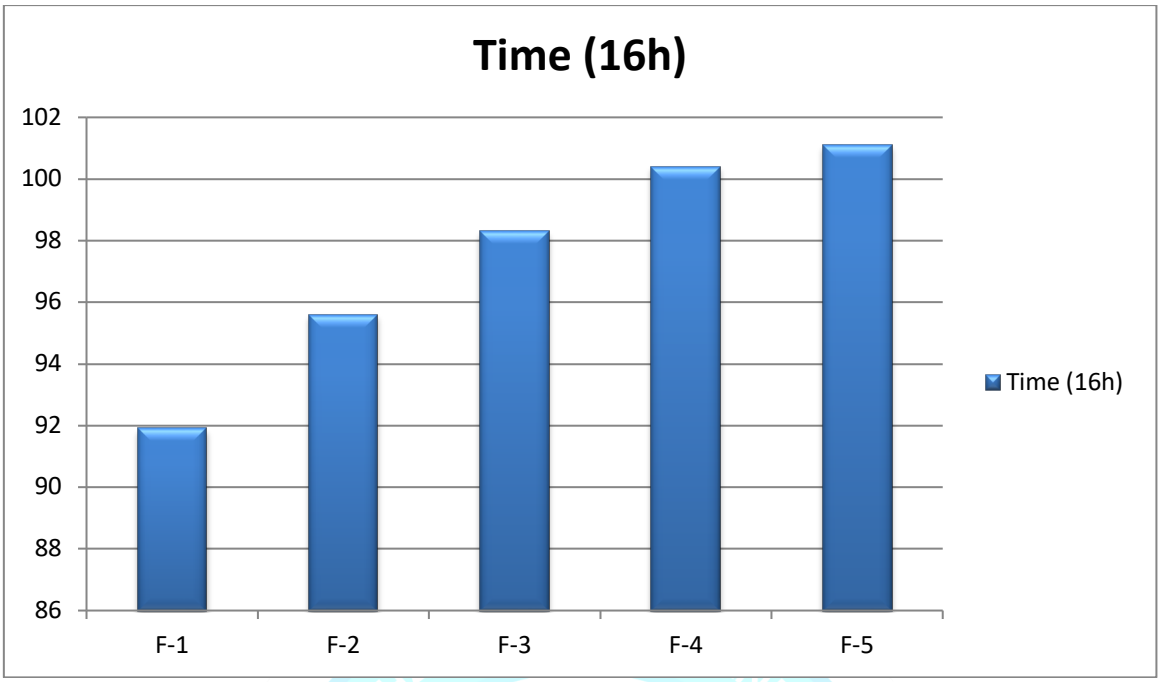

Fig 11: dissolution testing data

3.9 Open field apparatus test

The Open Field Apparatus Test is used to assess locomotor activity, exploration, and anxiety in rats. Each rat is placed in a marked arena, and its behavior is observed for 5–10 minutes. Key parameters recorded include line crossings, rearing, grooming, and time spent in the center versus periphery. Increased movement and central activity indicate reduced anxiety, while reduced activity suggests sedation. The apparatus is cleaned between trials to avoid scent bias. This test helps evaluate the neuropharmacological effects of *Ocimum sanctum* extract.

Table 9: Open field apparatus test

Group No.	Treatment	Dose (mg/kg)	Ambulation (N)	Activity in Centre (N)	Rearing (N)	Fecal Dropping (N)
1	Control	–	74.4 ± SD	16.9 ± SD	12.6 ± SD	3.3 ± SD
2	Diazepam	10	36.6 ± SD	7.6 ± SD	5.4 ± SD	1.2 ± SD
3	*O. sanctum* Extract	100	55.1 ± SD	10.8 ± SD	8.6 ± SD	2.2 ± SD
4	*O. sanctum* Extract	200	67.5 ± SD	14.7 ± SD	10.5 ± SD	2.2 ± SD

4. Summary

The present study successfully demonstrated the development and evaluation of self-nanoemulsifying tablets containing *Ocimum sanctum* extract as a promising approach to enhance its therapeutic efficacy and patient acceptability. The tablets exhibited desirable physical characteristics including uniform weight, acceptable hardness, low friability, and rapid disintegration. Phytochemical screening confirmed the presence of key bioactive constituents such as alkaloids, flavonoids, glycosides, saponins, and phenolics, supporting the pharmacological relevance of the formulation. The use of SNEDDS technology significantly improved the solubility and dissolution profile of the extract, potentially overcoming bioavailability challenges commonly associated with herbal drugs. Moreover, preliminary in vivo studies, such as the open field apparatus test, suggested neuropharmacological potential, aligning with the traditional use of *Ocimum sanctum* in stress and anxiety management. Overall, the findings support the application of modern pharmaceutical strategies like SNEDDS in herbal drug development to ensure consistent quality, efficacy, and enhanced therapeutic outcomes.

5. Acknowledgement

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6. Conflict Of Interest

No authors declared Conflict of Interest.

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