

**ADVANCED SELF-NANO EMULSIFYING DRUG DELIVERY
SYSTEM USING EXTRACT FROM ANNONA SQUAMOSA**

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ABSTRACT

Annona Squamosa L. (Annonaceae), a multipurpose, evergreen tree with commonly referred to the "custard apple" is its medicinal plants with extensive of Bio-active compound such bioactive compound found. This compound has demonstrated significant bioactivity. However, it's had bad bioavailability limits its effectiveness when used directly, so preparing SNEDDS are used for enhancing solubility of drug. This study was used *Annona Squamosa L.* leave extract with using ethanol from Soxhlet extractor then check the compatibility study between differen6 formulas from the D- optimal design software consisting of Corn oil, Tween 80, and Transcutol HP and prepared SNEDDS were characterized for transmittance, emulsification time, and phase separation. For verification one sample was t-test, then optimum SNEDDS formula was evaluated against particle size, PDI, zeta potential, stability studies. Optimized SNEDDS made up of 22.00% oil: 64.34%

surfactant: 13.66% co-surfactant that show desirability value 1.00. SNEDDS was produce

25.0 mg of *Annona Squamosa L.* leaf extract with particle size 172.9nm, poly-dispersion index 0.198, and zeta potential -19.6 mV. And then Compile all the data.

KEYWORDS: *Annona Squamosa L.* (Annonaceae), Self-Nano emulsifying, Formulation.

INTRODUCTION

1.1 *Annona Squamosa L.*

Natural treatments derived from medicinal plants have been shown to be both safe and effective. Many plant species have been employed in folklore medicine to cure a variety of conditions. Compounds derived from plants are still used extensively in basic health care as medicinal treatments in many underdeveloped nations. Plants are an excellent source of secondary metabolites with intriguing biological activity. In general, these secondary metabolites constitute a significant source with a wide range of structural and functional features. Flavonoids, phenols, phenolic glycosides, saponins, and cyanogenic glycosides are some notable examples of these chemicals. Natural products derived from microbial sources have traditionally been the primary source of antibiotics; however, with the growing recognition of herbal medicine as an alternative form of health care, screening medicinal plants for active compounds has become increasingly important because these may serve as sources of antibiotic prototypes. **Vanitha et al.**^[45]

This is a tropical endemic species, *Annona Squamosa L.* (Annonaceae), commonly called the "custard apple," which grows in the West Indies, South and Central America, Ecuador, Peru, Brazil, India, Mexico, and Egypt. The Indian Council of Agricultural Research (ICAR) reports that *Annona Squamosa* is widely grown throughout several Indian states, including Maharashtra, Gujarat, Madhya Pradesh, Chhattisgarh, Assam, Uttar Pradesh, Bihar, Rajasthan, Andhra Pradesh, and Tamil Nadu. *Annona Squamosa* fruit and leaf are shown in Figure 1.1 **Kumar et al.**^[32]

In the past years, many compounds have been reported and have gained organic chemist's and biochemist's attention due to their novel structure and wide range of bioactivity. Much research works prove that every part of *Annona Squamosa* possesses medicinal properties. Roots are used as internally in depression of spirits and spinal diseases. Bark has a strong astringent reputation. According to Ayurveda, fruits are a good tonic because they improve blood quality, can be used as an expectorant, strengthen muscles, cool down the body, reduce burning and biliousness, calm the heart, and stop vomiting. Mature fruit is used to make a

mixture that, when combined with salt, accelerates suppuration in malignant tumors. Dried, unripe fruit is ground into a powder and combined with gramme flour to create a suppurative environment. **Rajsekhar Saha.**^[38]

Annona Squamosa has been used as a natural medicine as well as in a variety of other food uses. For example, its pulp is used as a flavouring component in ice cream, and 50-80% of custard apple fruit is edible and may be pulped for juice. It includes a significant amount of vitamin C (35-42 mg per 100 g), as well as dietary Fiber, vitamin B1 (thiamine), and potassium. **Zahid et al.**^[35]

Phytochemical studies have shown that custard apple contains a high amount of phenol-based compounds, for example, proanthocyanidins, with 18 different phenolic compounds mainly consisting of alkaloids or flavonoids. Apart from fruit, during pruning, large amounts of leaves are generated and pose serious complications related to their disposal for farmers. *Annona Squamosa* leaves offer value addition potentiality as they possess broad pharmacological properties and biological activities such as antioxidant, antimicrobial, antidiabetic, antiviral, anticancer, and hepatoprotective activities. **Kumar et al.**^[32]

Extracts from various portions of the *Annona Squamosa* plant, such as its bark, roots, leaves, stem, fruit, peel, and seeds, have been used in traditional pharmaceutical uses in numerous nations. **Anaya-Esparza et al.**^[3]

Annona Squamosa leaves show antioxidant, anthelmintic, hepatoprotective, antibacterial, wound healing, anti-arthritic, anti-inflammatory, analgesic, antimicrobial, hypoglycaemic and anti-nociceptive activities. **Kumar et al.**^[32] This section was chosen for the study because of the leaves' special ability to treat a variety of illnesses. The current study's goal was to identify the primary phytonutrients that support a range of biological processes as well as the physical, chemical, and phytochemical characteristics of *Annona Squamosa* leaves. **Varadharajan et al.**^[44]



(A)



(B)

Fig 1.1 *Annona Squamosa* L. (A) Fruit, (B) Leaf.

1.2 Botanical Description

ASL is an evergreen tree that grows to a height of 3–8 meters. Leaf oblong lanceolate or lanceolate, 6–17 cm long and 3–5 cm wide, alternately arranged on short petioles; bark thin, gray; flower greenish, fleshy, drooping, extra-axillary, more on leafy shoot than on older wood and tending to open as the shoot elongates; fruit round, heart-shaped, ovate, or conical, 5–10 cm in diameter, with many round protuberances; seeds 1.3–1.6 cm long, oblong, smooth, shiny, blackish, or dark brown. Flowers are solitary or in groups of 2–4 and grow on short, woody stalks. Each bloom measures around 2.5–4 cm in diameter and has three fleshy, greenish-yellow petals that are mildly aromatic. **Chengyao Ma et al.**^[11]

1.3 Phytochemical Compounds and their Pharmacological Activities

Annona Squamosa leaves are a rich source of various bioactive phytochemicals that contribute to their wide range of pharmacological activities. The primary classes of phytochemicals found in *Annona Squamosa* include alkaloids, flavonoids, tannins, and essential oils. Below, we'll delve into the specific phytochemicals and their associated pharmacological activities. (Table 1.1) **Nugrahaet al.**^[7]

Table 1.1: Phytochemical Compounds and their Pharmacological Activities in *Annona Squamosa* leaves Extract.

Sr No.	Phytochemical Compounds	Pharmacological Activities
1.	Alkaloids	
1a.	Liriodenine	Antimicrobial, Anti-plasmodial, Anticancer
1b.	Anonaine	Antitumor, Antifungal, Antimicrobial
1c.	Norcorydine	Antimalarial, Cytotoxic
2.	Flavonoids	
2a.	Kaempferol	Anti-inflammatory, Anticancer, Neuroprotective,
2b.	Rutin	Anti-inflammatory, Anticancer Cardioprotective
2c.	Quercetin	Antioxidant, Anti-inflammatory, Antiviral, Anticancer
3.	Essential Oils	
3a.	Caryophyllene	Anti-inflammatory, Analgesic, Anticancer
3b.	Pinene	Antimicrobial, Anti-inflammatory, Bronchodilator
3c.	Linalool	Anxiolytic, Sedative, Antimicrobial, Anti-inflammatory
4.	Tannins	
4a.	Ellagic Acid	Antioxidant, Anticancer, Antiviral,
4b.	Gallic Acid	Antioxidant, Anticancer, Antimicrobial,

1.4 Self-nanoemulsifying drug delivery systems (SNEDDS)

Emulsions created from nanoscale droplets that have dimensions less than 300 nm, often between 20 and 200 nm, are known as nano emulsions. Transparent, there is an uneven thermodynamic difference between the two liquids. These systems exhibit kinetic stability, as destabilization kinetics are extremely slow, often taking many months to materialize. Approximately four years ago, it was revealed that they could boost bioavailability. Brownian motion, droplet deformation, nano emulsions, and low gravity are all referred to as "nearly thermodynamically stable" since the droplets exhibit strong resistance to emulsification or solution, agglomeration, and coalescence. **Verma et al.**^[5]

A drug delivery system for self-nano emulsification was created by combining a mixture of neutral triglycerides with non-ionic surfactants that are important for oral medication administration. Self-nanoemulsifying drug delivery systems (SNEDDS) are isotropic mixtures of active pharmaceutical substances in natural or manufactured lipids or oils, surfactants, and water-soluble cosolvents or cosurfactants. **Anuja et al.**^[18] Increase the oral bioavailability of medicines with low solubility. SEDDS are often used. The nano emulsion of SEDDS is known as SNEDDS. A typical droplet size of 20–200 nm has these emulsions, which are composed of two immiscible liquid dispersions, either o/w or w/o. **Verma et al.**^[5] Mechanism of action of SNEDDS Show in Figure 1.2

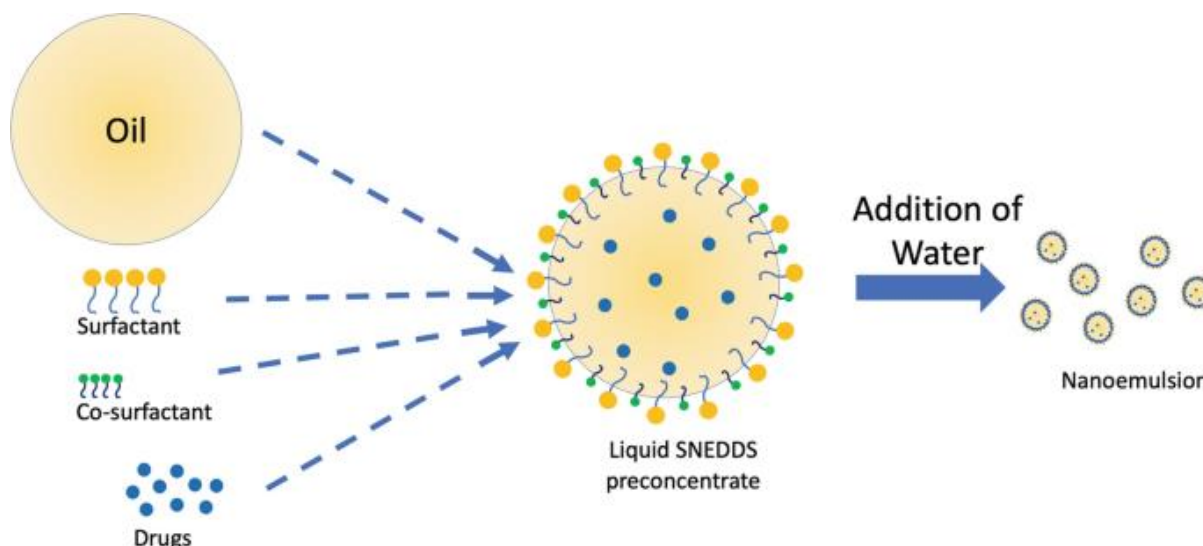


Figure 1.2: Representation of self-emulsifying drug delivery system.

1.5 Important aspects for selection of SNEDDS

- Droplet size of Nano emulsion
- Self-emulsification time
- Self-emulsifying region in the phase diagram
- Thermodynamic stability
- Solubility of drug in the excipients **Reddy and Sravani.**^[30]

1.6 Properties of SNEDDS

- Able to self-emulsify quickly in GI fluids and make fine form in oil-in-water emulsion with gentle agitation from peristaltic movements.
- can effectively integrate hydrophobic or hydrophilic drugs into oil surfactant mixtures.
- Both solid and liquid forms are used as dosage forms and require a lower medicine dose compared to traditional dosing forms. **Verma et al.**^[5]

1.7 Advantages

- No effect on digestion process of lipid.
- SNEDDS can protect drugs from degradation in the gastrointestinal tract.
- enhancing the stability of sensitive compounds
- it can be designed to provide controlled and sustained release of the drug, leading to improved therapeutic efficacy and patient compliance.
- Enhanced drug loading capacity with SNEDDS. **Anuja et al.**^[18]

1.8 Limitations

- **Limited Drug Loading:** SNEDDS may have a limited capacity to load drugs, especially hydrophilic drugs.
- **Stability Issues:** The physical and chemical stability of the formulation can be challenging to maintain over time.
- **Irritation Potential:** Some surfactants in SNEDDS can result in gastrointestinal irritation or even be toxic. **Anuja et al.**^[18]

1.9 Formulations Components

SNEDDS mainly present drugs, oil, surfactant, and co-surfactant. These excipients are mainly selected based on compatibility and solubility between drug and oil, surfactant, and co-surfactant. **Verma et al.**^[5] **Reddy and Sravani.**^[30]

1.9.1 Drug (Active pharmaceutical ingredient)

As per the Biopharmaceutical Classification System (BCS), drugs that are not very soluble in water will be chosen for the SNEDDS. It offers a potential platform for enhancing the overall bioavailability of drugs, particularly those belonging to BCS class II and BCS class IV. The log p value should be greater than 4, and the melting point should be lower. **Reddy and Sravani.**^[30]

1.9.2 Oil

The choice of the proper lipid moiety is so critical because it will influence the choice of other choice of the appropriate lipid moiety is crucial since it influences the selection of other nano emulsion components. Selection of the oil phase is based on the drug's solubility in the oil and its ability to form nano emulsions. Generally, the oil helps in achieving maximum drug concentration in nano emulsions. Triglycerides are naturally occurring fatty acids found in oils and fats. **Anuja et al.**^[18]

The concentration of oil in SNEDDS formulations varies according to the formulation and Drug being given. The concentration of oil in SNEDDS typically ranges from 20% to 60% of the overall all formulation. **Reddy and Sravani.**^[30]

Table 1.2: Example of oils.

Verity of Oil	Some Example
Medium chain triglycerides	Castor oil, Corn oil, Soybean oil, Olive oil,
Fatty acid esters	Ethyl oleate, Capryol 90
Long chain triglycerides	Capmul MCM, Capmul MCM, Akoline, Invitor 742
Glyceryl monolinoleate	Capmul GMO
Others	Solutol HS 15

1.9.3 Surfactants

In the SNEDDS formula, surfactants play a major role in solubilizing poor water-soluble drugs. The surfactant component that reduces surface and interface tension spontaneously undergoes bond formation. When the oil concentration is high, surfactants join the oil-water boundary, forming an emulsion in which the drug can dissolve in the lipid portion. **Anuja et al.**^[18]

Examples of Surfactants

- Cremophor RH 40
- Cremophor EL
- Plurololeique
- TWEENS and SPANS

1.9.4 Co-Surfactants

Co-surfactant or compound an agent added along with surfactant to improve the solubility ability of surfactant and reduce interfacial tension. Combination of surfactant will increase flexibility and effectiveness; selection of a proper post-surfactant will enhance the solubility of the drug and promote the design of the SNEDDS formulation. Which increases drug loading, decreasing surface or interface tension and reducing droplet size. **Reddy and Sravani.**^[30]

Examples of Co-Surfactants

- Ethanol
- Propylene glycol (PG)
- PEG-400
- Poloxomer 188

DRUG PROFILE

Annona Squamosahttps://en.wikipedia.org/wiki/Annona_squamosa assessed on 2024 March 12/10:25^[20]

- ❖ Scientific Name: *Annona Squamosa* L.
- ❖ Synonyms: *Annona asiatica* (L.), *Guanabanussquamosus* (L.).



Fig 6.3: *Annona Squamosa* Plant.

Taxonomical Classification

- Kingdom : Plantae
 - Order : Magnoliales
 - Family : Annonaceae
 - Species : *A. squamosa*
 - Genus : *Annona*
- Binomial Name** : *Annona Squamosa*

Common Names

- Hindi : Sharifa or Sitaphal
- English : Sugar apple, Custard apple
- Sanskrit : Krishnagaru

Habitat

Widely distributed in tropical and subtropical regions. Abundant in the eastern regions of India.

Morphology

A. Squamosa Linn. (Annonaceae) is a small, semi-deciduous plant, 3–7 meters in height, with a broad and open crown or inappropriately spreading branches. Widely accessible in India.

Chemical Constituents (Leaves)

Phytochemicals

Like linalool, borneol, eugenol, farnesol, geraniol tannins, phenolic compounds, polyphenols, annotemoyin-1, and -2-squamon, cholesteryl, glucopyranoside, and flavonoids.

Essential oils

Like for α -pinene, caryophyllene, and carvone, couch with diacetyl germacrene D β -elemenesabinenesizesbicyclogerm in all eight T-cadinol and t-murolols.

Other compounds glycosides, alkaloids, saponins, carbohydrates proteins, phytosterols, amino acids.

Parts of the Plant Used: Leaves

Uses: used as antioxidants, anti-inflammatory agents, and antimicrobials, Pain Relief, Fever Reduction, Anti-tumor, Anti-diabetic, Anti-ulcer, Insect Repellent, Wound Healing, Respiratory Aid, Skin Treatment, etc.

MATERIALS AND METHODS

7.1 PROCUREMENTS OF EXCIPIENTS

The corn oil was purchased Deve Herbes, New Delhi, the olive oil from Deoleo India Pvt. Ltd., Mumbai, and V. Coconut oil from Apex Coco & Solar Energy Limited, Tamil Nadu, Solutol HS 15, Cremophor RH 15, Propylene Glycol, and Polyethylene Glycol-400 were given as gift samples from BASF Pharmaceutical India, and Tween 80 was given by Goel Institute of Pharmacy and Sciences.

All Required Glassware's and Apparatus were provided by Goel institute of Pharmacy and sciences, Lucknow.

Table 7.1: Procurements of Excipients.

Sr. No.	EXCIPIENTS	Manufacturer/Supplier	
1.	Corn Oil	Deve Herbes, New Delhi	Purchased
2.	Olive oil	Deoleo India PVT. LTD, Mumbai	Purchased
3.	V. Coconut oil	Apex Coco & Solar Energy Limited, Tamil Nadu	Purchased

4.	Solutol HS 15	BASF India Limited, Navi Mumbai	Gift sample
5.	Tween 80	Goel institute of Pharmacy and sciences, Lucknow	
6.	Cremophor RH 15	BASF India Limited, Navi Mumbai	Gift sample
7.	Propylene Glycol	BASF India Limited, Navi Mumbai	Gift sample
8.	Polyethylene Glycol-400	BASF India Limited, Navi Mumbai	Gift sample
9.	Transcutol HP	Alpha Chemika, Mumbai	Purchased

7.2 PREFORMULATION STUDIES

7.2.1 Plant Collection and Preparation of Powder

Newly fresh leaves of the *Annona Squamosa L.* plant were gathered locally in Lucknow, U.P., India, during the month of January. The collected leaves were dried in the shade at room temperature, using a mechanical grinder to make fine powder and a sieve of 40 mesh, and then preserved in an airtight container.

7.2.2 Identification and Authentication of the plant material

Dr. Lal Babu Chaudhary, NBRI-CSIR, Lucknow, Uttar Pradesh, India, identified and verified the plant.

7.2.3 Extract Preparation

Conventional Soxhlet extraction was employed to prepare an ethanol extract. 50 grams of powdered *A. squamosa* leaves were extracted with 200 millilitres of ethanol at a temperature of 65 °C for a duration of 3 days. After the extraction process was completed, the filtered extract was concentrated by distilling off the solvents using a water bath. And then calculating the yield. Joyti *et al.*^[23]

$$\text{Yield (\%)} = \left(\frac{\text{Weight of Plant Material (g)}}{\text{Initial Weight of Extracted Material (g)}} \right) \times 100$$

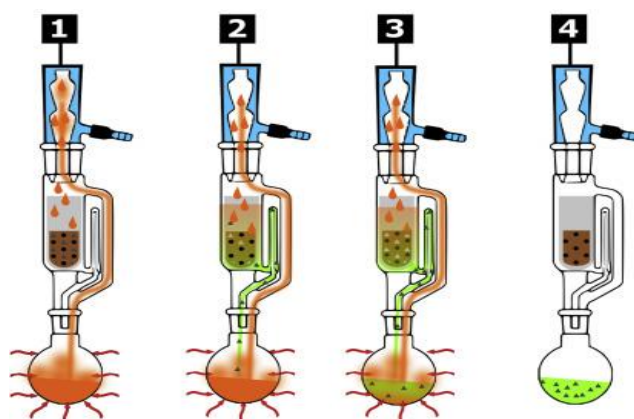


Fig 7.1: Soxhlet Extraction Process.

7.2.4 Phytochemical Screening of Extract

7.2.4.1 Test for Phenols

A small amount of the ethanolic extract was taken with 1 ml of water in a test tube and 1 to 2 drops of ferric chloride (FeCl₃) was added. Formation of green color indicated the presence of phenols. Chandraiah G.^[10] Joyti et al.^[23] Varadharajan et al.^[44]

7.2.4.2 Test for Flavonoids

In excess amount of the extract, 1 ml of a 1 percent ammonia solution was added. If yellow colour appearance that indicate the presence of flavonoids. Joyti et al.^[23]

7.2.4.3 Test for Tannins

In excessive amount of plant extract and take 2 ml of 5% ferric chloride were added. Then of the formation of greenish black indicated the presence of tannins. Varadharajan et al.^[44]

7.2.4.4 Test for Saponins

2 ml of crude extract was mixed with 5 ml of distilled water in a test tube, and it was shaken vigorously. Add some drops of olive oil. Indication of the presence of saponins when the Formation of a 1-cm layer of Form. Joyti et al.^[23], Varadharajan et al.^[44]

7.2.4.5 Test for Alkaloids Mayer's test

Approximately 1 ml of crude extract was mixed with 2 ml of Wagner's reagent. If the reddish-brown precipitate indicates it means the presence of alkaloids. Joyti et al.^[23]

7.2.5 Functional group analysis by FT-IR: To know the functional group in extraction of Custard Apple i.e. *Annona Squamosa* Leave. They identify the functional group and their corresponding bio active compound. The FTIR spectra of Extract employee FTIR spectrometers (PerkinElmer Spectrum Version 10.03.06), CDRI Lucknow, U.P. India.

7.2.6 Solubility Study of The Extract

1 mg of extract was taken into every Eppendorf tube containing different components: oil (corn oil, olive oil, and virgin coconut oil), surfactants (Tween 80, Solutol HS15, and Cremophor RH40) at a ratio of 1:1 by weight, and co-surfactants (Transcutol HP), PEG-400, and Propylene Glycol). The mixture was vortexed on a Vortex mixer at 2.0 min, incubated in a water bath of 45 ± 1 °C for 10 minutes, sonicated at 15 min, and allowed to stand at room temperature for two days. The separating, non-dissolvable part was accumulated by centrifugation at 3000 rpm for 20 min. The visual selection of components, which were able

to dissolve a relatively larger amount of extract, was optimized. **Sulkhan et al.**^[1], **Ermawati et al.**^[16], **Pratiwi et al.**^[28]

7.2.7 Compatibility Study of Drug & Excipient

Based On FT-IR spectra analysis, Draw the conclusion about the compatibility of the crude drug with excipients. The interaction of crude drug and excipient is measured visually and by FTIR spectroscopy. In which mixer [1:1] of crude drug and other excipients like the oil surfactant and co-surfactant. The FTIR spectra of all sample employee FTIR spectrometers (PerkinElmer Spectrum Version 10.03.06), CDRI Lucknow, U.P. India.

7.3 FORMULATION STUDIES

7.3.1 SNEDDS Formula with D-Optimal Mixture Design

Three independent variables were studied: oil (A), surfactant (B), and co-surfactant (C) against three response variables: transmittance (%), emulsification time (second), and phase separation using D-Optimal mixture design. Oil, surfactant, and co-surfactant that showed the maximum solubility of the extract from the previous study were chosen as independent variables. Table 7.2 shows the independent variables, and the levels selected for optimization. **Sulkhan et al.**^[1]

Table 7.2: Levels of Components Used in SNEDDS formulation.

Code	Selected Components	Low	High
A	Oil	12.00%	22.00%
B	Surfactant	60.00%	70.00%
C	Co-Surfactant	12.00%	22.00%

7.3.2 Prepared SNEDDS and Drug Loading

The formulation was optimized using Design Expert 23 Trial software and obtained 16 formulations with oil phase (corn oil), surfactant (Tween 80), and co-surfactant (Transcutol HP) and added ASL extract (100mg, 50mg, 25mg) was added in 1.0 gm of SNEDDS then homogenized with a vortex for 2.0 minutes, sonicated for 15 minutes, and incubated at 45 ± 1 °C for 15 minutes in a water bath. **Artanti et al.**^[4]

7.4 CHARACTERIZATION AND EVALUATION OF SNEDDS

7.4.1 Transmittance value (% T)

A 1.0-ml SNEDDS was added with aquabides until the volume reached 50 ml. The mixture was homogenized with a vortex for 1.0 minute. The percentage of transmittance was then analyzed by a UV-Vis spectrophotometer at 650 nm. **Sulkhan et al.**^[1] **Artanti et al.**^[4]

7.4.2 Emulsification Time

The emulsification time was done on SNEDDS ASL extract in an artificial gastric fluid (AGF) medium. Each 1-liter AGF medium contains 2 grams of NaCl and 7 ml of 37% HCl with a pH value of 1.5. Then, 50 ml of AGF media was conditioned at body temperature 37 °C above the magnetic stirrer at 100 rpm. SNEDDS is dripped as much as 200µL into the medium-fast. Visual monitoring was done until it became a nano-emulsion. It is then done by looking at the AGF media solution's transparency. **Artanti et al.**^[4]

7.4.3 Phase Separation

SNEDDS was added into a microcentrifuge tube of 1.5 ml, then centrifuged at 3000 rpm for 3×10 minutes. **Restu et al.**^[46] After that, the phase separation was calculated based on what was visually observed by comparing the height of the emulsion that remained stable with the total height of the emulsion. **Sulkhan et al.**^[1]

7.4.4 Determination of Optimum Formula of SNEDDS and Verification

The stylish expression results are also optimized using the D-optimal system in the state Ease Expert 23 trial design operation with sludge Corn oil, tween 80, Transcutol HP with transmittance value, separation phase, and emulsification time. **Sulkhan et al.**^[1] The optimal expression of SNEDDS ASL leaves attained from optimization using the d-optimal system was tested for its physical stability based on transmittance response, emulsification time in artificial gastric fluid (ASL), and phase separation. The optimum expression of vaticination was also validated by performing three SNEDDS phrasings using a single sample t-test. However, it means it is valid, and a p-value is set up to be If the verification results aren't significantly different from the prognosticated results. **Artanti et al.**^[4]

7.4.5 Particle Size and Zeta Potential

SNEDDS formulation 1000 times, dilute with water [1:1000]. 1 ml of the SNEDDS formula was diluted with 1000 ml of water, then homogenized with a vortex for 1.0 minute, and 3.0

ml of the sample was evaluated against particle size, polydispersity index, and zeta potential using a particle size analyzer in NIPER, Raebareli, Lucknow, U.P **Reddy and Sravani**.^[30]

7.4.6 Data Analysis

Using Design Expert 23 Trial software, the SNEEDS formula is determined by analyzing the effects of co-surfactant, oil, and surfactant. The outcome was found to be statistically significant, and a one-sample t-test was used to confirm the p-value. The ideal composition was found using the D-Optimal method, and the predicted and observed values were compared. **Artanti et al.**^[4]

CHAPTER 8

RESULTS AND DISCUSSION

8.1 PRE-FORMULATION STUDY

Studying pre-formulation characteristics is essential for developing pharmaceutical formulations that are reliable, safe, stable, and effective. This process involves detailed analysis and characterization of the *Annona Squamosa* leaf extract to gather valuable information necessary for creating a stable and bioavailable dosage form. By understanding the solubility and compatibility with excipients.

8.1.1 Extract Preparation

The purpose of the extract is to receive an *Annona squamosa* leaf extract used as an SNEDDS active compound. The initial stage is the sampling of Anonna Squamosa leaves in the local area of Lucknow, U.P., in January. Using conventional Soxhlet extraction and an ethanol solvent of 200 ml, After the extraction process was completed, the filtered extract was concentrated by distilling off the solvents using a water bath. The total yield of *Annona Squamosa* leave extract is 17.0%, or 8.5 g.

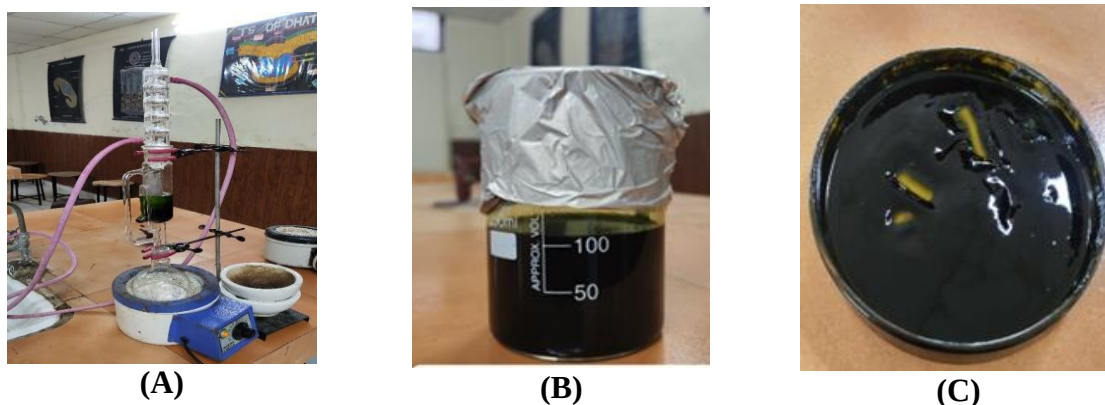


Fig 8.1: (A) Soxhlet Extraction Assembly (B) After extraction (C) After concentration.

Table 8.1: Percent extractability of ethanolic extract of *Annona Squamosa* leaves.

Plant	<i>Annona Squamosa</i>
Part of the plant extracted	Leaves
Solvent for extraction	Ethanol
Method of extraction	Soxhlet
Weight of the plant material (g) before extraction	50 g
Weight of extract (g)	8.5 g
Percent extractability	17.00%

8.1.2 Phytochemical Screening of Extract

The ethanolic leaf extract of *Annona Squamosa* was analyzed using specific reactions, revealing the presence of alkaloids, phenols, flavonoids, tannins, and saponins. Phytochemical screening confirmed the presence of alkaloids, phenols, and tannins, with phenols, flavonoids, and tannins notably abundant in the extract, highlighting their significant presence in *Annona Squamosa* leaves, which may be responsible for the anticancer, antidiabetic, and antimicrobial antioxidant activity.

Table 8.2: Phytochemical Analysis of ethanolic extract of ASL.

S. No.	Phytochemical	Test	Result
1	Phenols	FeCl ₃ Test	+
2	Flavonoids	Shinoda Test	+
3	Tannins	FeCl ₃ Test	+
4	Saponins	Foam Test	-
5	Alkaloids	Mayer's Test	+

Keywords: Present: +, Absent: -

8.1.3 Functional group analysis by FT-IR

To identify the Functional group in the *Annona Squamosa* leaf extract from FTIR data. We need the match observation peaks (Wavelength in cm^{-1}), with known characteristic absorption bands of functional group and their corresponding bioactive compound.

Table 8.3: FT-IR Analysis of *Annona Squamosa* Leave Extract.

Reference Range (cm^{-1})	Observed Peak (cm^{-1})	Bond Type	Functional Group	Bioactive Compounds
3200-3600	3921.48, 3777.36, 3697.41, 3398.60	O-H Stretching	Alcohols, Phenols	Phenolic Compounds, Flavonoids
2850-3000	2923.50, 2856.77	C-H Stretching	Alkanes	Essential Oils, Terpenoids
2100-2260	2370.96, 2281.67	C $\equiv$ C Stretching	Alkynes	Acetogenins

1680-1750	1735.17	C=O Stretching	Carbonyl Compounds (Ketones, Aldehydes, Esters)	Flavonoids, Tannins
1600-1680	1617.69	C=C Stretching	Alkenes, Aromatic Rings	Flavonoids
1580-1650	1511.47	N-H Bending	Amines	Alkaloids, Amino Acids

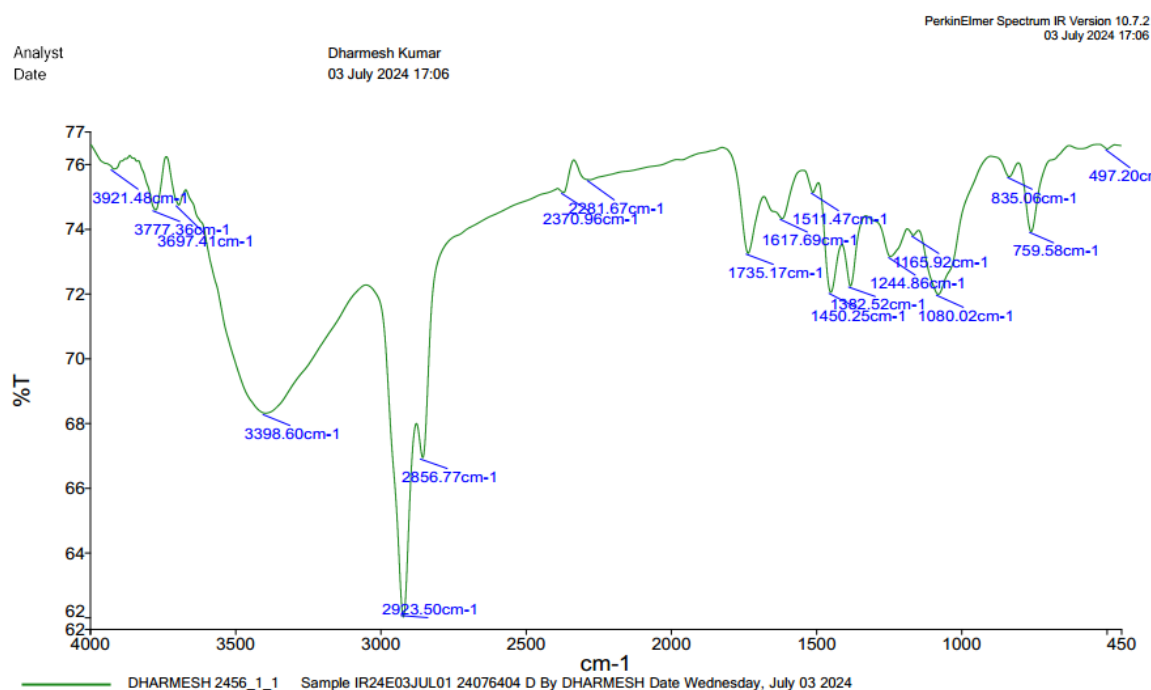


Fig8.2: FT-IR Graph of *Annona Squamosa* Leave Extract.

8.1.4 Solubility Study of The Extract

The selection of the right component is an important prerequisite for the formulation of stable SNEDDS. The drug should have good solubility in each component so that the precipitation of the drug during the shelf life of the formulation and after dilution in the GI lumen can be avoided.

Venkatesh and Mallesh^[34]

The oil phase has Most importance in the formulation of SNEDDS. Solubility of extract in the oil phase is an essential basis for the selection of oil. In selection of oily phase for the formulation of SNEDDS the oil, has maximum solubilizing potential for the selected drug candidate. This helps to achieve maximal drug loading in the SNEDDS. The solubility of the extract in various oils was determined. The solubility of *Annona Squamosa* leaf extract is freely soluble in corn oil then v. coconut oil and slightly soluble in olive oil. The extent to

which it demolishes highly soluble in corn oil and wear is chosen as the oil phase for further formulation. After the selection of the oil phase, the target was identified as the surfactant having a highly soluble ability for oils and extracts. The choice of surfactant was also critical for the formulation of SNEDDS. Surfactants have a great influence on the nano-emulsification process, the self-nano-emulsification region, and the droplet size of the nano-emulsion. Extract was most soluble in Tween 80, then in Solutol HS 15, and slightly soluble in Cremophor RH 40. So, after all visual inspection, Tween 80 is chosen as a surfactant in future formulations. Solubilized the ASL extract in propylene glycol, PEG-400, and Transcutol HP. Then the result of the extract that is highly soluble in Transcutol HP, then propylene glycol, and slightly soluble is PEG-400. So Transcutol HP is finalized as a co-surfactant that helps to improve the drug loading and modulate the self-nano emulsification time of the SNEDDS.

Table 8.4: Solubility of ASL Extract in different oils, Surfactant and Co-Surfactant.

Phase Type	Excipients	Solubility
Oils	Corn Oil	Highly Soluble
	Olive Oil	Less Soluble
	Virgin Coconut Oil	Less Soluble
Surfactant	Tween 80	Highly Soluble
	Solutol HS 15	Less Soluble
	Cremophor RH 40	Less Soluble
Co-Surfactant	Transcutol HP	Highly Soluble
	PG	Less Soluble
	PEG 400	Less Soluble

8.1.5 Compatibility Study of Drug & Excipient

To determine the compatibility of excipient (corn oil, Tween 80, Transcutol HP) with crude drug *Annona Squamosa* Leaves extract. We can analyse with FT-IR spectra. Drug and Excipient mixed separately, compatibility is generally inflated by examining the characteristic peak of component and notice nothing any sufficient shift or change when they are mixed. As Show below table the FT-IR Spectra of mixture of Corn Oil + ASL extract, Tween 80 + ASL Extract, Transcutol HP + ASL Extract.

Table 8.5: Sample detail of FT-IR spectroscopy for Compatibility.

Sample Code	Sample Name
DCO	ASL extract + Corn Oil
DT80	ASL extract + Tween 80
DTHP	ASL extract + Transcutol HP

FS1 Graphical Report obtained from FT-IR and their FT-IR analysis report for functional group is given below

Table 8.6: FT-IR Analysis of Sample DCO.

Reference Range (cm ⁻¹)	Observed Peak (cm ⁻¹)	Bond Type	Functional Group
3400-3850	3852.46, 3741.39, 3466.76	O-H stretching	Alcohols, Phenols
3000-3100	3005.78	=C-H stretching	Alkenes
2850-2960	2925.93, 2857.79	C-H stretching	Alkanes
2500-2700	2679.83	O-H stretching	Carboxylic Acids
1600-1680	1654.05	C=C stretching	Alkenes
1350-1480	1455.89, 1376.62	C-H bending	Alkanes
1000-1300	1234.58, 1163.1, 1104.56	C-O stretching	Alcohols, Ethers

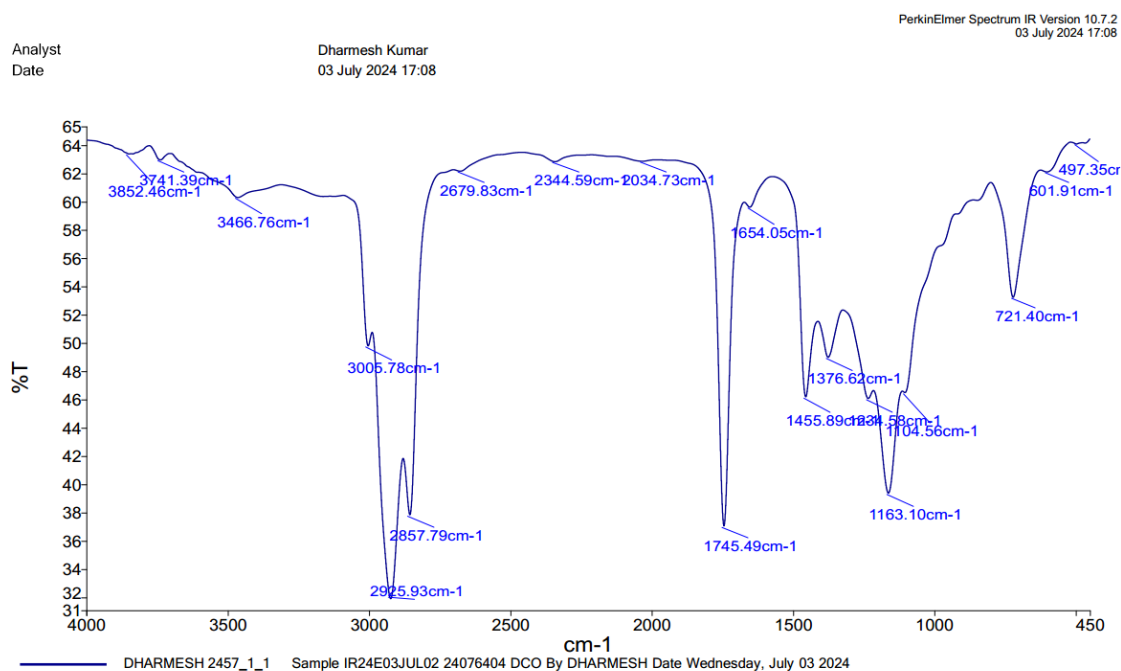


Fig8.3: FT-IR graph of sample DCO obtained from FT-IR spectroscopy.

FS2 Graphical Report obtained from FT-IR and their FT-IR analysis report for functional group is given below.

Table 8.7: FT-IR Analysis of Sample DT80.

Reference Range (cm ⁻¹)	Observed Peak (cm ⁻¹)	Bond Type	Functional Group
3200-3600	3911.25, 3775.47, 3430.92	O-H stretch	Alcohol, phenol
2850-2960	2920.85, 2873.24	C-H stretch	Alkane
2100-2260	2346.62, 2100.9	C≡C stretch	Alkyne

1670-1820	1963.71, 1734.4	C=O stretch	Carbonyl
1620-1680	1643.99	C=C stretch	Alkene
1350-1470	1459.7, 1353.25	C-H bend	Alkene
1000-1300	1293.51, 1250.56, 1105.48	C-O stretch	Ether, alcohol

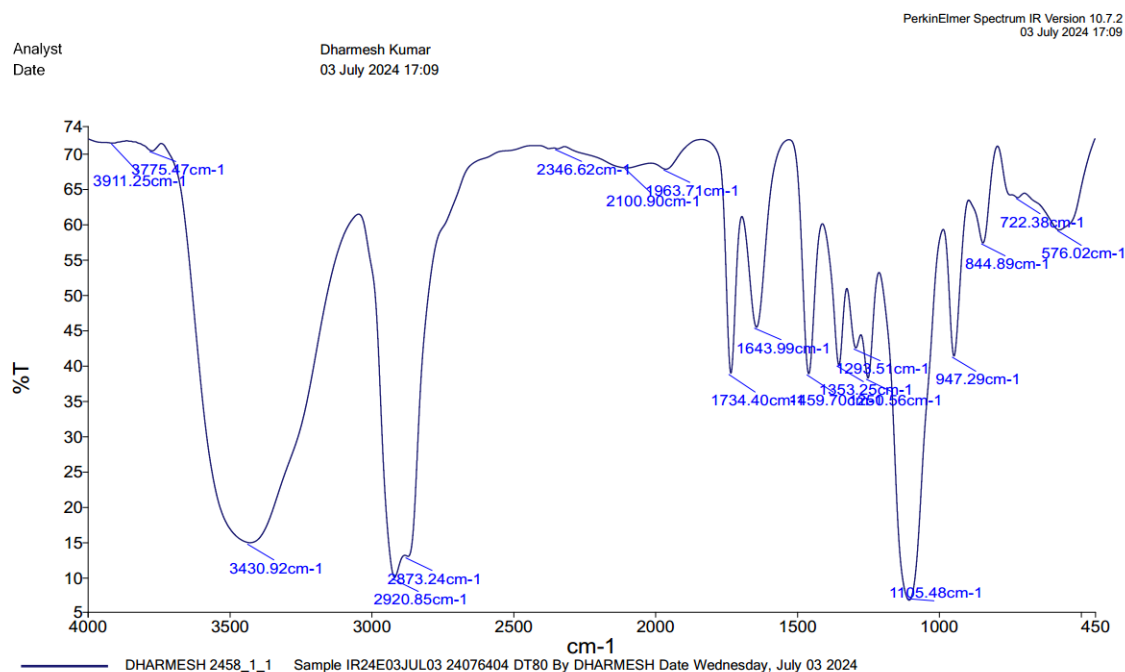


Fig8.4: FT-IR graph of sample DT80 obtained from FT-IR spectroscopy.

FS3 Graphical Report obtained from FT-IR and their FT-IR analysis report for functional group is given below

Table 8.8: FT-IR Analysis of Sample DTHP.

Reference Range (cm ⁻¹)	Observed Peak (cm ⁻¹)	Bond Type	Functional Group
3200-3600	3920.54, 3775.71, 3417.97	O-H stretch	Alcohol, phenol
2850-2960	2878.44	C-H stretch	Alkane
2100-2260	2130.23,	C≡C stretch	Alkyne
1670-1820	1956.7	C=O stretch	Carbonyl (general)
1620-1680	1649.01	C=C stretch	Alkene
1350-1470	1454.84, 1380.19	C-H bend	Alkane
1000-1300	1294.85, 1246.26, 1110.29	C-O stretch	Alcohol, ether
720-1000	933.92, 886.44, 832.93	C-H Bend	Alkane

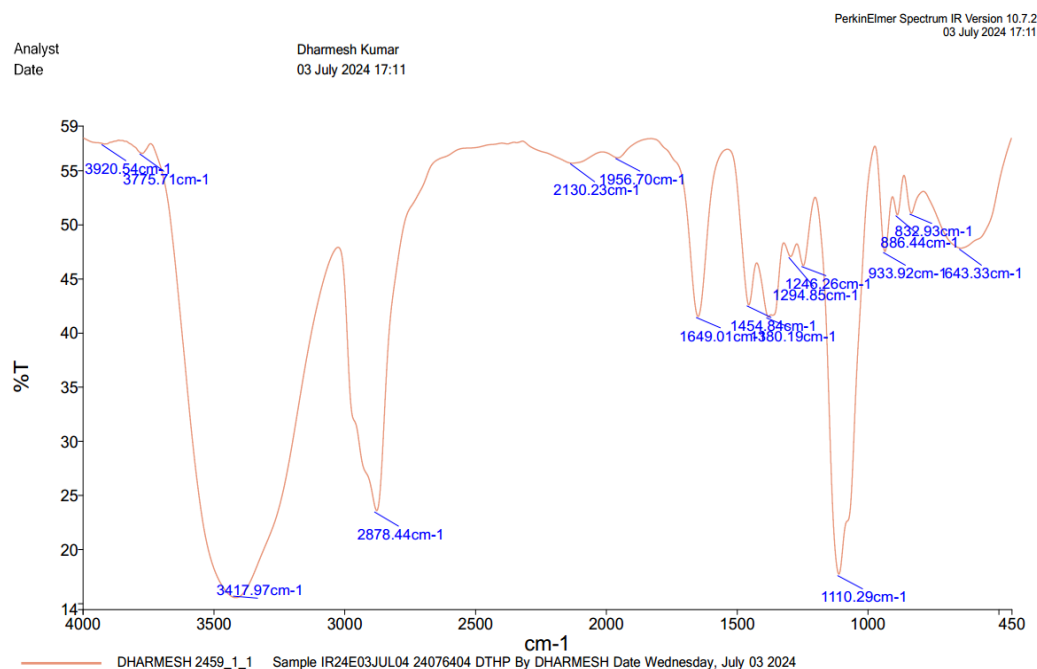


Fig8.5: FT-IR graph of sample DTHP obtained from FT-IR spectroscopy.

8.2 FORMULATION STUDIES

8.2.1 SNEDDS Formula with D-Optimal Mixture Design

The D-Optimal mixture design of Design Expert 23 Trial software showed 16 formulas with various concentrations of oil, surfactant, and co-surfactant. These 11 formulas from the software have different compositions, with five formulas replicated. This is in order to reduce any possible errors that may occur.

The composition used was corn oil as the oil phase, tween 80 as a surfactant, and Transcutol HP as a co-surfactant. The total concentration is 100%, percentage of upper and lower limits of Excipient composition were that why gained the SNEDDS formulation had lower limit and upper limit range in oil 12 % - 22%, surfactant 60% -70%, co surfactant is 12% - 22%. The use of oil and co-surfactants to produce a good SNEDDS formula was less than 22% and surfactants minimum 60%. **Cerpnjak et al.**^[26] The total weight of SNEDDS formula was 5.0 gm.

Table 8.9: SNEDDS formulation of *Annona Squamosa* Leave Extract using State-Ease Design Expert 23 Trial Version.

Formula	Percentage of Oil [A], Surfactant[B] and Cosurfactant [C] based on D-Optimal			Result of SNEDDS Physical Stability Parameters SNEDDS of <i>Annona Squamosa</i> Extract		
	A: Oil %	B: Surfactant %	C: co-Surfactant %	Transmittance (%)	Emulsification Time (Second)	Phase Separation (F)
1	22.00	66.00	12.00	85.00	24.00	0.908
2	12.00	69.34	18.65	87.50	15.00	0.994
3	15.02	70.00	14.98	93.70	19.00	0.885
4	17.99	67.28	14.72	85.30	13.00	0.942
5	18.00	70.00	12.00	81.60	25.00	0.91
6	18.00	60.00	22.00	84.90	17.00	0.905
7	18.00	60.00	22.00	86.20	13.00	0.923
8	12.00	66.00	22.00	85.90	11.00	0.942
9	22.00	60.00	18.00	91.50	7.00	0.885
10	22.00	66.00	12.00	90.60	18.00	0.963
11	17.65	64.70	17.63	79.30	23.00	0.885
12	18.00	70.00	12.00	85.20	20.00	0.923
13	21.31	63.39	15.28	81.80	15.00	0.945
14	15.03	62.96	22.00	91.30	23.00	0.924
15	12.00	66.00	22.00	78.70	14.00	0.968
16	22.00	60.00	18.00	86.70	10.00	0.885

8.2.2 Prepared SNEDDS and Drug Loading

16 formulas were prepared following the D-Optimal mixture design. The drug loading measurement aimed to determine the maximum concentration of the extract that could be uniformly dissolved in the SNEDDS system. The results from the drug loading test at 25 mg/g showed that the SNEDDS system could dissolve the extract effectively. Solubility testing parameters were assessed by visual observation for clarity, homogeneity, and the absence of precipitation in the SNEDDS system.



(A)



(B)



(C)

Fig 8.6 All 16 formulas with various concentrations of oil, surfactant, and co-surfactant Prepared SNEDDS

(A) 1-6 No. Formula

(B) 7-12 No. Formula

(C) 13-16 No. Formula

8.3 CHARACTERIZATION AND EVALUATION OF SNEDDS

8.3.1 Transmittance value (% T)

The percent transmittance test was used to evaluate the purity of the emulsion produced by SNEDDS formulation of *Annona Squamosa* Leave extract. If transmitter value of emulsion is greater than 90%, it is considered to be nanoscale. Gupta [2016].

Between 78.70% to 93.70% was the transmittance value obtained from the 16 run formulae. (Table No. 8.9)

8.3.2 Emulsification Time

The emulsification time is a crucial metric for evaluating the effectiveness of SNEDDS in forming a spontaneous nano-emulsion in GI media with modest agitation. An ideal SNEDDS formulation will have a viewable nano-emulsion that disperses completely and rapidly in less than 1.0 minute. Pratiwi et al.^[27] This study shows that 16 SNEDDS formulations had clear appearances and an emulsification time of less than 1.0 minute. Emulsification times from all 16 formula runs ranged from 7.00 to 25.00 seconds. (Table No. 8.9)



Fig8.7: (A) Formulation No 7,8,9 (B) Formulation No 14,15,16 (left to right).

8.3.3 Phase Separation

In addition, nano-emulsions are kinetically stable systems that require a significant amount of time to separate into different phases. In order to reduce the observation period, accelerated stability testing using the centrifugation method was conducted. Stirring at 3000 rpm for 3×10 minutes could be compared to gravity for ± 1.0 year. **Cicilia and Septi**^[12] The oil separates as a result of the interface layer being destroyed by the centrifugal force's pressure on the SNEDDS system. The SNEDDS system's separation level provided the phase separation value (F). The phase separation value will increase with the height of the remaining stable layer. Higher Phase Separation value (F) is 0.994 and Lower Value is 0.908. (Table No.8.9)

8.3.4 Determination of Optimum Formula of SNEDDS and Verification

The experimental mixture design technique is applied for optimization for independent variables when response variables are dependent only on the proportions of the ingredients of mixture. D-Optimal mixture design is often used to reveal the main effects and interaction effects of independent variables against response variables; it is illustrated by a polynomial equation from the Design Expert 24 Trial software, as shown in Table 8.9. **Bolton et al.**^[42]

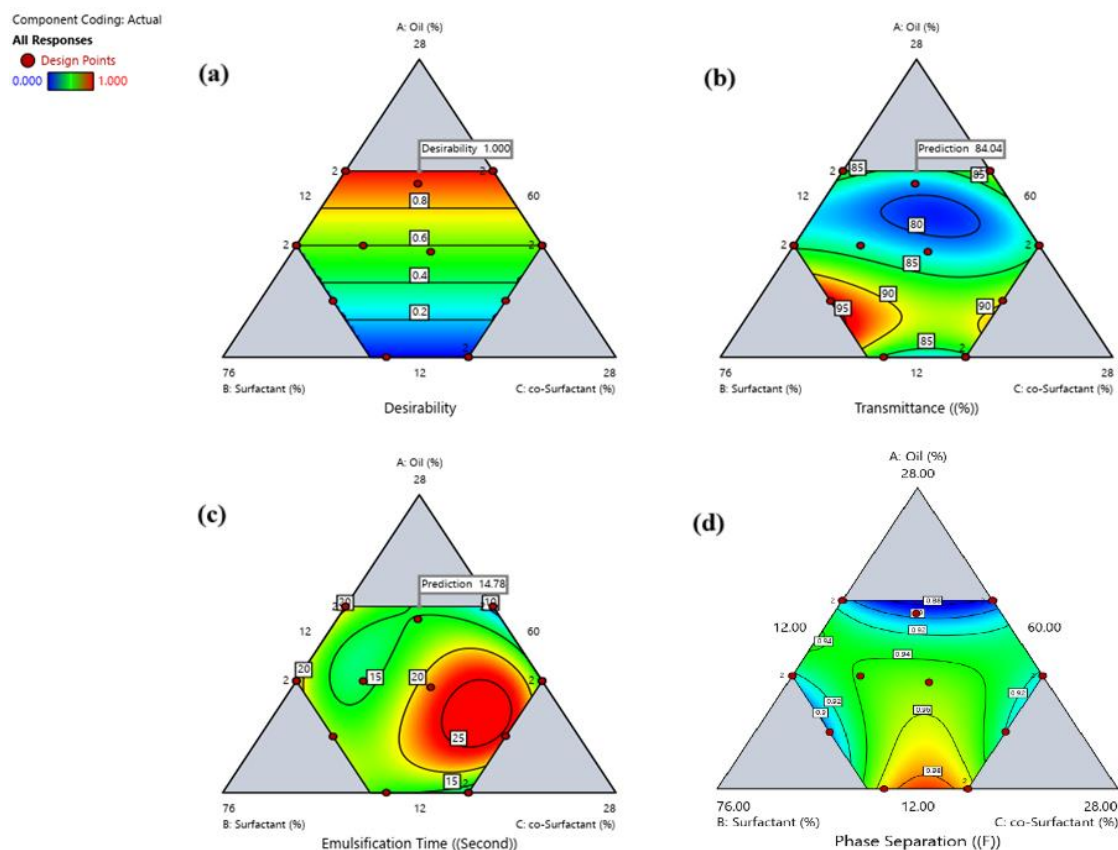


Fig 8.8: Contour plot diagram physical stability responses of SNEDDS: (a) Superimpose diagram showed the optimum area of the oil: surfactant: co-surfactant component. (b) transmittance, (c) emulsification time, (d) phase separation.

The interaction of oil (corn oil), surfactant (tween 80), and co-surfactant (Transcutol HP) against each of the responses was displayed in a contour plot diagram. The red area in Figure 8.8 represents the highest response, while the areas of yellow, green, light blue, and dark blue represent the lowest response. The chosen SNEDDS optimum formula with the maximum desirability value (1.00) in the superimpose diagram was determined by the optimization result. The optimal characteristic value that corresponds with the intended value will be produced by this formula. The ideal formula has the following composition: 22.00% oil, 64.34% surfactant, and 13.66% co-surfactant.

Verification of the optimum formula compared observed values with predicted values from the Design Expert 23 Trial software. showed no statistically significant differences ($p > 0.05$) between predicted and observed values for transmittance, emulsification time, and phase

separation in a one-sample t-test (Table 8.10). The D-Optimal method demonstrated the software's predictive accuracy with a 95% confidence level.

Table 8.10: Verification of optimum formula of SNEDDS.

Response	Prediction	CI	Observation	p-value [p>0.05]
Transmittance (%)	79.29	84.36-74.19	78.89	0.0110
Emulsification Time (second)	15.97	21.90-10.04	15.00	0.03.1
Phase Separation (F)	0.882	0.911-0.854	0.894	0.0056

8.3.5 Particle Size and Zeta Potential

To make sure the nano-emulsion droplet size was less than 200 nm, the nano-emulsion droplet size was observed. Based on the test results droplet size is 172.9nm with a polydispersity index (PI) value of 0.198. This proved that nano-emulsion production is possible using SNEDDS preparations. As the concentration of surfactant increased the size of the drop reduced. **Kassem et al.**^[25] The size of the resulting nano-emulsion decreases with increasing surfactant to co-surfactant ratio. **Xi et al.**^[22] The oil content has an effect on the nano-emulsion's particle size as well. **Singh et al.**^[41] Oil was able to improve the drug-carrying capacity of SNEDDS, but it also increased the size of the nano-emulsion, requiring a constant ratio of oil to surfactants. **Fernandez et al.**^[37]

The research revealed a zeta potential value of -19.6 mV. The investigation produced nano-emulsion droplets with zeta potential that met the specifications. The zeta potential value within the ± 30 mV range was the limit value that could preserve the emulsion's stability since it was nearly neutral, which reduced the possibility of particles aggregating. **Honary and Zahir.**^[19]

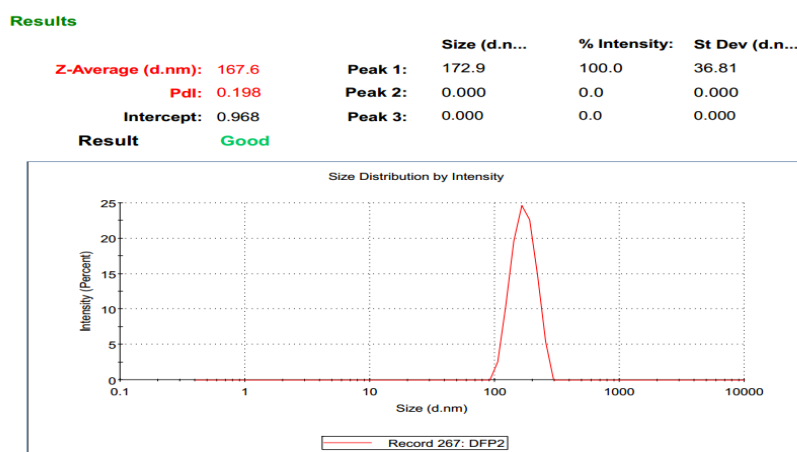


Fig 8.9: Partical Size & PDI of Optimized Formula.

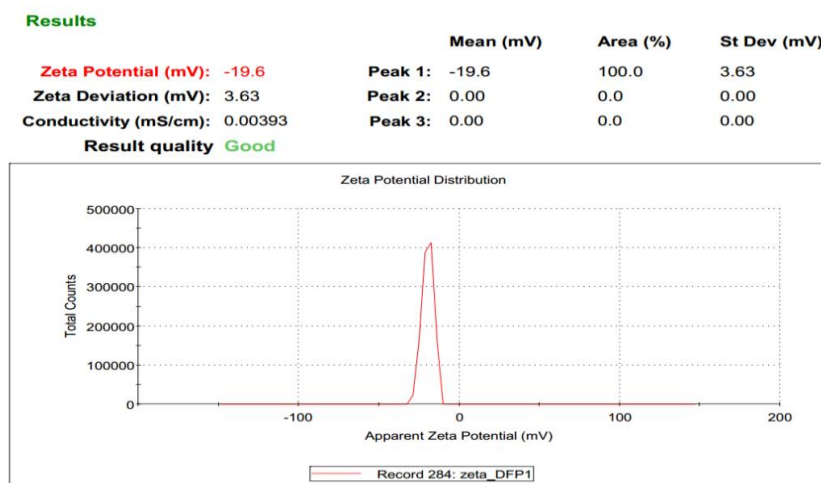


Fig 8.10: Zeta Potential of Optimized Formula.

CONCLUSION

In *Annona Squamosa* leaf extract, after preliminary phytochemical analysis, alkaloids, flavonoids, tannins, and phenols were found. The best SNEDDS formula was determined using the D-Optimal mixture design to be 22.00% oil (corn oil), 64.36% surfactant (tween 80), and 13.66% co-surfatant (Transcutol HP) with a drug extract loading of 25 mg/g. Verification test There was no significant difference, $p > 0.05$, between the observation and the prediction from the software. The optimum formula of SNEDDS fulfilled the criteria of nano-emulsion with 78.89% transmittance, 15.00 seconds of emulsification time, and 0.894 of phase separation; the particle size is 171.9 nm; PDI is 0.19; and the zeta potential is -19.6 mV.

ETHICAL STATEMENT

A pharmacist needs to act with integrity and honesty. A pharmacist refrains from actions that undermine their dedication to acting in their patients' best interests, such as discriminatory acts, inappropriate behaviour, and unfavourable working conditions that cloud judgment. A pharmacist keeps up their level of expertise.

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CONFLICT OF INTEREST

The authors attest that they are free of any known financial or personal conflicts of interest that would taint the findings of this study.

INFORMED CONSENT

Using websites, review articles, and other sources to produce research content.

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