Synergistic Potential of *Glycyrrhiza glabra* and *Annona muricata* Extracts in Optimized *Shrikhand*: A Study on Bioactive Enrichment and Shelf-Life Extension

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DECLARATION OF THE STUDENT

I hereby declare that the data presented in this Dissertation report entitled,"Synergistic Potential of *Glycyrrhiza glabra* and *Annona muricata* Extracts in Optimized *Shrikhand*: A Study on Bioactive Enrichment and Shelf-Life Extension" is based on the results of investigations carried out by me in the Botany Discipline at the School of Biological Science and Biotechnology, Goa University under the supervision ofDr. Aditi Naik (Asst. Prof, SBSB) and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand the Goa University or its authorities will not be responsible for the correctness of observations/ experimental or other findings given in the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation report "Synergistic Potential of *Glycyrrhiza glabra* and *Annona muricata* Extracts in Optimized *Shrikhand*: A Study on Bioactive Enrichment and Shelf-Life Extension" is bonafide work carried out by Ms. Shivani Vilas Aiya under my supervision in partial fulfilment of the requirements for the award of the degree of M. Sc. Botany in the Botany Discipline at the School of Biological Science and Biotechnology, Goa University.

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ABBREVIATIONS USED

Entity	Abbreviations
TLC	Thin Layer Chromatography
DPPH	1,1-diphenyl-2-picrylhydrazyl
BE	Before Encapsulation
AE	After Encapsulation
IC_{50}	Half-maximal inhibitory concentration
pH	Potential of Hydrogen
NaOH	Sodium Hydroxide
MRS agar	de Man, Rogosa and Sharpe agar
PDA	Potato Dextrose Agar
w/v	Weight/Volume

ABSTRACT

This research explores the integration of encapsulated Annona muricata leaf extract into *Jestamadh* stem extract-enriched shrikhand, a fermented dairy product. Two formulations were studied: T_1 containing 1% encapsulated extract and T_2 containing 2%. Comprehensive analysis encompassed sensory evaluation, microbial studies, total phenolic content, and antioxidant activity.

Sensory evaluation revealed that T_2 exhibited superior attributes in terms of taste, sweetness, texture, aroma, and appearance. Microbial analysis indicated an 8-day shelf life for both formulations when refrigerated, with no detectable presence of coliforms initially. Total phenolic content was notably higher in T_2 (2.157 ± 0.022 µg/mL) compared to T_1.

TLC analysis confirmed the presence of phenols, essential oils, and flavonoids in both formulations. The DPPH assay demonstrated stronger antioxidant activity in T_2 (IC_{50}: 281.71 µg/mL) compared to T_1 (IC_{50}: 154.47 µg/mL). These findings underscore the potential health benefits of incorporating encapsulated*A. muricata* extract into *Jestamadh*-enriched *Shrikhand*. Further research is necessary to validate its safety and efficacy for consumption.

Keywords: *Glycyrrhiza glabra*(liquorice); Encapsulated *Annona muricata* leaf extract; *Jestamadh* stem extract; *Shrikhand*; Antioxidant activity; Sensory evaluation

CHAPTER 1: INTRODUCTION

In recent years, the exploration of functional foods enriched with bioactive compounds has gained substantial traction in the pursuit of promoting health and wellness. This paradigm shift towards functional edibles has prompted an extensive investigation into the medicinal properties of natural botanical extracts. Among these, *Glycyrrhiza glabra* (*Jestamadh*) and *Annona muricata* (Soursop) have emerged as potent sources of diverse phytochemicals, garnering attention for their potential health benefits. The quest for healthier dietary options to combat lifestyle-related ailments has driven substantial research into incorporating natural extracts, such as those from *Glycyrrhiza glabra* (*Jestamadh*) and *Annona muricata* (Soursop), into dairy products. These extracts boast a spectrum of bioactive compounds known for their potential health benefits, as evidenced in a myriad of studies.

1.1 BACKGROUND

1.1.1 *Glycyrrhiza glabra*

One of the most often used herbs in Ayurvedic medicine's long history is *Glycyrrhiza glabra*, which is utilised as a flavouring as well as a medication. *Glycyrrhiza glabra* is also called *Jesthamadh* or Licorice. It is mostly found in the Mediterranean and certain parts of Asia. (Kaur *et al.*, 2013).

It has been revered in traditional medicine for its multifaceted bioactive compounds, including glycyrrhizin, glycyrrhizinic acid, isoflavones, and triterpene sterols, attributed to various pharmacological actions such as anti-inflammatory, antioxidant, antiviral, and anti-diabetic properties.

1.1.2 Scientific Classification

Kingdom: Plantae

Division: Angiospermae

Class: Dicotyledoneae

Order: Rosales

Family: Leguminosae

Genus: *Glycyrrhiza*

Species: *glabra*

Binomial Name: *Glycyrrhiza glabra* Linn.

1.1.3 Botanical Description

Licorice is a perennial plant or under-shrub, 50–150 cm tall, white hairy, thickly scaly, glandular punctate, and woody at base. The leaves are 5-14 cm long and 11-17-foliolate. The stipules are caducous and linear, measuring 1-2 mm. The petiole is densely yellow-brown glandular hairy and villous. The leaflets are ovate-oblong, oblong-lanceolate, or elliptic, measuring 1.7-4 × 0.8-2 cm. They are abaxially densely yellow scaly glandular punctate and pubescent on veins, adaxially glabrescent or pilose, with a rounded base, apex rounded or retuse.

At 2 metres tall, it grows erect. It has stunning oval leaves, flat pods, and clusters of purple white flowers. Reddish-brown, 1-3 cm long, 4-5 mm broad, are the pods, or fruits. Each pod bears 2-5 brown or blackish seeds. Flowers are 1 centimetre long, with flat pods that are oblong to linear, measuring 1-3 centimetres long by 6 mm broad. The pods are more or less densely echinate glandular, with numerous seeds or a shorter number of 2, 3, or 4

seeds. Dark green, smooth, about 2 mm in diameter, seeds number 2–8. Flowering occurs from May to June, followed by fruit: 2n = 16* July–Sep.

Its fibrous main taproot, which has a bright yellow interior, is utilised for medicinal purposes (Olukoga & Donaldson, 1998). Its root system is huge. Liquorice is the term used in commerce to refer to the plant's dried rhizome and root. The officinal portion consists of roots and rhizome, which are roughly cylindrical segments up to one metre and 5–20 mm in diameter. The bark's exterior is wrinkled longitudinally, ranging in colour from brownish grey to dark brown. Occasionally, it develops tiny black buds in rhizomes or tiny circular or transverse rootlet-scars in roots. According to the African Pharmacopoeia (1985), the European Pharmacopoeia (1995), and Akao *et al*. (1991), the roots are thick, multibranched, lengthwise, and cylindrical. The substance known as liquorice in commerce is made up of dried, peeled or unpeeled subterranean stems and roots (Badkhane *et al.,* 2014).

1.1.4 Chemical Constituents

More than 400 chemical compounds made up of triterpene saponins have been identified from the *Glycyrrhiza* species. Flavonoids, such as isoliquiritoside and libiritoside, are thought to be the cause of bioactivities found in liquorice. Due to differences in plant species and regional sources, these saponin and flavonoid concentrations might vary greatly. Glucose, pectins, polysaccharides, steroid hormones, amino acids, and mineral salts are reported in this plant. Triterpenoid saponins include Glycyrrhizin (2-15%), also known as Glycyrrhizic acid, which is found in calcium and potassium salts.Among these are glycyrrhetinic acid (18-beta-glycyrrhetinic acid, GA), tannins, progesterone, bitter principle (glycymarin), asparagine, starch, glucose, manitol, atropine, choline, betaine, steroid hormone, and others.

Glycyrrhizin, an oleanane-type triterpene saponin, is the main ingredient in the roots and stolons of *Glycyrrhiza* plants that gives them their sweet flavour. This compound is a combination of glycyrrhizic acid salts with potassium, calcium, and magnesium contents that range from 2 to 25%. Glycyrrhizic acid is a naturally occurring saponin that consists of two glucuronic acid molecules, a hydrophilic portion, and a fragment of glycyrrhetic acid.

1.1.5 Traditional Uses of *Glycyrrhiza glabra*

The plant *Glycyrrhiza glabra*Linn. is a very commercially significant target species that has been utilised extensively in traditional medicine. The traditional medical system uses the rhizomes and roots of *Glycyrrhiza glabra* for their anti-inflammatory, antibacterial, antiulcer, expectorant, and anxiolytic properties in the treatment of allergic (Badkhane *et al.*, 2014 & Korhalkar *et al.*, 2012).

It has also been used to treat Addison's illness, alleviate rheumatism, osteoarthritis, and arthritis, and control low blood sugar. It is used to treat oral ulcers and arthritis. Additionally, it has been used to treat respiratory, gastrointestinal, cardiovascular, eye, and skin ailments as well as antifungal, demulcent, emollient, antiallergic, and antiviral properties.

The root extract contains modest estrogenic properties and has been shown to help some people with menopausal symptoms, cramping during menstruation, and menstrual regulation. *Glycyrrhiza glabra* roots and rhizomes have been used in clinical practice for centuries to treat liver diseases and are a major component of polyherbal formulations for the cure of hepatotoxicity, antiallergic, demulcent, emollient, fungicide, peptic ulcer, to prevent liver toxicity, and to treat tuberculosis and adrenocorticoid insufficiency (Bradley, 1992;Schambelan, 1994).

1.1.6 *Annona muricata*

Annona muricata L., a species of the Annonaceae family, is proudly referred to as ***Corossolor Soursop*** and has a large pantropical range. It is endemic to Central America and is a widely distributed small tree. *Annona muricata* Linn. fruit has been proven to be edible in Yunnan province, China, and is commercially utilised to make juice, candies, and sherbets. Numerous acetogenins have been identified *via* extensive chemical analysis of this species' leaves and seeds.

The isolated compounds exhibit certain noteworthy biological or pharmacological effects, such as antitumoral, cytotoxic, antiparasitic, and pesticidal characteristics. The antiparasitic and pesticidal qualities of these plants' roots make them useful in traditional medicine. (Rajeswari *et al.*, 2012). *Annona muricata*, known as Soursop, has been a subject of scientific interest due to its rich phytochemical composition, encompassing flavanol Tri glycosides, alkaloids, phenolics, annonaceousacetogenins, and essential oils. These compounds have shown promise in exhibiting anticancer, antioxidant, and antidiabetic effects.

1.1.7 Classification

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida

Order: Magnoliales

Family: Annonaceae

Genus*: Annona*

Species: *muricata*

Binomial name: *Annona muricata* L.

1.1.8 Botanical Description

Approximately 130 genera and 2300 species make up the Annonaceae family, which includes *A. muricata* L., often known as soursop, graviola, guanabana, pawpaw, and sirsak. Originally from the warmest tropical regions of South and North America, *A. muricata* is now found in many tropical and subtropical nations across the world, such as Africa, Australia, Malaysia, Nigeria, and India.

Annona muricata is an evergreen terrestrial tree that reaches a height of 5 to 8 metres. Its canopy is wide, round, glossy, and dark green. This tree has bigger individual yellow blooms on woody stalks. The tree produces huge, edible fruits that are oval or heart-shaped, green in colour, and weigh more than 4 kg. The fruits have a diameter of 15 to 20 cm. The fruit pulp is composed of white, juicy, fibrous segments that resemble an extended container. Fruits can have anywhere from five to two hundred seeds. The skin has small spines and is reticulated, giving it a leathery appearance. Its soft pithy base gets easily separated from its creamy, granular inner surface.

1.1.9 Chemical Constituents

Numerous phytochemical analyses conducted on diverse *Annona muricata* plant sections have revealed the existence of a variety of phytoconstituents and chemicals, such as phenolics, cyclopeptides, megastigmanes, flavanol triglycosides, alkaloids, and essential oils. *Annona* species, on the other hand, have been demonstrated to be a typically rich source of annonaceousacetogenin compound; this includes *Annona muricata*. The inclusion of many major minerals such as potassium, sodium, calcium, copper, iron, and magnesium suggest that frequent eating of the *Annona muricata* fruit can assist deliver important nutrients and elements to the human body.

1.1.10 Traditional uses of *A. muricata*

Similar to other *Annona* species, like *Annona squamosa* and *Annona reticulata*, all of the parts of the *Annona muricata* tree are often used as traditional remedies against a number of human health problems and diseases, including cancer and parasitic infestations (Moghadamtousi*et al.*, 2015).

The leaves have been used to treat cystitis, diabetes, headaches, and sleeplessness. Additionally, the decoction of the leaf offers anti-rheumatism and neuralgic properties when administered internally, while the boiled leaves are applied topically to cure rheumatism and abscesses. The fruit is used to improve a mother's milk after giving birth and is used as a natural remedy for rheumatism, neuralgia, arthritis, diarrhoea, dysentery, fever, malaria, parasites, and rheumatism. It is proposed that the crushed seeds contain anthelmintic properties that inhibit both internal and external worms and parasites. The plant is used to cure coughs, pains, and skin conditions in tropical Africa. It is also used as an astringent, pesticide, and piscicide. The root bark and leaves are said to have anthelmintic and antiphlogistic properties, while the fruit and flower are used as treatments for catarrh in India. To prevent fainting, a combination of crushed *A. muricata*, *A. squamosa*, and *Hibiscus rosa- sinensis* leaves is applied topically to the head is practiced in Malaysia.

Annona muricata leaves are used as an ethnomedicine to treat cancer and tumours throughout South America and tropical Africa, particularly Nigeria. The leaves, barks, and roots of *Annona muricata* are additionally considered to have anti-inflammatory, hypoglycaemic, sedative, smooth muscle relaxant, hypotensive, and antispasmodic properties. Along with their traditional therapeutic use, fruits are extensively used in the making of beverages, candies, frozen desserts, shakes, and syrups (Patel & Patel, 2016).

1.1.11 *Shrikhand*

Fermented milk and milk products occupy a place in satisfying nutritional requirements of human being since the time antiquity. Fermented milk products have been well recognized to have therapeutic, anticholesterolemic, anticarcinogenic properties (Boghra and Mathur 2000).The dairy matrix is nutrient-dense and contains bioactive minerals, proteins, lipids, and carbohydrates. Dairy consumption is recommended as a component of healthy dietary patterns.

Shrikhand is a fermented and sweetened milk product from India, derived from the Sanskrit word 'shrikhirni' which means sugared curd, flavouring agents (Saffron), fruits and nuts. It is popular in Gujarat, Maharashtra, and some parts of Karnataka, Madhya Pradesh, and Rajasthan.

Shrikhand is a semi-soft, sweetish-sour milk product whole milk product prepared from lactic fermented curd. The curd (Dahi) is partially strained through a muslin cloth to remove the whey and thus produce a solid mass called Chakka, the basic ingredient for *Shrikhand* (Singh *et. al*., 2014).

It is consumed as desert.*Shrikhand* is known for its high nutritive, characteristic flavour, taste, palatable nature and possible therapeutic value. It is very refreshing particularly during summer months. Lactic acid fermentation is used to make it.

The integration of these potent botanical extracts, that are *Jestamadh* and *Annona muricata*, into dairy-based products, such as *Shrikhand*, a traditional Indian dessert made from strained yogurt, presents an innovative approach to developing functional foods with enhanced nutritional profiles and potential health benefits.

1.2 AIMS AND OBJECTIVE OF THE PRESENT WORK

This study aims to harness the therapeutic potential of *Jestamadh* and *Annona muricata* extracts by enriching *Shrikhand* with their bioactive constituents. By leveraging these natural resources, the objective is to create a novel functional food product that aligns with the increasing demand for health-conscious dietary options.

The main objectives of the present study are as follows:

1. To investigate the ideal proportions of *Jestamadh* (*Glycyrrhiza glabra*) extract in enriched *Shrikhand* to optimize its bioactive constituents.
2. To analyse the biochemical, physiochemical, and functional characteristics of *Jestamadh* stem extract-enriched *Shrikhand* to understand its properties.
3. To assess the shelf life of the optimized *Jestamadh* stem extract-enriched *Shrikhand* formulation under varied storage conditions.
4. To evaluate the impact of incorporating encapsulated *Annona muricata* leaf extract into *Jestamadh* stem extract-enriched *Shrikhand* on its properties and potential synergies.

1.3 HYPOTHESIS

It is hypothesized that there are significant differences in the bioactive constituents of *Shrikhand* enriched with different proportions of *Jestamadh* (*Glycyrrhiza glabra*) extract. Additionally, the biochemical, physiochemical, and functional characteristics of *Jestamadh* stem extract-enriched Shrikhand are influenced by the proportion of extract used. Furthermore, there are significant differences in the shelf life of *Shrikhand* enriched with *Jestamadh* stem extract under varied storage conditions. Lastly, the incorporation of encapsulated *Annona muricata* leaf extract into *Jestamadh* stem extract-enriched Shrikhand results in significant changes to its properties and potentially leads to synergistic effects.

1.4 SCOPE

The scope of the research encompasses a comprehensive investigation into the synergistic potential of *Glycyrrhiza glabra* (*Jestamadh*) and Annona muricata extracts in optimized *Shrikhand*. The study aims to explore various aspects, including the determination of optimal proportions of *Jestamadh* extract to enrich *Shrikhand*, analysis of the biochemical, physiochemical, and functional characteristics of the enriched product, assessment of its shelf life under different storage conditions, and evaluation of the impact of incorporating encapsulated Annona muricata leaf extract. Additionally, the research extends to understanding potential synergies between the two extracts in enhancing the bioactive constituents and overall properties of *Shrikhand*. Through systematic experimentation and analysis, the study seeks to provide insights into the development of a novel functional food product that aligns with the growing demand for health-conscious dietary options.

CHAPTER 2. REVIEW OF LITERATURE

2.1 OPTIMIZATION OF *JESTAMADH* EXTRACT PROPORTIONS

Heema *et al*. (2020) investigated the physicochemical properties, sensory parameters, antioxidant studies, and antibacterial studies of *Shrikhand* combined with an aqueous and ethanol extract of dried and fresh pomegranate fruit peel. The functional *Shrikhand* infused with pomegranate peel ethanol extracts showed more antioxidant activity than the aqueous extracts, according to the results. The overall counts of bacteria, yeast, and mould were lower than the control due to the antibacterial action of the additional fruit peel extracts.

In order to make herbal *Shrikhand*; David in 2015 added aqueous basil extract at 1 %, 2 %, 3 %, and 4 %. These amounts were represented as T_1, T_2, T_3, and T_4, respectively, with T_0 serving as the control sample and being assessed based on a variety of physico-chemical, organoleptic, and microbiological aspects. In the study, five treatment combinations were employed and duplicated five times. According to the findings of the physico-chemical examination, the control sample (T_0) showed the highest total solids, fat content, and pH, whereas treatment 4 (T_4) revealed highest moisture, protein, ash content, antioxidant activity, and acidity.

Heema*et al.*,2022 assessed the role of liquorice as a prebiotic in promoting growth of *Lactobacillus rhamnosus*GG and yoghurt starters by combining liquorice (*Glycyrrhiza glabra*) aqueous extract to yoghurt at several concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 %). This was conducted in order to make herbal yoghurt. The incorporation of liquorice extract at different concentrations had a favourable effect on the bio-yoghurt's total viable count. The antioxidant content of the herbal bio-yoghurt ranged from 39.26 ± 0.02 to 68.13 ± 0.04 percent, according to analysis. The control yoghurt's antioxidant content was 22.08 ± 0.03%. The microorganism’s physico - chemical, functional, and survival were taken into consideration while determining the optimal amount of liquorice extract to incorporate. The

2% level of herbal extract incorporation was the best among the five levels, according to physico -chemical and sensory criteria.

2.2 DETERMINATION OF THE BIOACTIVE COMPOUND IN THE PLANT SAMPLE

Using HPTLC fingerprinting in combination with pharmacogenetic research, Husain *et al*. (2015) determined the total phenolic and flavonoid contents of *G. glabra* Linn roots to assess the plant's potential as an antioxidant and developed quality control parameters for the standardisation of this significant medicinal plant. Using standard procedures, a variety of pharmacogenetic analyses were performed on *G. glabra* Linn roots, including extractive values, total ash, water soluble ash, acid insoluble ash, moisture content, loss on drying, pH, and phytochemical screening.Also, the contents of total phenolics, flavonoids, pesticide residues, aflatoxin, and heavy metals were assessed. Studies on phytochemicals showed that different solvent extracts contained phenolic substances, proteins, carbohydrates, alkaloids, flavonoids, saponins, lipids, sterols, and tannins. The total amounts of flavonoids and phenolics in the methanolic extract were determined to be 2.25 µg and 7.47 mg/gm, respectively. The amounts of heavy metals were determined to be below the recommended levels.

2.3 SENSORY EVALUATION

In order to develop *Shrikhand*, Chavan *et al*. (2019) used Jamun pulp at 10 %, 20 %, and 30 % with 40 % sugar. The aim of the study was to extract and preserve the therapeutic qualities of Jamun, which is particularly useful as an antidiabetic and a significant and affordable source of manganese, calcium, iron, potassium, and salt. The colour and appearance scores for treatments T0, T1, T2, and T3 were found to be 7.63, 7.50, 8.00, and 8.50 respectively. The flavour score was 8.13, 8.00, 8.25, and 8.38respectively. Taste scores

were 8.13, 7.75, 8.00, and 8.25 respectively. The consistency scores were 8.25, 7.75, 7.75, and 7.75, respectively. Overall acceptance values for sensory were 8.03, 7.75, 8.00, and 8.22.

2.4 BIOCHEMICAL AND PHYSIOCHEMICAL ANALYSIS

The physicochemical characteristics of two formulations - Soursop juice (SJ) and Soursop nectar (SN)—that were added to yoghurt formulations were examined by Dias & Jayasooriya (2017). Physicochemical characteristics were examined, including protein content, pH, and titratable acidity. Microbial numbers and antioxidant activity were also investigated. The results revealed that yoghurts containing soursop juice (SJ) and nectar (SN) had enhanced physicochemical and antioxidant qualities in addition to favourable sensory attributes.

In accordance with Pankaj *et al*. (2022), probiotic yoghurt blended with varying amounts of jaggery has been studied for its physicochemical, microbiological, and sensory differences. The antioxidant qualities of the probiotic yoghurt were assessed, and the alterations that occurred over the course of 21 days of storage at 4°C were explored. During storage, it was found that there was an increase in titratable acidity and a decrease in pH.

2.5 SHELF-LIFE ASSESSMENT

In 2016, Dhotre and Bhadania prepared *Shrikhand* using a thermization machine. The sensory characteristics of this type of thermized *Shrikhand* were examined while it was refrigerated at 8 ± 2°C. The titratable acidity, consistency, and sensory characteristics of the thermized shrikhand were compared to those of the unthermized *Shrikhand*, which was used as a control and treated in the same way as the thermization stage. It was discovered that the thermized *Shrikhand* kept its sensory qualities for a greater period of time than the control. It was also discovered that throughout the storage time, thermized Shrikhand maintained titratable acidity and consistency (Jadhav *et al*., 2019)).

2.6 PHYTOCHEMICAL STUDIES ON *GLYCYRRHIZA GLABRA*

Tiwari & Alim, (2020) conducted phytochemical analysis and evaluated the*in vitro* antidiabetic, and antioxidant activities of traditional medicinal plant *Glycyrrhiza glabra*. The phytochemical analysis revealed that *Glycyrrhiza glabra* root showed presence of secondary metabolites flavonoids, Saponin, glycosides, terpenoids etc.

Damle, (2014) identified significant phytoconstituents in *Glycyrrhiza glabra*, including isoflavones, glabrin A and B, highlighting its multifaceted medicinal properties like anti-inflammatory, anti-diabetic, and anti-viral, anti-ulcer, antitussive, anti-oxidant, skin whitening, anti-diuretic agent.

2.7 TRADITIONAL USE OF *GLYCYRRHIZA GLABRA*

Sharma & Agrawal, (2013) explored the historical use of *Glycyrrhiza glabra* in traditional medicine, emphasizing its diverse therapeutic properties against ailments like hepatitis, coughs, and even SARS and cancer. It is found that *Glycyrrhiza glabra* can be used as a mild laxative, anti-arthritic, anti-inflammatory, anti-biotic, anti-viral, anti-ulcer, anti-tussive, anti-oxidant, estrogenic, anti-diuretic and hypolipidemic agent. It is reported to contain important phytoconstituents such as glycyrrhizin, glycyrrhizinic acid, glabrin A&B, triterpene sterols, saponin, and isoflavones.

2.8 BENEFITS OF NATURAL SWEETING AGENTS

Priya, (2011) detailed the types and benefits of natural sweeteners derived from plants for diabetic patients, highlighting their active sweet principles of terpenoids, steroidal saponins, dihydroisocoumarins, dihydrochalcones, proteins, polyols, volatile oils, etc. stored in plants. Further sweeteners along with their properties, chemical structure of sweet principle and pharmacological applications were evaluated.

Gandhi *et al.*, (2018) explored the health benefits of *Stevia*, a natural sweetener, emphasizing its potential advantages in comparison to artificial sweeteners. Studies found that *Stevia*-

incorporated products possess better sweetening potency and maximum consumer acceptability, when compared with other sugar substitutes. They also explored the effect of incorporation of stevia on physicochemical, rheological, and nutritional food properties.

2.9 PHYTOCHEMICAL STUDIES ON *ANNONA MURICATA*

Vijayameena*et al.*, (2013) assessed the phyto-chemicals, antioxidants, and antibacterial activity in different sections of *Annona muricata*, highlighting its potential pharmacological applications. They found that the aqueous leaf extract contains a high protein and phenol content of 36.66 mg % and 134.28 mg % respectively. The ethanolic root extract showed highest calcium content of 22mg %and the aqueous extract of seed contains high carbohydrate content of 11.025 mg % respectively. The ethanolic extract of leaf shows highest antibacterial activity towards *Pseudomonas aeruginosa* and *Staphylococcus aureus.*

2.10 NUTRITIONAL ADVANTAGES OF YOGURT

Fernandez *et at.,* (2017)explored the nutritional advantages of yogurt and lactic acid bacteria (LAB) in promoting gastrointestinal health, highlighting their positive impact on gut microbiota and intestinal function. They also found that Certain disease with gastrointestinal tract such as, lactose intolerance, diarrhoea, Colon Cancer, inflammatory bowel disease and other bacterial infection were inhibited through high consumption of yogurt as based diary food product.

2.11 ENCAPSULATION AND DEVELOPMENT OF FUNCTIONAL FOOD

A review work by Routh *et al.,* (2022) examined the process of extracting and encapsulating a few Indian plants and examined their antiviral properties. A few chosen herbs shown excellent efficacy and efficiency. Various methods of extraction were employed to maintain its efficacy. Herbs were encapsulated to preserve their qualities and prolong their shelf life. A

variety of functional foods have been created with a significant concentration of bioactive compounds.

CHAPTER 3. METHODOLOGY

This chapter outlines the materials, chemicals, and methodology employed in the investigation titled, "Synergistic Potential of *Glycyrrhiza glabra*and *Annona muricata* Extracts in Optimized *Shrikhand*: A Study on Bioactive Enrichment and Shelf-Life Extension." The research was conducted at the Discipline of Botany, School of Biological Sciences and Biotechnology, Goa University, situated in Taleigao Plateau, Panjim, in collaboration with Goa Dairy, Ponda Goa.

3.1 MATERIALS

3.1.1 Materials and Chemicals

All chemicals and reagents utilized in this study were of analytical grade and sourced from reputable suppliers such as Sigma Chemicals Co. (St. Louis, MO, USA), HiMedia Laboratories Pvt. Ltd. (Bombay), and Sisco Research Laboratories Pvt. Ltd. (Bombay). Additionally, milk used for the research work was taken from Goa Dairy Curti Ponda Goa.

3.2 OPTIMIZATION OF *JESTAMADH* EXTRACT PROPORTIONS

3.2.1 Preliminary trials

Preliminary trials involved the replacement of sugar with various substitutes, including jaggery, stevia, date powder, and *Jestamadh* powder. The trials aimed to determine the optimal proportions of *Jestamadh* extract in the *Shrikhand* formulation. Specifically, the trials investigated the individual and combined effects of *Jestamadh* extract, jaggery, Stevia, date powder, and dates on the sensory attributes and bioactive enrichment of the *Shrikhand* product.

Further details regarding the proportions and combinations of these components were explored to identify the most effective formulation for bioactive enrichment and shelf-life extension of the *Shrikhand*.

Specifically, different proportions of *Jestamadh* water extract used included varying concentrations, such as 1%, 2%, and 3%, to assess their impact on the final product's sensory properties and bioactive content. Similarly, different concentrations of sugar substitutes like jaggery, stevia, and date powder were tested, ranging from 5% to 20%, to determine their optimal levels for enhancing sweetness and bioactive enrichment in the *Shrikhand* formulation.

Additionally, variations in the type and quantity of dates used, such as whole dates, date paste, or date syrup, were investigated to explore their potential synergistic effects with *Jestamadh* extract.

3.2.2 Preparation of aqueous herbal extract

Aqueous herbal extract preparation involved slight modifications to the method outlined by Hasneen*et al*. (2020). *Jestamadh*stem powder was dissolved and extracted in hot distilled water (60°C) at a ratio of 1:10 (w/v) for 15 minutes.

Subsequently, the mixture underwent centrifugation at 6000 rpm for 15 minutes and was filtered through Whatman No. 1 filter paper. The resulting clear aqueous extract was utilized promptly for further experimentation.

3.2.3 Preparation of experimental *Shrikhand*

The experimental *Shrikhand* was prepared following a standardized procedure. Initially, milk was heated to 90°C for 20 minutes and then cooled to 30°C. After the incubation period, the resulting curd, characterized by a well-developed body and texture, was broken and enveloped in muslin cloth. Subsequently, it was hung for eight hours to facilitate whey drainage. The chakka,(hunged curd) obtained after draining, was weighed to determine the yield and stored in a refrigerator for subsequent analysis.

For experimental purposes, the chakka was divided into three portions to accommodate different treatments. Each portion was subjected to a specific treatment protocol, with one portion serving as the control and the remaining two portions treated with varying concentrations of the aqueous extract of *Jestamadh* stem powder.

The treated chakka portions were thoroughly kneaded with the respective extract concentrations until a homogenous mixture was achieved. Finally, the prepared *Shrikhand* samples were refrigerated to maintain freshness and stability for further analysis.

Figure 3.1 Preparation of aqueous herbal extract

Figure 3.2 *Jestamadh*extract enriched Herbal Shrikhand

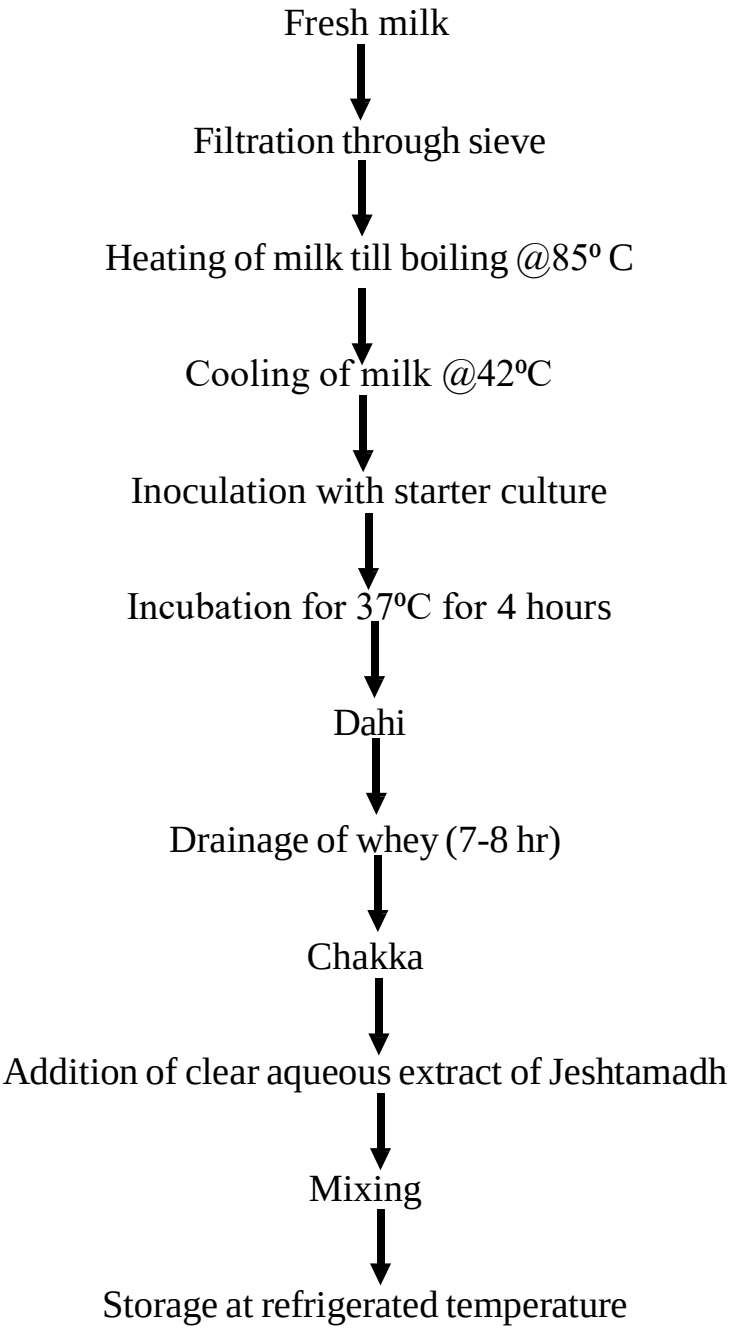

Figure 3.3 Flowchart for preparation of Herbal *Shrikhand*

3.3 PREPARATION OF *GLYCYRRHIZA GLABRA*PLANT EXTRACT FOR PHYTOCHEMICAL ANALYSIS

The *Glycyrrhiza glabra* plant extract was prepared for phytochemical analysis following a standardized procedure. Initially, *G. glabra* stem powder was dried in a hot air oven at temperatures below 60°C and subsequently sieved to ensure uniform particle size. A total of 100 grams of the dried powder was then subjected to a continuous hot extraction process using methanol in a Soxhlet apparatus. The powder was immersed in methanol for a duration of six hours to facilitate efficient extraction of bioactive constituents.

Following extraction, the resulting extracts were dried and filtered using a rotary evaporator to remove excess solvent and impurities. The final polar extract obtained after filtration was stored at a low temperature to maintain its integrity and stability for subsequent phytochemical analysis. This standardized extraction method ensured the extraction of a wide range of phytochemicals from *G. glabra stem*, laying the groundwork for further investigation into its bioactive constituents and potential therapeutic properties.

3.2 PREPARATION OF SHRIKHAND POWER ANDEXTRACT FOR PHYTOCHEMICAL ANALYSIS

The preparation of *Shrikhand* powder and extract involved several key steps to ensure the extraction of bioactive compounds. Initially, the *Shrikhand* product was evenly spread onto a petri plate and dried in a hot air oven at a temperature below 60°C, resulting in a powdered form suitable for further analysis. For the extraction process, a continuous method using methanol was adopted, with 100 grams of the dry powder being agitated in a shaking incubator with methanol for six hours. Following this extraction period, the polar extract was obtained after filtration, and it was stored at a low temperature to preserve its integrity and facilitate subsequent research aimed at establishing standard parameters. This meticulous extraction process aimed to extract a wide range of bioactive compounds from the *Shrikhand*

product, paving the way for further investigation into its potential health benefits and applications.

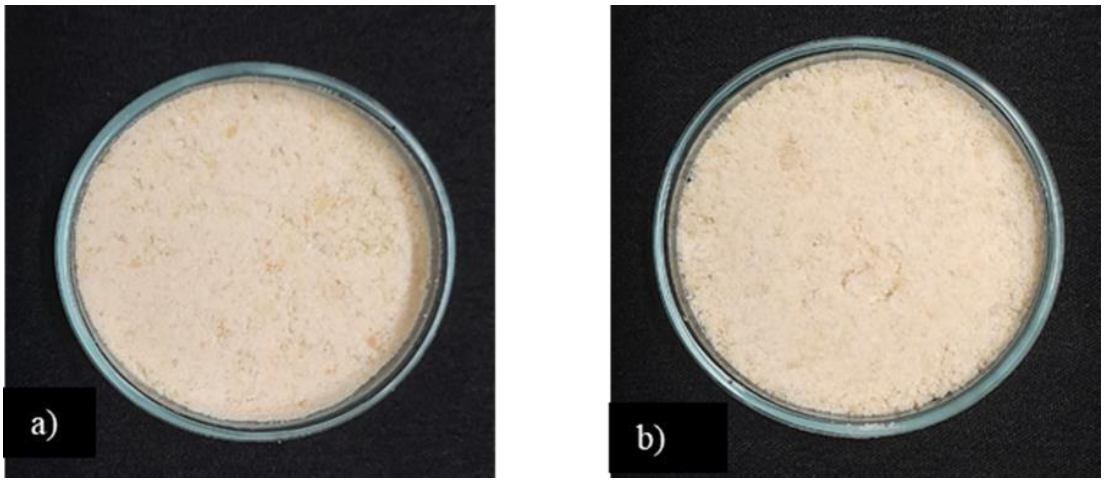

Figure 3.4 Dried powder of Shrikhand Prototype: a) T_1(1% w/v Jestamadh extract)and b) T_2 (2% w/v Jestamadh extract)

3.3 THIN LAYER CHROMATOGRAPHY (TLC) ANALYSIS OF *GLYCYRRHIZA GLABRA*

To identify the bioactive constituents, present in the crude plant extract, a qualitative chemistry analysis was conducted using thin layer chromatography (TLC). The extracts were examined for phenolic compounds, flavonoids, and essential oils, following the methodology outlined by Husain *et al*. (2015). Aluminium foil-backed silica gel plates with dimensions of 10 cm x 5 cm were utilized for the experiment. Different solvent systems, selected based on polarity, were employed to evaluate various classes of compounds present and to separate the bioactive constituents such as phenolic compounds, flavonoids, and essential oils.

The methanolic extract was spotted as concentrated bands onto a single TLC plate, positioned 1.5 cm from the edge, and allowed to dry. Subsequently, the spots were examined under visible light, long UV, and short UV both before and after spray drying with a derivatizing agent, as per the methodology described by Naik and Sellappan (2020).

This comprehensive TLC analysis aimed to provide insights into the composition of bioactive compounds present in the Glycyrrhiza glabra extract, laying the foundation for further characterization and identification of its potential therapeutic properties.

3.4 SENSORY EVALUATION

The sensory evaluation of the product aimed to assess its attributes, including colour, aroma, flavour, texture, and overall acceptability, utilizing a 5-point hedonic scale (where 5 denotes extremely liked and 0 represents extremely disliked). To ensure unbiased evaluation, samples of the yogurt were coded and arranged randomly before presentation in clear plastic cups under daylight conditions, as outlined by Kumar *et al*. (2022). Panellists were instructed to evaluate each sample individually, considering its appearance, scent, taste, mouthfeel, and overall impression. Prior to the evaluation, panellists were provided with clear instructions on

how to use the hedonic scale and were encouraged to cleanse their palate between samples with water or unsalted crackers. The sensory evaluation process was conducted in a controlled environment to minimize external influences on the panellists' judgments, ensuring the reliability and accuracy of the sensory data collected. **(Table 4.5)**

3.5 STORAGE STUDY

During the storage study, the probiotic *Shrikhand* samples were maintained at a consistent refrigeration temperature of 5 ± 1°C to simulate typical storage conditions. Over a period of ten days, the samples underwent daily analysis to monitor any changes or alterations in their properties. Parameters such as microbial growth, pH levels, sensory attributes, and overall product stability were assessed at regular intervals to evaluate the impact of storage on the quality and shelf-life of the probiotic *Shrikhand* formulation.

This controlled storage study provided valuable insights into the product's stability and allowed for the determination of an optimal storage duration to maintain its freshness and efficacy.

3.6 PHYSICO-CHEMICAL ANALYSIS OF OPTIMIZED PROBIOTIC SHRIKHAND

The physicochemical analysis of the optimized probiotic *Shrikhand*, including parameters such as titratable acidity, moisture content, and total solids, was conducted following established methodologies outlined in Indian Standards (1980; 1981). The pH measurement was performed using a digital pH meter, adhering to standard procedures as described in previous studies (Jadhav & Khedekar *et al.*, 2019).

These standardized methods ensured accurate and reliable determination of key physicochemical characteristics, allowing for comprehensive assessment of the probiotic *Shrikhand's* quality and composition.

3.6.1 Total Titrable Acidity

For the determination of total acidity, 10 grams of the Shrikhand samples were diluted with 30 mL of water and titrated against 0.1N NaOH solution, with phenolphthalein employed as the indicator.

The total acidity was calculated using the formula:

$$Total Acidity = \frac{\text{Titre value X N of alkali X volume made up X equivalent wt.of acid X 100}}{\text{volume of sample taken X 100}}$$

This calculation allowed for the determination of the total acidity of the Shrikhand samples, expressed as a percentage.

3.6.2 Moisture

The moisture percentage of yogurt was determined using the formula recommended by AOAC (1998, No: 990.20) and as described by Kiros *et al*. (2016):

$$Moisture\ \% = 100 - Total\ solid\ \%$$

This calculation provided the moisture content of the yogurt samples as a percentage.

3.7 MICROBIAL STUDIES OF THE *SHRIKHAND*

The *Shrikhand* prototypes were stored at a controlled temperature of 5±1°C throughout the 15-day testing period. Probiotic counts were conducted at the beginning and end of the storage period to assess microbial viability. To determine coliform, yeast, and mould counts, samples were diluted to 10^3 and plated on specific media: L. acidophilus-MRS agar for probiotic counts, Violet Red Bile agar for coliform counts, and Potato Dextrose Agar (PDA) for yeast and mould counts. Anaerobic conditions were maintained for the corresponding product sets during the storage studies, ensuring accurate microbial assessments (Jadhav & Khedekar *et al.*, 2019).

3.8 INCORPORATION OF ENCAPSULATED *ANNONA MURICATA* LEAF EXTRACT IN *JESTAMADH* STEM EXTRACT ENRICHED *SHRIKHAND*

The yogurt was divided into two portions: T_1 and T_2, containing 1.25 g and 2.5 g, respectively, of encapsulated Annona muricata leaf extract in *Jestamadh* stem extract- enriched *Shrikhand*. Encapsulation of the Annona muricata leaf extract was achieved using the optimized method developed by Miss Priya Velip.

For encapsulation, a 5% (w/v) solution of sodium alginate was prepared in distilled water, and the Annona muricata leaf extract was added to this solution. The mixture was homogenized using a high-speed homogenizer until a homogeneous dispersion was obtained. Subsequently, this dispersion was dropwise added to a 2% (w/v) solution of calcium chloride, acting as the crosslinking agent, under constant stirring using a magnetic stirrer.

The resulting gel beads were allowed to cure for 30 minutes, followed by thorough washing with distilled water to remove any excess calcium chloride. Finally, the encapsulated Annona muricata leaf extract was added to the *Jestamadh* stem extract-enriched *Shrikhand* samples. All yogurt samples were then stored at 4°C for 15 days to assess the antioxidant activity of the experimental *Shrikhand*, as described by Hashim *et al*. (2022).

3.9 TOTAL PHENOLIC CONTENT OF *SHRIKHAND* PROTOTYPE WITH ENCAPSULATED *A. MURICATA*

The total phenolic content of the samples was determined using the Folin-Ciocalteu method, as described by Dudonné*et al*. (2009). The method involved the preparation of solutions of each extract (50 µL; 1 mg/mL) in test tubes, followed by the addition of distilled water to reach a final volume of 0.5 mL. Subsequently, 2.5 mL of a 10-fold diluted Folin- Ciocalteu reagent was added to each solution, and the flasks were thoroughly shaken. After 1 minute, 2.0 mL of a 7.5% Na_2CO_3 solution was added to the mixtures, which were then

allowed to stand for 30 minutes at 25°C with intermittent shaking. The absorbance of the solutions was measured at 760 nm. All tests were conducted in triplicate to ensure accuracy and reproducibility of the results.

3.10 ANTIOXIDANT ACTIVITYWITH ENCAPSULATED *A. MURICATA*

The antioxidant activity of the *Shrikhand* samples was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical inhibition assay, following the method described by Dudonné*et al*. (2009). The antiradical activity against 2,2-diphenyl-1-picrylhydrazyl (DPPH) was assessed according to the protocol outlined by Nishino *et al*. (2000), with slight modifications in the sample preparation and reaction volumes. DPPH solution was prepared by dissolving 8 mg of DPPH in 100 mL of absolute ethanol. Subsequently, 2.0 mL of the DPPH solution was mixed with 1 mL of the diluted extract solution. The resulting solutions were then incubated for 30 minutes at 37°C. Following the incubation period, the absorbance of the solutions was measured at 517 nm against ethanol using a UV-Vis spectrophotometer.

The proportion of the DPPH radical inhibition by the sample was calculated by the following formula:

$$\text{DPPH scavenging activity (\%)} = \frac{(AT0 - ATx)}{(AT0)} \text{x}100\%$$

Where, AT_0 = absorbance value of the control reaction AT_x = absorbance value of each extract.

CHAPTER 4. RESULTS AND DISCUSSION

4.1 OPTIMIZATION OF *JESTAMADH* EXTRACT PROPORTIONS

In the optimization of *Jestamadh* extract proportions, various treatments were evaluated by incorporating different concentrations of aqueous herbal extract of *Jestamadh* into *Shrikhand*. It was found that the addition of jaggery and date powder did not significantly alter the taste of the *Shrikhand*. However, upon adding 1% and 2% aqueous herbal extract of *Jestamadh*, the resulting products were deemed acceptable based on sensory parameters and were selected for further analysis (**Table 4.1**).

This suggests that the incorporation of *Jestamadh* extract at these concentrations does not adversely affect the overall sensory characteristics of the *Shrikhand*, indicating its potential for further development as a functional food product. These results are in line with previous findings, which have also highlighted the compatibility of *Jestamadh* extract with dairy-based products.

Further investigation into the biochemical, physicochemical, and functional properties of the optimized formulations is warranted to better understand their potential health benefits and applications(Singh *et al.*, 2022).

Table 4.1. Details of different treatment

Treatment	***Jestamadh* stem extract enriched *shrikhand* [1: 10 (w/v)]**
T_1	1%
T_2	2%

4.2 IDENTIFICATION OF BIOACTIVE COMPOUNDS (PHENOLS, FLAVONOIDS AND ESSENTIAL OILS) IN *JESTAMADH* STEM EXTRACT ENRICHED *SHRIKHAND* USING TLC

In the quantification of bioactive compounds, TLC analysis was conducted to detect the presence of phenolic compounds, essential oils, and flavonoids in the enriched *Shrikhand* samples. The TLC study revealed the presence of dark blue, fluorescent compounds, indicating the presence of these bioactive constituents. Various compositions of the mobile phase were tested to optimize the separation of compounds on the TLC plates.

Based on the TLC profiles, the retention factor (Rf) values were calculated, which allowed for the identification of spots corresponding to phenols, essential oils, and flavonoids with different Rf values. The detailed results, including the Rf values for each compound, are presented in **Table 4.2, Table 4.3,** and **Table 4.4**, respectively. These findings provide valuable insights into the composition of bioactive compounds present in the enriched *Shrikhand* formulations, laying the foundation for further quantitative analysis and exploration of their potential health benefits.The bands were identified for essential oils using vanillin sulphuric acid reagent as a spraying agent which showed blue colour bands.

The TLC analysis was performed using different solvent systems to identify possible compounds present in *Jestamadh* extract and *Jestamadh* stem extract enriched *Shrikhand* samples. For the *Jestamadh* extract, the TLC analysis revealed the presence of four distinct spots with Rf values ranging from 0.33 to 0.82. These spots exhibited varying colors upon treatment with 10% ferric chloride solution, indicating the presence of unknown compounds and phenolic compounds.

Similarly, for the *Jestamadh* stem extract enriched Shrikhand samples (1% w/v), three distinct spots were observed with Rf values ranging from 0.37 to 0.8. These spots also

displayed different colors upon treatment with the ferric chloride solution, suggesting the presence of unknown compounds and phenolic compounds(**Plate 4.1, Plate 4.2** and **Plate 4.3**).

In the analysis for flavonoids, the TLC results showed three distinct spots for both the *Jestamadh* extract and the *Jestamadh* stem extract enriched *Shrikhand* samples (1% w/v and 2% w/v). These spots had Rf values ranging from 0.17 to 0.84 and exhibited a fluorescent colour when treated with 10% methanolic sulphuric acid, indicating the presence of flavonoids.

Additionally, the TLC analysis for essential oils revealed the presence of distinct spots with Rf values ranging from 0.21 to 0.8 for the *Jestamadh* extract and *Jestamadh* stem extract enriched Shrikhand samples (1% w/v and 2% w/v). These spots appeared dark blue or yellow when treated with vanillin sulphuric acid, suggesting the presence of essential oils and other unidentified compounds.

Among the formulations tested, the *Jestamadh* stem extract enriched Shrikhand with a concentration of 1% w/v and 2% w/v exhibited the highest number of spots for phenolic compounds, flavonoids, and essential oils, indicating a greater diversity of bioactive compounds compared to other formulations. This formulation showed three distinct spots for phenolic compounds, three spots for flavonoids, and two spots for essential oils, suggesting a rich profile of bioactive constituents with potential health benefits.

Overall, the TLC analysis provided valuable insights into the composition of bioactive compounds present in *Jestamadh* extract and *Jestamadh* stem extract enriched Shrikhand samples, laying the groundwork for further characterization and quantification of these compounds.

Table 4.2. Retention factor (Rf) values for samples showing possible compounds tested for phenolic compound

Solvent system	Sample	No. of distinct spots	Rf value	Colour with 10% Ferric chloride solution	Possible compound
Tetrahydrofuran: toluene: formic acid: water (16:8:2:1) v/v/v/v	*Jestamadh* extract	4	0.33	Light – yellow	Unknown
			0.53	Light – yellow	Unknown
			0.77	Dark blue	Phenolic compound
			0.82	Light – yellow	Unknown
	Jestamadh stem extract enriched shrikhand (1% w/v)	3	0.37	Light – yellow	Unknown
			0.69	Light – yellow	Unknown
			0.8	Dark blue	Phenolic compound
	Jestamadh stem extract enriched shrikhand (1% w/v)	3	0.37	Light – yellow	Unknown
			0.6	Light – yellow	Unknown
			0.77	Dark blue	Phenolic compound

Table 4.3. Retention factor (Rf) values for sample showing possible compounds tested for flavonoids

Solvent system	Sample	No. of distinct spots	Rf value	Colour with 10% methanolic sulphuric acid	Possible compound
Tetrahydrofuran: toluene: formic acid: water (16:8:2:1) v/v/v/v	*Jestamadh* extract	3	0.17	Fluorescent compound	Flavonoid
			0.25	Fluorescent compound	Flavonoid
			0.73	Fluorescent compound	Flavonoid
	Jestamadh stem extract enriched shrikhand (1% w/v)	3	0.2	Fluorescent compound	Flavonoid
			0.78	Fluorescent compound	Flavonoid
			0.84	Fluorescent compound	Flavonoid
	Jestamadh stem extract enriched shrikhand (2% w/v)	3	0.26	Fluorescent compound	Flavonoid
			0.77	Fluorescent compound	Flavonoid
			0.84	Fluorescent compound	Flavonoid

Table 4.4. Retention factor (Rf) values for sample showing possible compounds tested for essential oils

Solvent system	Sample	No. of distinct spots	Rf value	Colour with vanillin sulphuric acid	Possible compound
Toluene: Ethyl acetate (9.3:0.7) v/v	*Jestamadh* extract	1	0.21	Dark blue	Essential oils
			0.42	Dark blue	Essential oils
			0.8	Yellow	Unknown
	Jestamadh stem extract enriched shrikhand (1% w/v)	2	0.46	Dark blue	Essential oils
			0.73	Dark blue	Essential oils
	Jestamadh stem extract enriched shrikhand (2% w/v)	2	0.50	Dark blue	Essential oils
			0.8	Dark blue	Essential oils

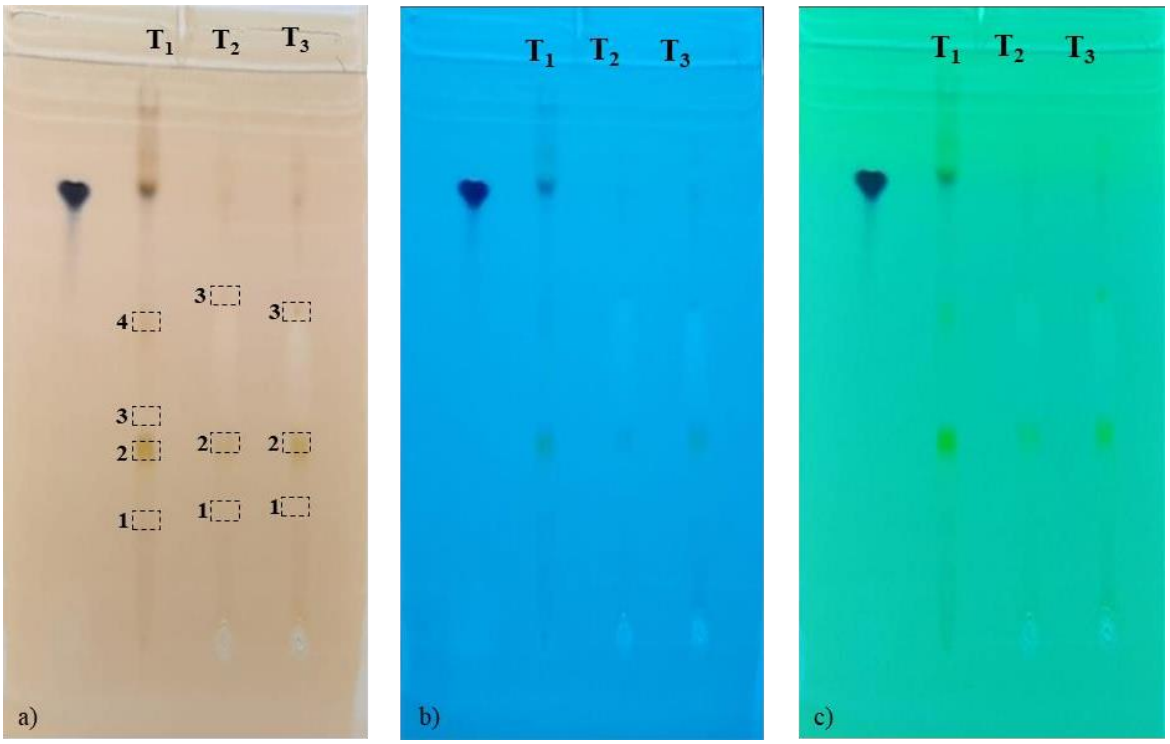

Plate 4.1 TLC: Isolation of phenolic compound after derivatization a) under visible light b) under short UV c) under long UV

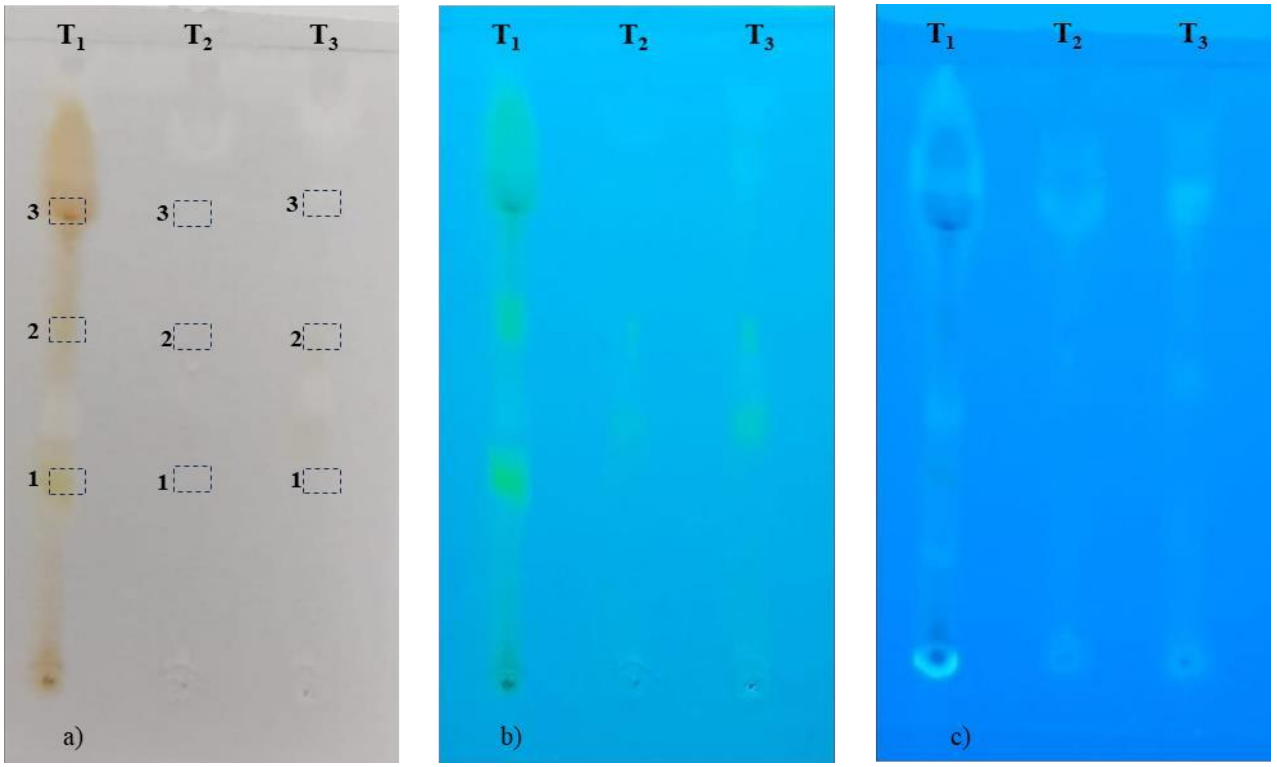

Plate 4.2 TLC: Isolation of flavonoids after derivatization a) under visible light b) under short UV c) under long UV

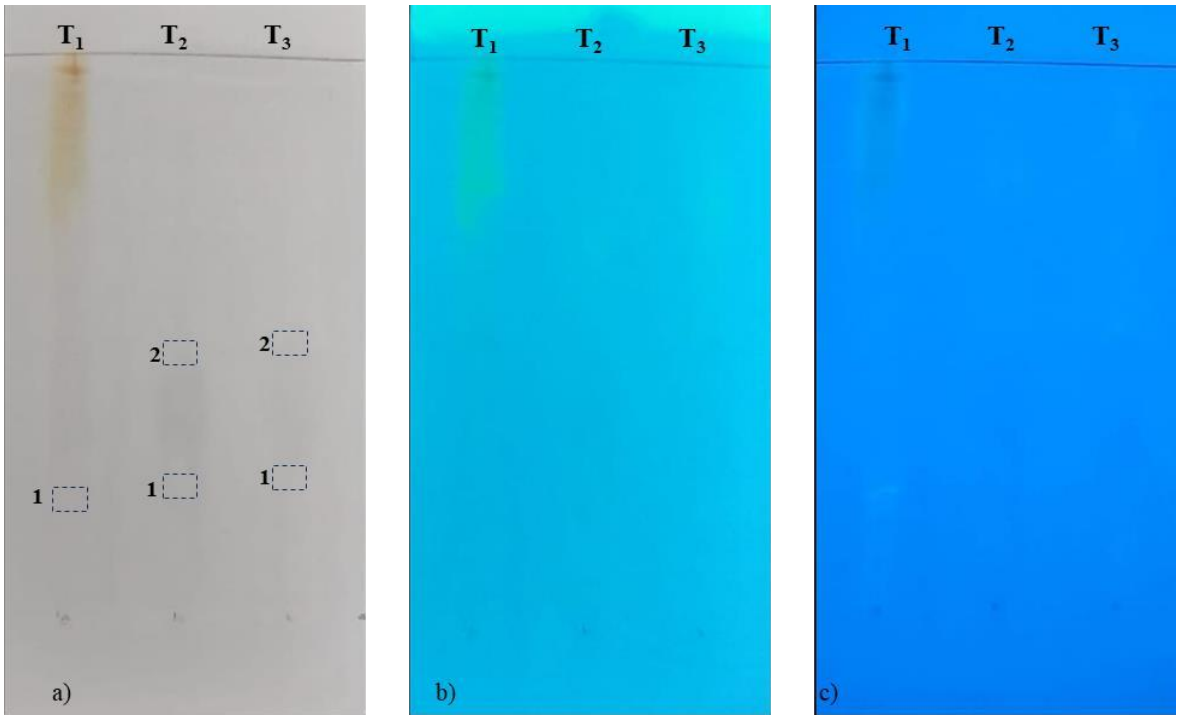

Plate 4.3 TLC: Isolation of essential oil after derivatization a) under visible light b) under short UV c) under long UV

4.3 EVALUATION OF SENSORY ATTRIBUTES IN HERBAL *SHRIKHAND*

The sensory evaluation scores revealed that the *Jestamadh* stem extract enriched *shrikhand* exhibited the highest scores for all sensory attributes, including taste (3.85 ± 0.35), sweetness (3.76 ± 0.43), texture (3.66 ± 0.48), aroma (3.09 ± 0.76), and appearance (2.57 ± 0.67). In comparison, shrikhand incorporated with date powder and jaggary scored lower on these attributes. Therefore, *Jestamadh* stem extract enriched shrikhand was selected for further analysis. The sensory evaluation scores are summarized in **Table 4.5**. The results indicate that the *Jestamadh* stem extract enriched shrikhand not only exhibited superior sensory attributes but also maintained consistency across multiple sensory parameters. This suggests that the incorporation of *Jestamadh* stem extract enhances the overall quality of the shrikhand, making it a promising candidate for further development as a functional food product.

Table 4.5. Sensory attributes of herbal *Shrikhand*

Treatment	Appearance	Aroma	Taste	Sweetness	Texture
T_1	2.57 ± 0.67	3.09 ± 0.76	3.85 ± 0.35	3.76 ± 0.43	3.66 ± 0.48
T_2	2.42 ± 0.5	2.85 ± 0.65	3.38 ± 0.74	3.38 ± 0.49	2.95 ± 0.74
T_3	2.19 ± 0.4	2.52 ± 0.6	3.14 ± 0.65	2.95 ± 0.66	2.33 ± 0.79

T_1 = *Jestamadh* stem extract enriched *shrikhand*; T_2 = Date powder enriched *shrikhand*; T_3 = Jaggary enriched *shrikhand*. Results are as reported as average ± standard deviation of triplicate measurement.

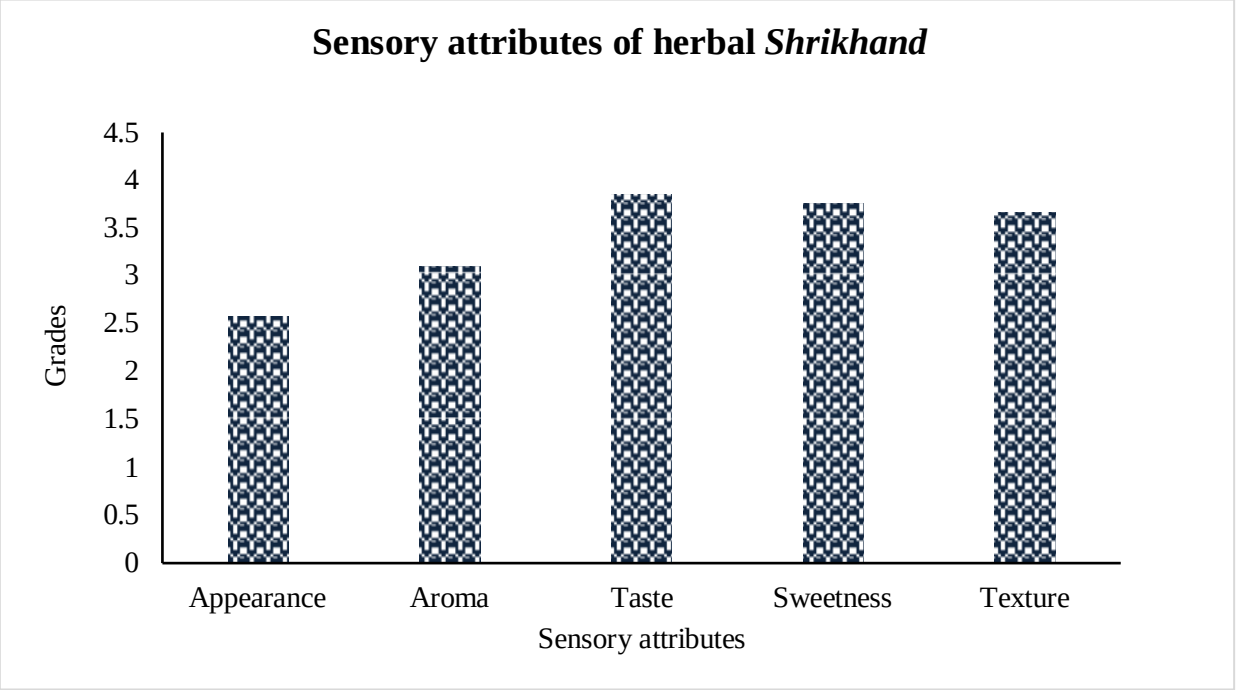

Figure 4.1. Sensory attributes of herbal shrikhand

4.3 PHYSICO-CHEMICAL ANALYSIS OF OPTIMIZED PROBIOTIC SHRIKHAND PROTOTYPE

The moisture content and total solids of herbal *Shrikhand* formulated with different levels of *Jestamadh* extract were analyzed. Sample T2, containing *Jestamadh* extract at a rate of 2%, was selected based on its appealing sensory attributes. The mean values for the parameters are summarized in **Table 4.6**.

The analysis reveals that both formulations of *Jestamadh* stem extract enriched *Shrikhand* exhibit similar moisture content and total solids. However, the sample containing 2% *Jestamadh* extract shows a higher total phenolic content compared to the 1% extract formulation. This suggests that increasing the concentration of *Jestamadh* extract enhances the bioactive compounds present in the *Shrikhand*, potentially contributing to its health benefits.

Additionally, both formulations exhibit comparable pH and titrable acidity, indicating similar levels of acidity and overall stability. (Singh *et al*., 2022)

4.5 pH AND TITRATABLE ACIDITY

The pH and titratable acidity of herbal *Shrikhand* enriched with *Jestamadh* stem extract were investigated. The pH values (**Figure 4.2**) and titratable acidity of the *Shrikhand* were found to be higher in the sample containing 2% *Jestamadh* stem extract compared to the sample with 1% extract.

This observation is consistent with previous findings by David (2015), who reported increased pH and titratable acidity in herbal *Shrikhand* fortified with basil extract.

The mean pH values for the herbal *Shrikhand* formulations are 3.784 ± 0.053 for T1 (1% *Jestamadh* stem extract) and 3.785 ± 0.08 for T2 (2% *Jestamadh* stem extract).

Regarding titratable acidity, the mean value for T1 is 1.441 ± 0.265, while for T2, it is 1.327 ± 0.146 (**Table 4.6**).

These results suggest that the incorporation of a higher concentration of *Jestamadh* stem extract leads to an increase in both pH and titratable acidity of the *Shrikhand*. This could be attributed to the presence of bioactive compounds in the extract, which may influence the acidity of the product. Further studies are needed to elucidate the specific mechanisms underlying these observations.

Table 4.6.Physico-chemical parameters of herbal Shrikhand

Parameters	Treatment	
	T_1	T_2
Moisture content (%)	94.070 ± 1.038	94.048 ± 2.069
Total solid (%)	5.951 ± 2.069	5.929 ± 1.038
pH	3.784 ± 0.053	3.785 ± 0.08
Titrable acidity (% lactic acid)	1.441 ± 0.265	1.327 ± 0.146
Total phenolic content (µg/mL)	1.267 ± 0.021	2.157 ± 0.022

T_1: *Jestamadh* stem extract enriched shrikhand 1% (w/v); T_2: *Jestamadh* stem extract enriched shrikhand 2% (w/v). Results are as reported as average ± standard deviation of triplicate measurement.

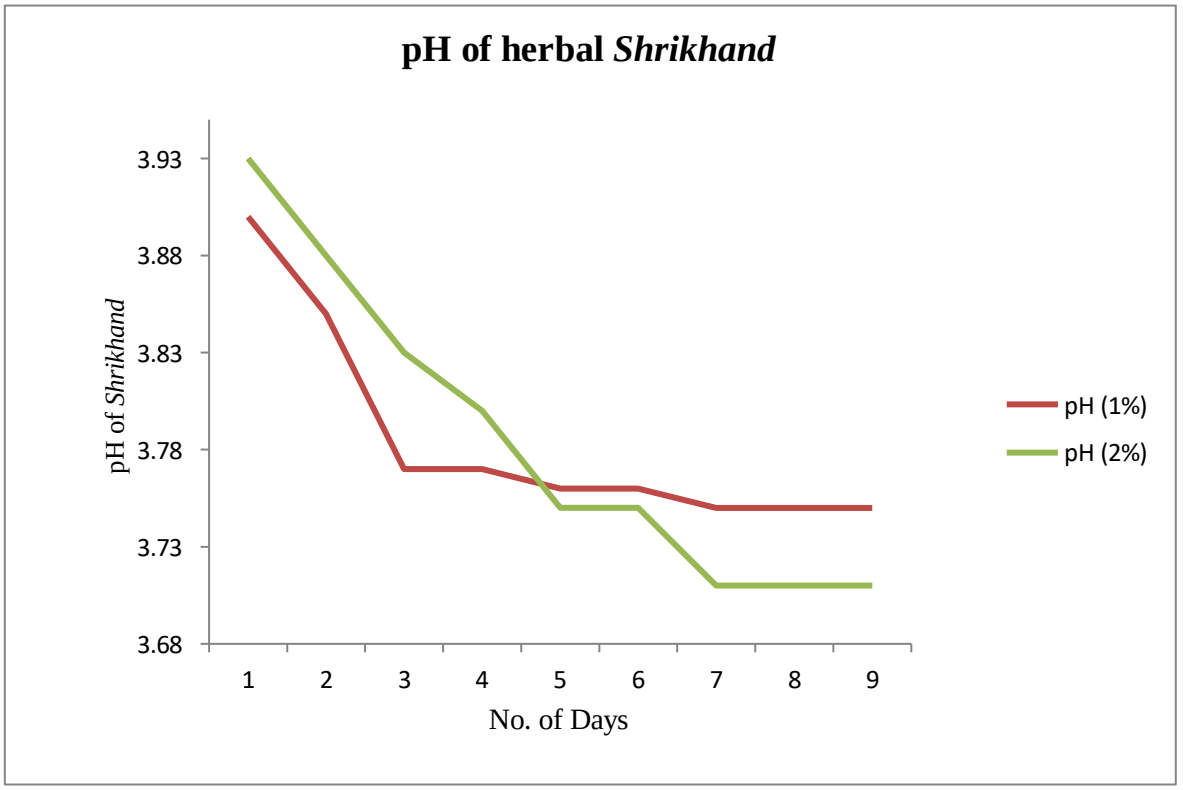

Figure 4.2. Effect on pH of herbal shrikhand

4.6 MICROBIAL STUDIES OF THE OPTIMISED SHRIKHAND PROTOTYPE

Microbial analysis was conducted on herbal *Shrikhand*, and based on the sensory evaluation scores, sample T2 was selected for further microbiological analysis due to its superior acceptance. The microbial load in sample T2 was found to be lower compared to sample T1, indicating better microbial stability. The samples remained acceptable for consumption for up to 8 days, with spoilage observed after 9 days of storage, prompting termination of further microbial analysis (**Table 4.7, Plate 4.4**).

At the initial assessment (Day 0), none of the experimental samples showed the presence of yeast, molds, or Lactobacillus acidophilus. However, by Day 9, the microbial counts increased slightly, with L. acidophilus detected in both T1 and T2 samples. Interestingly, the presence of *L. acidophilus* suggests the retention of probiotic activity in the *Shrikhand* samples even after storage.

The extended shelf life of the herbal *Shrikhand* may be attributed to the antimicrobial activity of the herb extract added during preparation. Similar findings were reported by Ojha *et al*. (2018) in herbal *Shrikhand* fortified with basil and turmeric powder, which exhibited acceptability for 10 days, followed by spoilage after 15 days.

These results highlight the potential of herbal extracts in extending the shelf life of dairy products like *Shrikhand* by inhibiting microbial growth, thereby enhancing their safety and quality. Further research is warranted to elucidate the specific antimicrobial mechanisms of the herbal extracts and their effects on the overall microbial stability of dairy products.

Table 4.7. Microbial attributes of herbal Shrikhand

Days Intervals	Treatment	Yeast	Moulds	*L. acidophilus*
DAY 0	1%	ND	ND	ND
	2%	ND	ND	ND
DAY 6	1%	ND	ND	ND
	2%	ND	ND	ND
DAY 9	1%	ND	ND	1.333 ± 1.154
	2%	2 ± 1.732	ND	1.333 ± 1.154

ND: Not detected; Results are as reported as average ± standard deviation of triplicate measurement.

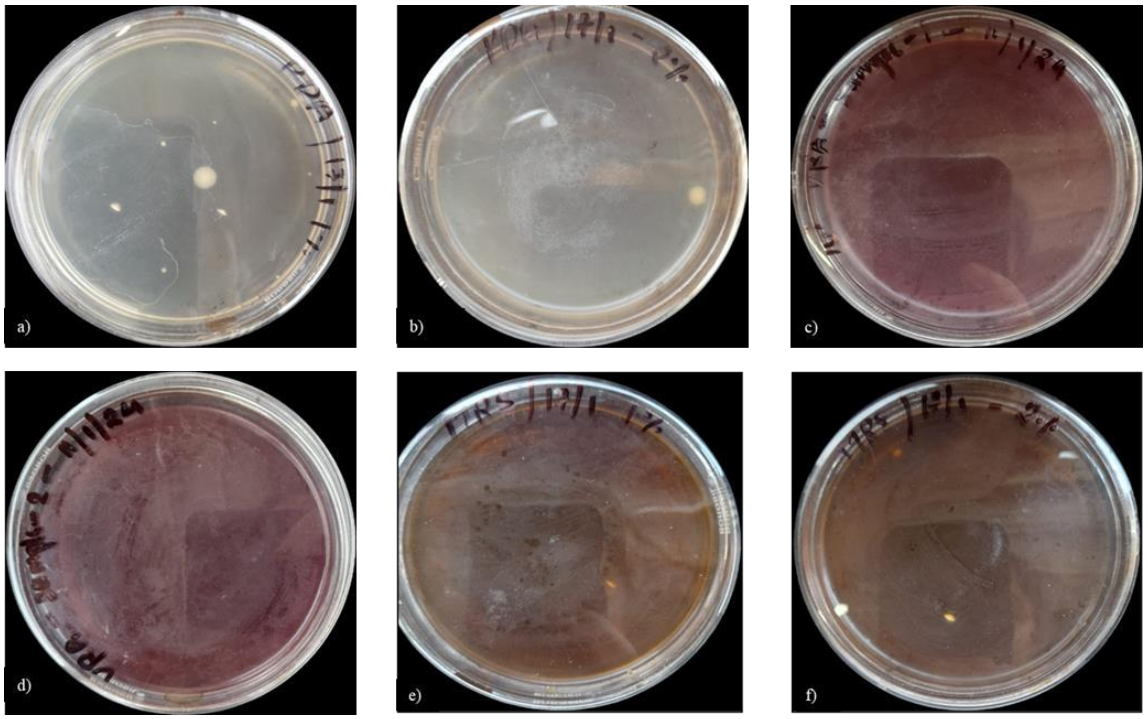

Plate 4.4 Microbial analysis: a) yeast count b) yeast count c) moulds count d) moulds count e) *L. acidophilus* countf) *L. acidophilus count*

4.7 INCORPORATION OF ENCAPSULATED ANNONA MURICATA LEAF EXTRACT IN *JESTAMADH* STEM EXTRACT ENRICHED *SHRIKHAND*

The encapsulation of *Annona muricata* leaf extract in *Jestamadh* stem extract-enriched *Shrikhand* was successfully achieved using the optimized method developed by Miss Priya Velip. Two different concentrations of encapsulated extract, designated as T_1 and T_2, were prepared and added to the *Shrikhand*prepared samples. The encapsulation process resulted in the formation of gel beads containing *Annona muricata* leaf extract dispersed within the *Shrikhand* matrix. Both T_1 and T_2 samples were stored at 4°C for 15 days to evaluate their stability and sensory attributes.

The successful incorporation of encapsulated *Annona muricata* leaf extract into *Jestamadh* stem extract-enriched *Shrikhand* holds promise for enhancing the functional properties of the product. Encapsulation provides a means to protect the bioactive compounds present in the *Annona muricata* leaf extract from degradation or loss of activity during processing and storage. The gel beads formed during encapsulation serve as carriers for the extract, allowing for controlled release and improved stability. This method offers a convenient way to introduce bioactive compounds into dairy products like *Shrikhand*, enhancing their nutritional and functional value.

Furthermore, the use of *Annona muricata* leaf extract adds potential health benefits to the *Shrikhand* due to its reported antioxidant and antimicrobial properties. By encapsulating the extract, these beneficial properties can be preserved and delivered effectively to consumers. The incorporation of *Annona muricata* leaf extract may also contribute to the development of unique flavour profiles in the *Shrikhand*, offering consumers a novel sensory experience.

Overall, the successful incorporation of encapsulated *Annona muricata* leaf extract in *Jestamadh* stem extract-enriched *Shrikhand*may open up opportunities for the development of functional dairy products with enhanced nutritional value and potential health benefits.

However, thorough clinical studies are necessary to investigate the impact of encapsulated extract on the physicochemical properties, shelf life, and consumer acceptance of the product.

The incorporation of encapsulated *Annona muricata* leaf extract in *Jestamadh* stem extract-enriched *Shrikhand* resulted in visually appealing enriched Shrikhand prototype with a creamy texture and uniform distribution of gel beads throughout the matrix. The *Shrikhand* samples exhibited a smooth texture and a pleasing appearance, indicative of their high quality and potential consumer acceptance. Additionally, the gel beads added a subtle crunchiness to the overall texture, enhancing the sensory experience of the product.

Although, the incorporation of encapsulated *Annona muricata* leaf extract in *Jestamadh* stem extract-enriched *Shrikhand* resulted in visually appealing samples with a smooth and creamy texture. However, it is crucial to emphasize that sensory evaluation, a cornerstone of food product development, was not conducted in this study. Sensory analysis is imperative to assess attributes such as taste, aroma, and overall acceptability, which are vital for consumer satisfaction. Additionally, further experimental validation is required to evaluate the stability, shelf life, and safety aspects of this prototype before considering it suitable for consumption. Such comprehensive assessments would contribute to a more robust understanding of the potential of this product in the food industry.

4.8 TOTAL PHENOLIC CONTENT ANALYSIS OF HERBAL SHRIKHAND

The total phenolic content of the produced herbal shrikhand was analyzed, revealing significant levels of phenolic compounds, as depicted in **Table 4.8**. Phenolic molecules are well-known for their antioxidant properties, as they act as effective hydrogen donors, neutralizing harmful free radicals in the body. This indicates a clear link between total phenolic content and antioxidant activity, as supported by previous studies (Tyagi *et al.*, 2020).

The herbal *Shrikhand*, enriched with *Jestamadh* extract, exhibited a high phenolic content, as evidenced by the mean value of 2.157 ± 0.022 in sample T_2. This suggests that *Jestamadh* extract, being rich in phenolic compounds, significantly contributes to the antioxidant potential of the *Shrikhand*. The presence of phenolic compounds in the *Shrikhand* makes it a valuable source of natural antioxidants, which are beneficial for human health. Further research could explore the specific phenolic compounds present in *Jestamadh* extract and their individual contributions to the antioxidant activity of the herbal *Shrikhand*. Moreover, additional studies could investigate the potential health benefits associated with the consumption of this phenol-rich *Shrikhand*, thereby elucidating its role in promoting overall well-being**(Figure 4.3 and Figure 4.4).**

Table 4.8 Total phenolic content in Shrikhand extract: T_1 andT_2

Parameters	**Treatment**	
	T_1	T_2
Total phenolic content (µg/mL)	1.267 ± 0.021	2.157 ± 0.022

Results are as reported as average ± standard deviation of triplicate measurement.

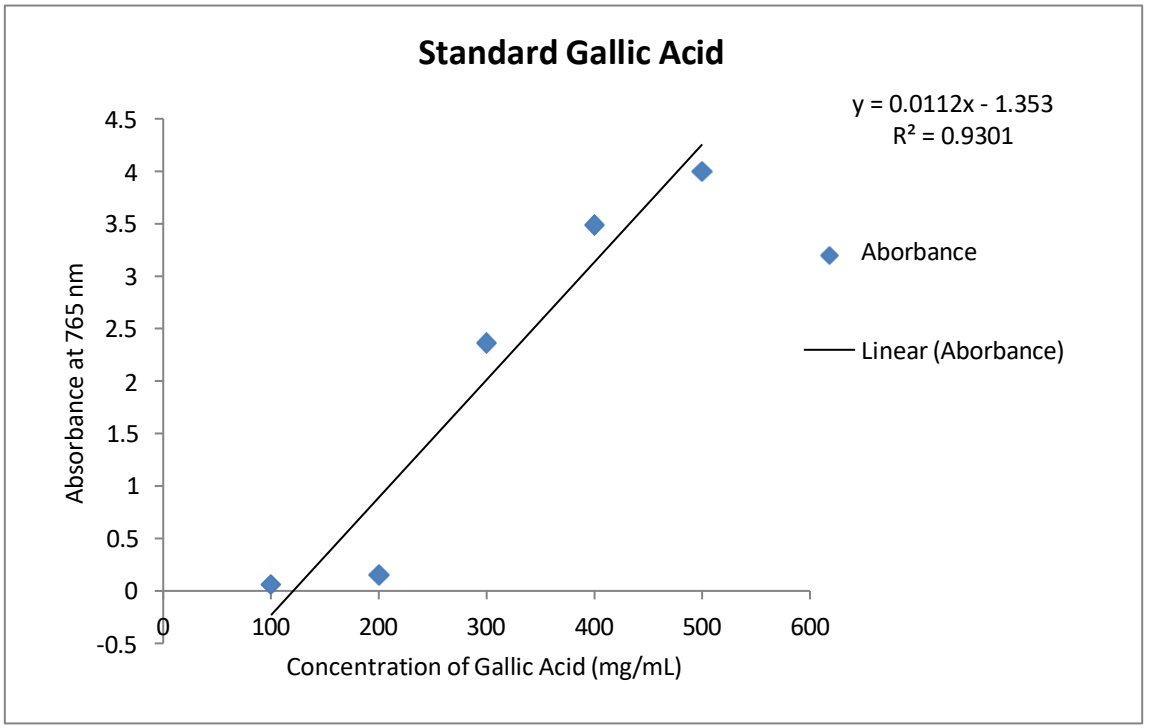

Figure 4.3. Calibration curve for gallic acid (mg/ml)

Figure 4.4. TPC (mg of GAE/g of extract)

4.9 ANTIOXIDANT ACTIVITY OF *JESTHAMADH* EXTRACT ENRICHED *SHRIKHAND* WITH AND WITHOUT ENCAPSULATED *ANNONA MURICATA* LEAF EXTRACT

The antioxidant activity of *Jesthamadh* extract enriched *Shrikhand* was evaluated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. The results, as shown in **Table 4.9**, **Figure 4.5**& **4.6**, indicate the percentage scavenging activity of DPPH by L-ascorbic acid and methanolic extract of *Jesthamadh* extract enriched *Shrikhand* before and after the incorporation of encapsulated Annona muricata leaf extract. It was observed that both concentrations of Jesthamadh extract enriched *Shrikhand* showed significant scavenging activity compared to the positive control (L-ascorbic acid), indicating the presence of antioxidant compounds in the *Shrikhand* samples. Additionally, the incorporation of encapsulated Annona muricata leaf extract appeared to enhance the antioxidant activity of *Jesthamadh* extract enriched *Shrikhand*, as evidenced by the increased percentage scavenging activity observed in the samples containing Annona muricata leaf extract.

In the DPPH free radical scavenging assay, the percent scavenging activity of DPPH by L-Ascorbic Acid and the Methanolic Extract of *Jestamadh* enriched *Shrikhand* (BE and AE) before and after the incorporation of encapsulated Annona muricata leaf extract was evaluated. The results indicated that the antioxidant activity varied depending on the concentration and the type of extract used. For instance, at a concentration of 100 µg/mL, the *Jestamadh* 2% (AE) formulation exhibited the highest percent inhibition (42.5 ± 0.001), followed by *Jestamadh*1% (AE) (54.166 ± 0.005), *Jestamadh* 2% (BE) (41.111 ± 0.001), *Jestamadh* 1% (BE) (35 ± 0.002), and L-Ascorbic Acid (0.011 ± 0.015).

Furthermore, the IC_{50} values, as presented in **Table 4.10**, provide a quantitative measure of the antioxidant potential of the *Shrikhand* samples. A lower IC_{50} value indicates higher

antioxidant activity. In this study, the IC50 values of Jesthamadh extract enriched Shrikhand with and without encapsulated *Annona muricata* leaf extract were compared with that of L-ascorbic acid, a known antioxidant. The results indicate that both concentrations of *Jesthamadh*extract enriched *Shrikhand* exhibited considerable antioxidant activity, with IC50 values lower than that of L-ascorbic acid. Moreover, the incorporation of encapsulated Annona muricata leaf extract further reduced the IC50 values of *Jesthamadh* extract enriched *Shrikhand,* suggesting enhanced antioxidant potential.

Moreover, the IC_{50} values, representing the concentration required to inhibit 50% of the DPPH radicals, were determined for each sample. L-Ascorbic Acid showed the lowest IC50 value of 56.347 µg/mL, indicating its high antioxidant potential. Among the *Jestamadh* extract enriched *Shrikhand* samples, *Jestamadh* 1% (AE) demonstrated the lowest IC50 value of 70.724 µg/mL, followed by *Jestamadh* 1% (BE) (154.471 µg/mL), *Jestamadh* 2% (BE) (281.708 µg/mL), and *Jestamadh* 2% (AE) (278.414 µg/mL).

These results suggest that the antioxidant activity of the *Shrikhand* formulations was enhanced after the incorporation of encapsulated *A. muricata* leaf extract. The observed antioxidant potential could be attributed to the synergistic effects of bioactive compounds present in both *Jestamadh*and *A. muricata* extracts.

indings are consistent with previous research by Pugazenthi*et al*. (2020), who evaluated the antioxidant activity of functional *Shrikhand* prepared with pomegranate (*Punica granatum*) fruit peel extract using the DPPH inhibition method. The agreement between the results of the current study and those reported by Pugazenthi*et al*. (2020) highlights the effectiveness of incorporating plant extracts into *Shrikhand* to enhance its antioxidant properties.

The observed differences in IC_{50} values among the *Jestamadh* extract enriched *Shrikhand* samples can be attributed to several factors, including the concentration of *Jestamadh* extract, the encapsulation process, and the interaction between bioactive compounds from *Jestamadh* and *Annona muricata* extracts**(Table 4.10, Figure 4.7)**.

Firstly, it is important to consider the concentration of *Jestamadh* extract in the *Shrikhand* formulations. The samples labeled as "1%" and "2%" refer to the concentration of *Jestamadh* extract used in the preparation of the *Shrikhand*. It's noteworthy that higher concentrations of bioactive compounds do not always correlate with increased antioxidant activity. In fact, at higher concentrations, certain compounds may exhibit pro-oxidant effects due to complex interactions with other components in the matrix. This phenomenon could potentially explain why *Jestamadh* 2% (BE) and *Jestamadh* 2% (AE) showed higher IC50 values compared to *Jestamadh* 1% (BE) and *Jestamadh* 1% (AE), respectively.

Secondly, the encapsulation process of *Annona muricata* leaf extract may have influenced the overall antioxidant activity of the *Shrikhand* samples. Encapsulation can alter the bioavailability and release kinetics of bioactive compounds, potentially affecting their interaction with free radicals in the DPPH assay. It's possible that the encapsulation of Annona muricata extract at higher concentrations (2%) resulted in a decrease in its antioxidant activity due to factors such as encapsulation efficiency and stability.

Furthermore, the interaction between bioactive compounds from *Jestamadh* and *A. muricata* extracts could have contributed to the observed differences in antioxidant activity. It is known that certain combinations of bioactive compounds can exhibit synergistic or antagonistic effects on antioxidant activity. In this case, the combination of *Jestamadh* and *A. muricata* extracts at different concentrations may have resulted in complex interactions, leading to varied antioxidant outcomes.

Overall, the differences in IC_{50} values highlight the importance of considering multiple factors, including extract concentration, encapsulation, and synergistic effects, when evaluating the antioxidant activity of herbal formulations. Further studies investigating the specific mechanisms underlying these observations would provide valuable insights into optimizing the formulation for enhanced antioxidant activity.

Table 4.9. DPPH free radical scavenging assay: % scavenging activity of DPPH by Ascorbic acid and Methanolic Extract of Jeshtmadh extract enriched shrikhand before and after incorporation of encapsulated *Annona muricata* leaf extract

Sr. No.	Concentration (µg/mL)	L-Ascorbic Acid	Methanolic Extract			
			Jestamadh 1% (BE)	*Jestamadh* 2% (BE)	*Jestamadh* 1% (AE)	*Jestamadh* 2% (AE)
1.	20	0.311 ± 0.003	12.5 ± 0.006	7.222 ± 0.001	41.296 ± 0.012	38.888 ± 0.001
2.	40	0.226 ± 0.001	22.037 ± 0.001	9.722 ± 0.001	45.74 ± 0.003	39.629 ± 0.001
3.	60	0.196 ± 0.002	24.444 ± 0.002	20.092 ± 0.059	48.518 ± 0.003	40.37 ± 0
4.	80	0.086 ± 0.002	30.277 ± 0.002	14.537 ± 0.006	51.759 ± 0.004	41.111 ± 0.001
5.	100	0.011 ± 0.015	35 ± 0.002	20.833 ± 0.003	54.166 ± 0.005	42.5 ± 0.001

Results are reported as % inhibition ± standard deviation of triplicate measurement.

Table 4.10. IC_{50} value of L-Ascorbic Acid and Methanolic Extract of Jeshtmadh extract enriched shrikhand before and after incorporation of encapsulated *Annona muricata* leaf extract

Sr. No.	Sample	IC_{50} value (µg/mL)
1.	L-Ascorbic Acid	56.3472926
2.	*Jestamadh* 1% (BE)	154.4718257
3.	*Jestamadh* 2% (BE)	281.7078652
4.	*Jestamadh* 1% (AE)	70.72418136
5.	*Jestamadh* 2% (AE)	278.4137931

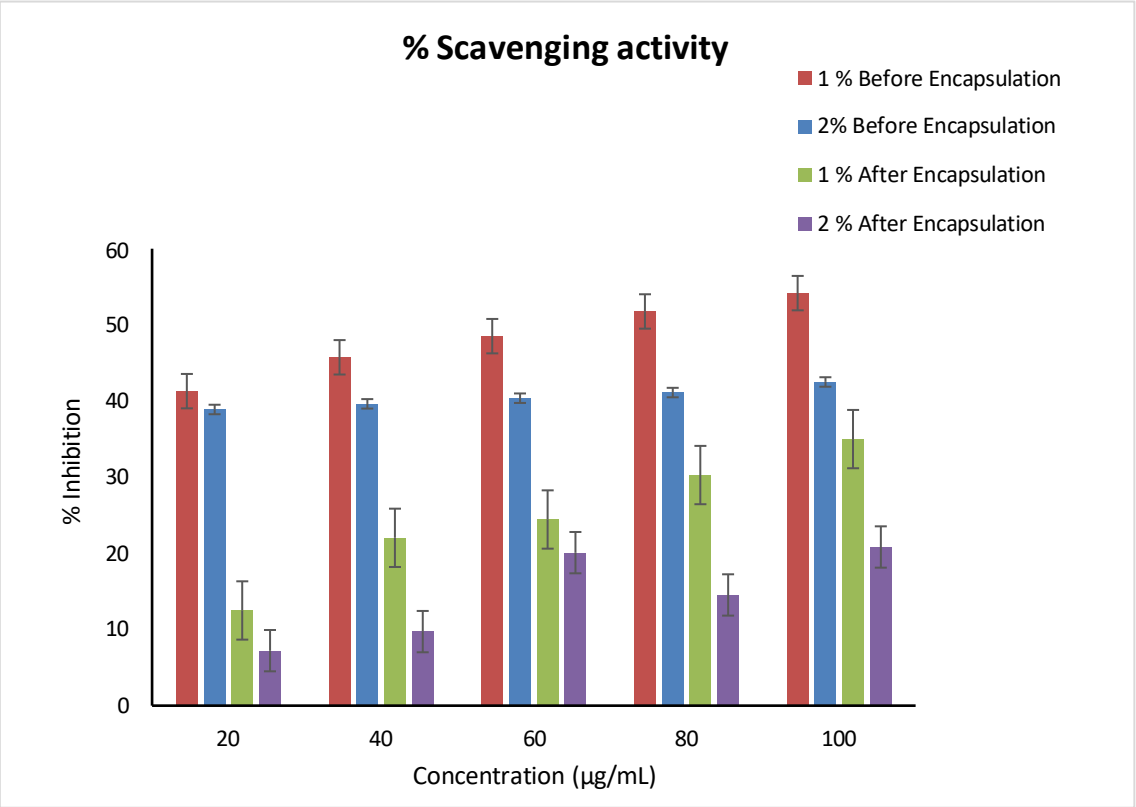

Figure 4.5. % scavenging activity of experimental shrikhand extract

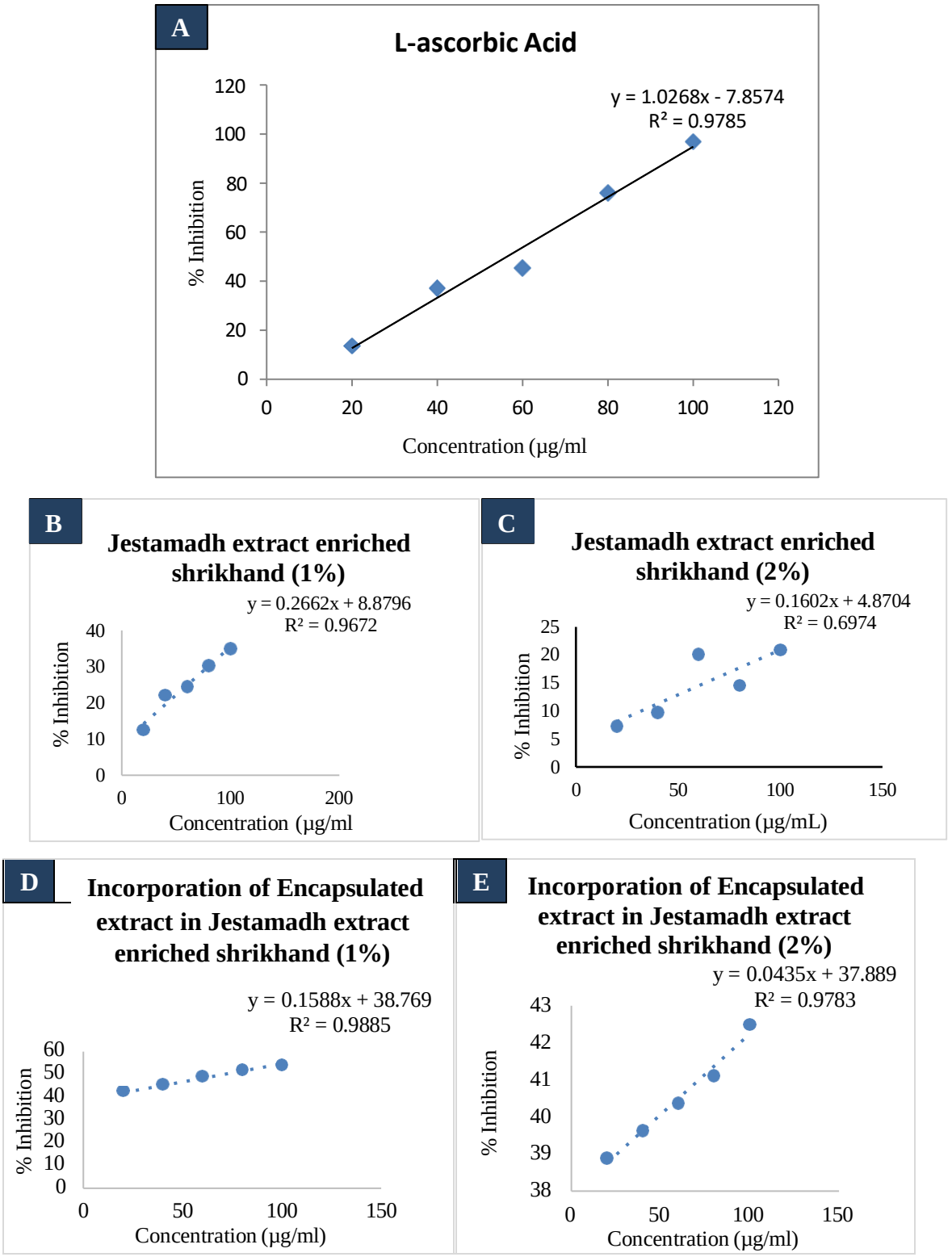

Figure 4.6. DPPH radical activity of (A) L-ascorbic Acid, (B) *Jestamadh* extract enriched shrikhand (1%) (C) *Jestamadh* extract enriched shrikhand (2%) (D) Incorporation of Encapsulated extract in *Jestamadh* extract enriched shrikhand (1%), (E) Incorporation of Encapsulated extract in *Jestamadh* extract enriched shrikhand (2%)

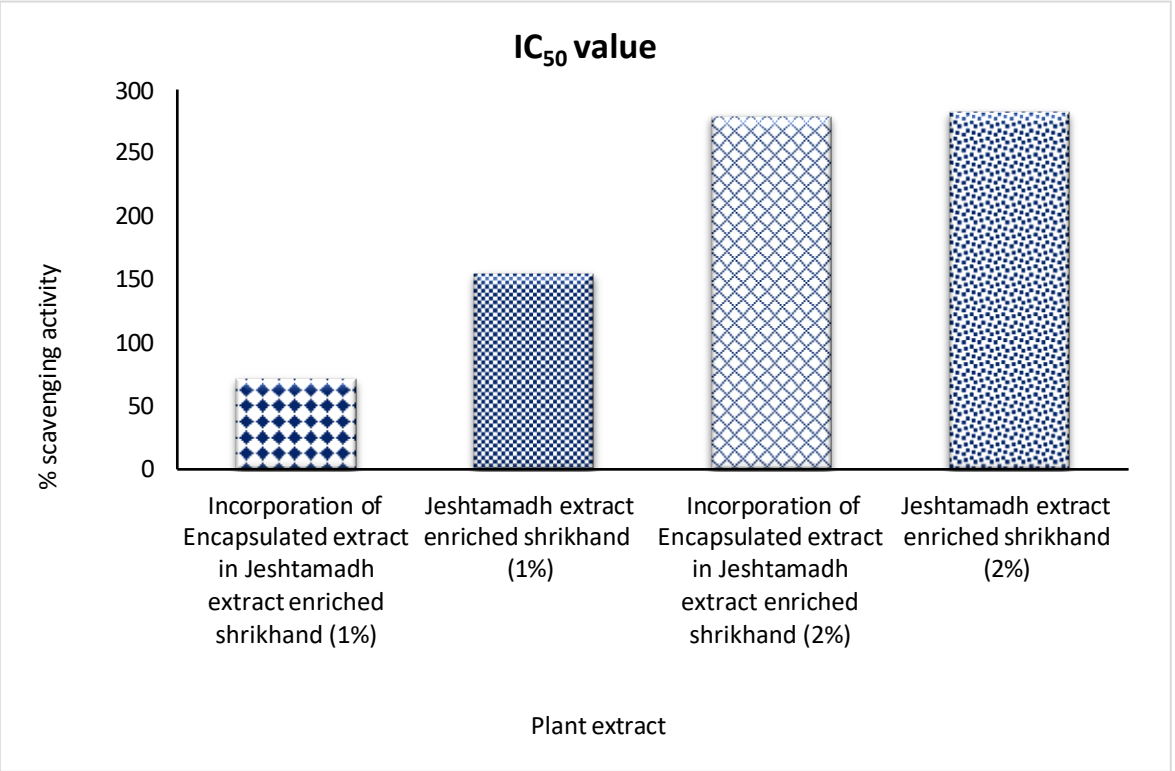

Figure 4.7. IC_{50} value of experimental shrikhand extract

CHAPTER 5. CONCLUSIONS

The investigation into herbal *Shrikhand* formulations enriched with *Jestamadh* stem extract yielded promising results. Treatment T_2, incorporating 2% *Jestamadh* extract, emerged as the optimal formulation, demonstrating superior sensory attributes compared to other formulations. With higher scores across multiple sensory parameters, including taste, aroma, and texture, the T_2 formulation underscored the efficacy of *Jestamadh* extract enrichment in enhancing the overall sensory experience of the *Shrikhand* product.

Furthermore, microbial analysis revealed that the T_2 formulation maintained acceptable microbial counts for up to eight days under refrigerated conditions, indicating a satisfactory shelf life for the developed product. These quantitative findings emphasize the practical feasibility and potential marketability of the optimized herbal *Shrikhand* formulation.

Moving forward, further research endeavours will be directed towards elucidating the safety and efficacy profiles of the developed *Shrikhand* formulation. Moreover, investigations into the optimization of encapsulated *A. muricata* leaf extract incorporation will be pursued to enhance the product's antioxidant properties. By combining quantitative assessments with comprehensive sensory evaluations, future studies aim to refine the formulation to meet consumer preferences while ensuring product safety and efficacy.

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