ULTRASOUND-ASSISTED EXTRACTION, OPTIMIZATION AND MICROENCAPSULATION OF BIOACTIVE COMPOUNDS FROM INDIAN BAEL LEAVES

BY

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KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND FOOD TECHNOLOGY

TAVANUR-679573,

MALAPPURAM, KERALA, INDIA

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PROJECT REPORT

Submitted in partial fulfilment of the requirement for the degree of

BACHELOR OF TECHNOLOGY IN AGRICULTURAL ENGINEERING

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DEPARTMENT OF PROCESSING AND FOOD ENGINEERING

KELAPPAJI COLLEGE OF AGRICULTURAL ENGINEERING AND FOOD TECHNOLOGY

TAVANUR - 679573,

MALAPPURAM, KERALA, INDIA

2025

DECLARATION

We hereby declare that this project report entitled **"ULTRASOUND-ASSISTED EXTRACTION, O P T I M I Z A T I O N A N D M I C R O E N C A P S U L A T I O N O F BIOACTIVE COMPOUNDS FROM INDIAN BAEL LEAVES"** is a bonafide record of research work done by us during the course of research and that the report has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

Place: Tavanur

Date: 02-06-2025

FATHIMA RINSHA K (2021-02-011)

NAYANTHARA S R (2021-02-033)

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CERTIFICATE

Certified that this project entitled **"ULTRASOUND-ASSISTED EXTRACTION, OPTIMIZATION AND MICROENCAPSULATION OF BIOACTIVE COMPOUNDS FROM INDIAN BAEL LEAVES"** is a bonafide record of project work jointly done by FATHIMA RINSHA K (2021-02-011), NAYANTHARA S R (2021-02-033), ARYA P (2021-02-036) and NIRANJAN P J (2021-02-038) under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to them.

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LIST OF SYMBOLS AND ABBREVIATIONS

%	:	Percentage
&	:	And
/	:	Per
+	:	Plus
=	:	Equal to
±	:	Plus or minus
°C	:	Degree Celsius
3D	:	Three-Dimensional
a*	:	Greenness or redness
ANOVA	:	Analysis of variance
aw	:	Water activity
b*	:	Blueness or yellowness
µm	:	micrometre
DPPH	:	2, 2-Diphenyl-1-picrylhydrazyl
et al.	:	And others
etc	:	Etcetera
Fig	:	Figure
g	:	Gram
GAE/g	:	Milligrams of gallic acid equivalents per gram
min	:	Minutes
i.e	:	That is
h°	:	Hue angle
KCAEFT	:	Kelappaji College of Agricultural Engineering and Food Technology

W	:	Watt
L*	:	Lightness or darkness
C*	:	Chroma
rpm	:	Rotation per minute
RSM	:	Response surface methodology
viz.,	:	Namely
mm	:	Millimetre
ml	:	Millilitre
m^3	:	Meter cube
MHz	:	Mega hertz
KHz	:	Kilo hertz
cm^3	:	Centimetre cube
UAE	:	Ultrasound-assisted extraction
TPC	:	Total phenol content
AA	:	Antioxidant activity
ROS	:	Reactive oxygen species
RSA	:	Radical scavenging activity
FDA	:	Food and Drug Administration
GRAS	:	Generally regarded as safe

CHAPTER I
INTRODUCTION

Indian Bael tree (*Aegle marmelos*), also known as the bael fruit tree, has leaves that possess a variety of medicinal properties and play a crucial role in traditional medicine systems such as Ayurveda, Unani, and Siddha. The leaves are renowned for their diverse therapeutic uses, which include treatment for respiratory conditions like asthma, digestive disorders, and infectious diseases (Sharma *et al*., 2011). Medicinally, bael leaves contain several bioactive compounds such as skimmianine, aegeline, and various essential oils, contributing to their analgesic, antifungal, and antioxidant activities (Patil, 2009; Rana *et al*., 1997). Bael leaves are not only utilized for their health benefits but also hold cultural significance, as they are often used in religious rituals.

The tree is well adapted to dry and semi-arid region thriving in marginal soils. The data from National Horticulture Board (NHB) during the year 2023 shows that major producing states include Uttarpradesh, Bihar, Jharkhand, Odisha, Madhyapradesh and Chhattisgarh. India is estimated to have approximately 25000 hectares under bael cultivation making it the largest producer globally. Uttarpradesh being the largest producing state in India with around 4000-5000 hectares of land dedicated to bael cultivation.

The extraction of bioactive compounds from plant leaves holds significant importance in the fields of pharmacognosy and nutraceuticals. Leaves, such as those from the Indian Bael tree (*Aegle marmelos*), are rich in phytochemicals, including flavonoids, polyphenols, and essential oils, which are known for their medicinal properties (Ramnik, 2008). These compounds exhibit antioxidant, anti-inflammatory, antimicrobial, and anticancer activities, making them valuable for health applications (Kaur *et al*., 2009).

Extraction poses significant challenges when isolating bioactive compounds from natural sources due to the complexity of raw materials, the necessity for efficient recovery methods, and concerns regarding environmental and health impacts. Traditional solid-liquid extraction (SLE) techniques, such as maceration, infusion, and Soxhlet extraction, are often time-consuming and require substantial amounts of solvents. However, issues regarding safety, toxicity, solvent residues, and low yields

have prompted increased interest in green extraction technologies aimed at minimizing or eliminating the use of organic solvents. These green methods are transforming the recovery of valuable compounds from natural resources by reducing environmental impacts and promoting sustainability. One notable approach within green extraction methodologies is ultrasound-assisted extraction (USAE), which effectively facilitates the extraction of high-value bioactive compounds from plant sources. This method offers several advantages, including improved extraction efficiency that conserves energy and the ability to operate at moderate temperatures, protecting heat-sensitive compounds.

Successful application of USAE requires optimization of process parameters like temperature, time, etc. Response Surface Methodology (RSM) has emerged as a valuable tool for optimizing such processes, particularly when independent variables exhibit non-linear and interactive effects. Applied across the chemical, pharmaceutical, and food industries.

Microencapsulation, particularly through techniques like spray drying, is a promising method to protect and stabilize these bioactive compounds (Ghangale *et al*., 2008). Spray drying involves converting a liquid solution containing the extracted bioactive substances into a fine powder by rapidly evaporating the solvent. This process offers numerous benefits, including enhanced stability, controlled release, and improved solubility of bioactive compounds, which can often be sensitive to environmental factors such as light, heat, and oxygen (Arul *et al*., 2005).

The present study entitled "Ultrasound-assisted extraction, optimization and microencapsulation of bioactive compounds from Indian bael leaves" was undertaken with following objectives:

- To characterize the physicochemical and bioactive properties of Indian bael (*Aegle marmelos*) leaves.
- To optimize ultrasound-assisted extraction (UAE) of bioactive compounds from bael leaves using Response Surface Methodology (RSM).
- To develop a spray dried microencapsulated product of bael leaf extract and evaluate its physicochemical properties.

CHAPTER II
REVIEW OF LITERATURE

This chapter reviews the previous research works carried out by research workers, scientists and students. Review related to Indian bael and its composition, therapeutic values, extraction methods, ultrasound assisted extraction, spray drying technology, microencapsulation, carrier materials, operating parameters of spray drying are included in this chapter.

2.1 INDIAN BAEL

Bael (*Aegle marmelos*), a member of the Rutaceae family, is a significant minor fruit crop native to the Indian subcontinent. It is renowned by various local names, including Bengal quince, bilva, Indian quince, golden apple, holy fruit, bel, belwa, sriphal and stone apple throughout the region (Singh *et al*., 2019). Bael tree, is a moderately sized, slender, and aromatic tree that reaches heights of 6.0 to 7.5 meters and has a girth of 90 to 120 cm (Sharma *et al*., 2011). It features a somewhat fluted trunk that can extend between 3.0 to 4.5 meters. This tree thrives in the deciduous forests of India and can be found at elevations up to 1200 meters in the western Himalayas, as well as on the Andaman Islands (Sharma *et al*., 2011).

The Bael tree originates from the Eastern Ghats and central India. It is native to the Indian subcontinent and predominantly thrives in tropical and subtropical regions. Additionally, it can be found growing wild in the lower Himalayan ranges at elevations of up to 500 meters. Bael is also present along the foothills of the Himalayas, in areas such as Uttarakhand, Jharkhand, Madhya Pradesh, the Deccan Plateau, and along the eastern coast (Sharma *et al.,* 2007). All parts of the Bael plant, including leaves, roots, bark, seeds, and fruits, serve as key components in various traditional formulations aimed at treating numerous diseases. Many bioactive compounds have also been extracted from these parts (Badam *et al.*, 2002). Bael (*Aegle marmelos*) is a significant medicinal plant in India, with its biochemical compounds found in leaves, fruits, and seeds being utilized for treating conditions such as diabetes, cardiovascular issues, and inflammation (Maity *et al.*, 2009). The primary active constituents in the plant include alkaloids, terpenoids, steroids, phenols, glycosides, and tannins.

2.1.1 ORIGIN

Bael is indigenous to India (Zeven & De Wet, 1982) and is prevalent throughout Southeast Asia. In India, this fruit is cultivated in the Indo-Gangetic plains and the Sub-Himalayan regions, reaching altitudes of up to 500 meters, especially in North-East India and the dry deciduous forests of central and southern India. *Aegle marmelos* is a subtropical species that can thrive up to 1,200 meters above sea level. It prefers dry forests and can be found in both hilly and flat areas. This plant is extensively distributed across various countries including India, China, Nepal, Sri Lanka, Myanmar, Pakistan, Bangladesh, Vietnam, Laos, Cambodia, Thailand, Indonesia, Malaysia, Tibet, Java, the Philippines, and Fiji. In India, it occurs in the Sub-Himalayan region from Jhelum to West Bengal and across central and southern states, being present in nearly all states of the country (Dhankar *et al*., 2011).

2.1.2 PRODUCTION

The data from National Horticulture Board (NHB) during the year 2023 shows that major producing states include Uttarpradesh, Bihar, Jharkhand, Odisha, Madhyapradesh and Chhattisgarh. India is estimated to have approximately 25000 hectares under bael cultivation making it the largest producer globally. Uttarpradesh being the largest producing state in India with around 4000-5000 hectares of land dedicated to bael cultivation.

2.1.3 BIOLOGICAL PROPERTIES AND NUTRITIONAL VALUE

Every part of the bael plant (*Aegle marmelos*), including the stem, bark, leaves, fruits, and roots, possesses a variety of uses and offers pesticidal, nutritional, and medicinal properties at all growth stages. In Ayurvedic medicine, bael is recognized for its therapeutic benefits, often referred to as the "panacea of stomach ailments." A significant component of the bael fruit, memelosin ($C_{13}H_{12}O_3$), serves as a diuretic and laxative. The fruit is rich in tannins about 20% in the rind and 9% in the pulp making it useful as a mild astringent for conditions like dysentery and diarrhea. The unripe fruit contains higher amounts of pectin and mucilage, both of which are beneficial for digestion. The leaves have applications in treating inflammatory conditions and acute bronchitis, while the roots are noted for their anti-inflammatory and anti-venom properties in traditional Indian medicine (Bhattacherjee *et al*., 2018).

In terms of nutritional value, the bael fruit is recognized for its significant contributions to dietary health. The pulp is an excellent source of carbohydrates, sugars (especially when used as an energy drink), and dietary fiber, offering nutrients that include vitamins A, B_1, and C. Analysis shows that the fruit contains macro-elements such as protein, fat and minerals like calcium and phosphorus. The fruit is also rich in bioactive compounds, including phenolic acids, flavonoids, alkaloids, tannins, and coumarins, which enhance its health benefits. Studies have highlighted bael's potential antidiabetic, hypolipidemic, and antioxidant properties, demonstrating relief from various conditions, including diabetes, cardiovascular issues, inflammatory diseases, and gastrointestinal problems. Additionally, bael has shown protective effects against depression, radiation, microbial infections, wounds, trauma, and oxidative stress (Choudhary & Grover, 2019).

2.1.4 BIOCHEMICAL PROPERTIES

The Indian bael (*Aegle marmelos*) is rich in numerous phytochemicals, including alkaloids, coumarins, steroids, and essential oils, which contribute to its therapeutic properties. Significant compounds identified include rutacine, aegelemine, and aegeline found in the leaves, alongside marmin and skimmianine detected in the bark and roots. Additionally, various coumarins such as umbelliferon, marmesin, scoparone, and scopoletin are present in the roots and fruits. The fruit also contains substantial amounts of fatty acids, with palmitic acid, and stearic acid (8.3%), linoleic acid (28.7%), and linolenic acid (15.6%). Polysaccharides like galactose, arabinose, and L-rhamnose are abundant, contributing to its medicinal capacity (Mujeeb *et al*., 2025).

Minor components of the bael plant include ascorbic acid, crude fibers, tannins, α-amyrin, carotenoids, and crude proteins, which together enhance its health benefits. Notably, the therapeutic activities of the bael plant are attributed to compounds like marmelosin, skimmianine, and umbelliferone. Aegeline, lupeol, cineole, citral, citronellal, cuminaldehyde, eugenol, and more than 100 other compounds have been discovered across various parts of the plant. These substances exhibit biological activities against a range of serious and mild illnesses, confirming bael's significance in both traditional and modern medicinal contexts (Mujeeb *et al*., 2025).

Table 2.1 Chemically active compounds present in different parts of bael

Plant Part	Chemically Active Compounds	Pharmaceutical Properties	Food Applications
Leaves	Alkaloids (e.g., marmeline, fagarine), Flavonoids, Tannins	Antimicrobial, Antidiarrheal, Antioxidant, Anti-inflammatory	Used in traditional medicine; teas or flavoring in cooking
Fruit Pulp	Alkaloids, Flavonoids, Phenolic compounds	Antioxidant, Anti-inflammatory, Antimicrobial, Antidiabetic	Eaten fresh; used in beverages, jams, candies
Seeds	Alkaloids (e.g., marmeline, fagarine), Essential oils	Antimicrobial, Antidiarrheal, Antioxidant	Dried and powdered for medicinal use; flavoring agent
Bark	Alkaloids, Tannins, Essential oils	Antimicrobial, Anti-inflammatory, Antidiarrheal	Used in traditional medicine; bark extracts for food preservation

(Source: Mujeeb *et al.*, 2025)

2.2 EXTRACTION METHODS: CONVENTIONAL AND GREEN EXTRACTION

Extraction is a crucial step in isolating bioactive compounds from their natural sources. The purpose of extraction is to obtain concentrated bioactive substances that can be further analysed, characterized, and used in pharmaceutical, nutraceutical, and food industries. There are several methods for extracting bioactive compounds, and each is suited to specific compounds and materials.

The purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue). The initial crude extracts using these methods contain complex mixture of many plant metabolites, such as alkaloids, glycosides, phenols, terpenoids and flavonoids. Some of the initially obtained extracts may be ready for use as medicinal agents in the form of tinctures and fluid extracts but some need further processing. Several of the commonly used extraction methods are discussed below.

2.2.1 CONVENTIONAL EXTRACTION METHODS

Extraction and recovery processes have surged in recent years due to trends in healthier lifestyles and the increasing use of antioxidants in diets. As a result, several extraction methods have been developed to enhance the yield of bioactive compounds. Techniques such as maceration, percolation, hydro-distillation, boiling, reflux, soaking, and Soxhlet have been proposed (Alara *et al.,* 2018). Soxhlet extraction is particularly

popular due to its simplicity, low cost, ease of maintenance, and lower solvent usage compared to methods like soaking, boiling, or maceration (Sharma and Janmeda, 2017). Various solvents, including ethanol, methanol, benzene, chloroform, and ethyl acetate, have been tested in this method to assess their impact on extraction yields.

In general, liquid–liquid and solid–liquid extractions are the most commonly employed techniques for extracting bioactive compounds. Despite being traditional methods, maceration and water infusion are still in use today (Cujic *et al.*, 2016). These techniques have expanded to incorporate solvents like ethanol, methanol, and acetone, in addition to water, to extract bioactive compounds (Albuquerque *et al.*, 2018). Conventional extraction methods often require large volumes of solvent, have lower yields, and take more time compared to newer techniques. It is also noted that when heat is applied during extraction, it can cause degradation of the extracted compounds, reducing their bioactivity. Factors such as time, particle size, solvent type, mass-to-volume ratio, and temperature have been studied in the context of traditional extraction methods (Bergeron *et al*., 2005). The choice of solvent plays a significant role in determining which compounds are extracted and their biological activity. Ethanol and methanol are the most frequently used solvents due to their ability to achieve higher yields of bioactive compounds (Yu *et al.*, 2019).

2.2.2 GREEN EXTRACTION METHODS

Green extraction refers to sustainable and eco-friendly methods of extracting valuable compounds from natural sources, aligning with the principles of green chemistry. These techniques aim to minimize the use of hazardous chemicals, reduce energy consumption, and lower environmental impact while maintaining efficiency and quality. Green extraction approaches include the use of renewable solvents, such as water, ethanol, or supercritical fluids like carbon dioxide, and innovative technologies such as ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), and enzymatic extraction. These methods improve extraction yields, reduce processing time, and eliminate or limit the generation of harmful waste. Additionally, green extraction emphasizes the valorization of by-products and biomass, promoting a circular economy. Advances in green extraction are increasingly applied in fields such as food, pharmaceuticals, and agriculture, enabling the isolation of bioactive compounds like polyphenols, essential oils, and pigments.

2.3 ULTRASOUND-ASSISTED EXTRACTION (UAE)

Ultrasound waves are akin to sound waves, but they possess a frequency exceeding 16 kHz, making them inaudible to the human ear (Fellows, 2000). The uses of ultrasonic technology are generally divided into two main categories: low-intensity ultrasonics and high- intensity ultrasonics (Fu *et al.,* 2020). Low-intensity ultrasonics are characterized by their high frequency, which ranges from 5 to 10 MHz, and their relatively low power output of less than 1 W/cm^2. This type of ultrasonic technology is non-destructive and is ideal for testing and characterizing various materials, which is why it is often referred to as diagnostic ultrasonics (Carreira *et al.,* 2021). High intensity ultrasonic technology, commonly known as power ultrasonics, functions at low frequencies between 20 and 100 kHz and operates at significant power levels ranging from 10 to 1000 W/cm^2. In contrast to low intensity ultrasonics, high intensity ultrasonics are noted for their destructive properties. This method is highly effective in speeding up chemical reactions through the process of cavitation. The collapse of cavitation bubbles generates energy that can produce a variety of effects, including extraction, crushing, and emulsification (Arvanitoyannis *et al.,* 2017). Ultrasound-assisted extraction is a non-thermal technique that utilizes acoustic energy to enhance the rates of release and diffusion of target substances through the cavitation of the solvent (Cui *et al*., 2021). It primarily operates on the principle of cavitation, which leads to the compression and expansion of the matrix, resulting in the permeabilization of the cell wall and an increased extraction yield of the desired compounds (Iqbal *et al*., 2021). In contrast to electromagnetic waves, ultrasonic waves are mechanical and can travel through solid, liquid, and gaseous mediums (Shen *et al*., 2023).

UAE is a part of power ultrasonic technology. Power ultrasonic is a field of ultrasonology dedicated to utilizing ultrasonic energy for matter processing. UAE employs thermal, mechanical, and cavitation effects to extract bioactive substances. The ultrasonic action disrupts cell walls, facilitating the release and diffusion of components contained within the cells (Chemat *et al*., 2017). The thermal impact of ultrasonic waves involves the absorption of their vibrational energy by the medium, which is then transformed into heat. As a result, the temperature of the medium rises correspondingly. The amount of heat produced is influenced by various factors, including the characteristics of the medium, the power of the ultrasonic waves, and the duration of

exposure (Qiu *et al.*, 2020). The mechanical effect of ultrasonic waves refers to the situation where applying ultrasonics to a medium causes the particles within the medium to vibrate in alignment with the mechanical wave. This leads to an enhancement in particle motion, thereby speeding up the mass transfer process (Wen *et al*., 2018). Among the various ultrasonic effects, cavitation is recognized as the most significant. Ultrasonic cavitation refers to the process where tiny bubbles, known as cavitation nuclei, within a liquid vibrate, expand, and accumulate energy from the acoustic field under the influence of ultrasound. When this accumulated energy exceeds a certain threshold, the cavitation bubbles collapse and close abruptly. Cavitation bubbles are generally categorized into two types: transient and stable. Transient cavitation bubbles have a very short lifespan, whereas stable cavitation bubbles persist for a longer duration (Shen *et al*., 2023). During cavitation, intense hydrodynamic shear forces are generated, leading to the formation of free radicals and a rise in both pressure and temperature (Constantino and Rojas, 2020).

UAE utilizes the thermal, mechanical, and cavitation effects generated by ultrasound in the extraction process. As illustrated in Fig. 2.1 ultrasound enhances the penetration of solvents into cells, thereby improving mass transfer. Furthermore, ultrasound disrupts cell walls, promoting the release of cellular contents. Consequently, efficient cell disruption and enhanced mass transfer are regarded as the two key factors contributing to the increased production achieved with UAE (Shen *et al*., 2023).

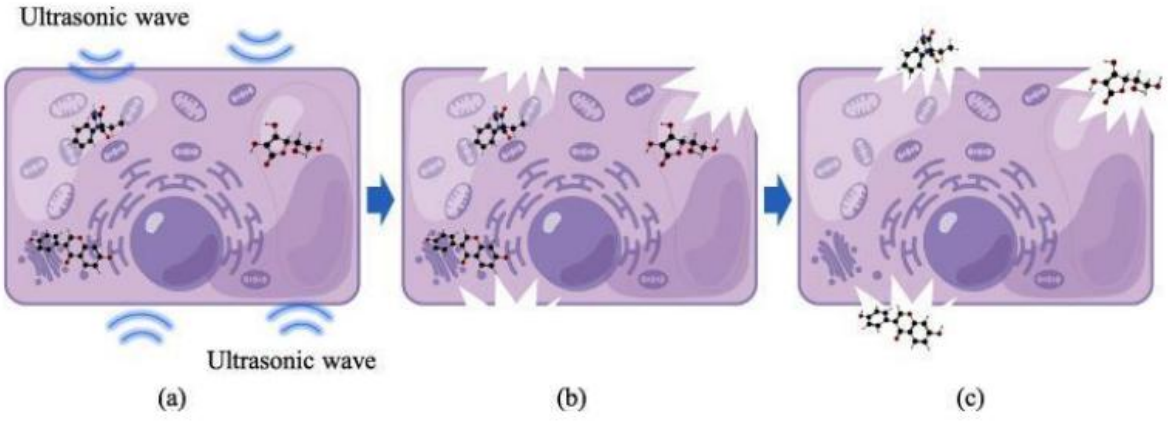


Fig.2.1 The mechanism of ultrasonic assisted extraction. (a) indicates that ultrasonic will act on intact cell (b) indicates cell rupture after ultrasonic treatment (c) indicates that the bioactive components are released. (Source: Shen *et al*., 2023).

The influence of UAE on various bioactive components varies depending on its application in the extraction process. The effect of UAE can be either beneficial or detrimental, based on the specific conditions and parameters utilized. The selection of extraction parameters plays a crucial role in determining the outcomes of the extraction. Additionally, these parameters can affect the physicochemical properties of bioactive components, highlighting the importance of careful optimization to achieve the desired results (Shen *et al*., 2023).Jorge *et al*. (2014) studied ultrasound assisted extraction (UAE) parameters, such as the liquid: solid ratio, solvent concentration and extraction time, using response surface methodology (RSM) for the extraction of polyphenols from desert plants including *Jatropha dioica, Flourensia cernua, Turnera diffusa* and *Eucalyptus camaldulensis*. The results indicated the ability of UAE to obtain polyphenolic antioxidant preparations from desert plants.

The ultrasound-assisted extraction technique was shown to be very efficient in the extraction of oil from grape seeds. Compared with Soxhlet, ultrasound-assisted extraction carried out at 20 kHz, 150 W for 30 min produced similar oil recovery. The fatty acid compositions of the oil was not affected significantly by the application of ultrasound (Carla *et al*. 2012). Samaram *et al*. (2013) evaluate the suitability of ultrasound-assisted extraction (UAE) for the recovery of oil from papaya seed as compared to conventional extraction techniques (i.e., Soxhlet extraction (SXE) and solvent extraction (SE)). Results indicated that both solvent extraction and ultrasound-assisted extraction (UAE) methods recovered relatively high yields.

Ma *et al*. (2009) investigated the effects of ultrasonic variables including extraction time, temperature, and ultrasonic power on the yields of seven phenolic acids. Results showed that the yields of phenolic compounds increased with both ultrasonic time and temperature increased, whereas the opposite occurred with increasing time at higher temperature to some certain. A positive effect on the yields of phenolic compounds was observed with increase of ultrasonic power. When ultrasonic power increased from 3.2 to 30 W, the yields of most phenolic compounds were significantly increased ($p < 0.05$), and then slowly after 30 W.

When appropriately applied with adequate power, ultrasound-assisted extraction offers several advantages over traditional heating methods. These benefits include

improved extraction efficiency, higher yields, and faster processing times. Additionally, ultrasound- assisted extraction is generally safer, consumes less energy, and requires smaller amounts of solvent. However, one limitation is the lack of uniformity in the process, as the intensity of ultrasound waves diminishes with increasing distance from the emitter (Cui *et al*., 2021).

2.3.1 UAE OF PLANT EXTRACTS

Wen *et al.* (2018) highlight that plant bioactive components, such as terpenes, flavonoids, and alkaloids, play a vital role in physiological processes, making their efficient extraction a focus for various industries. Betwal *et al.* (2018) emphasize that extraction serves as a cornerstone for industries like pharmaceuticals and food processing, where precise methods directly impact production efficiency and product quality. Calinescu *et al.* (2021) point out that traditional extraction methods face significant drawbacks, including long durations, high energy costs, and degradation of heat-sensitive components, which necessitate innovative solutions. Wei *et al.* (2015) advocate for green extraction technologies that not only address these challenges but also align with environmental sustainability by reducing waste, energy usage, and solvent reliance.

Chemat *et al.* (2017) explain that UAE leverages ultrasonic cavitation to enhance extraction rates, reduce processing time, and produce high-purity extracts, making it a leading green extraction method. Sun *et al*. (2020) stress that optimized UAE conditions ensure the preservation of biological activities, which is critical for functional applications in bioengineering, cosmetics, and nutraceuticals. Kadam *et al.* (2013) argue that UAE not only meets industrial demands for cost-effectiveness and high yields but also supports environmentally friendly practices, making it a transformative technology. They emphasize the need for further studies on UAE's integration with other technologies, such as microwave or enzymatic methods, to unlock its full potential and expand its applications across diverse sectors.

Xue *et al.*, (2017) found that low-frequency ultrasonic treatments (20, 28, and 35 kHz) have a more pronounced effect on the secondary structure of proteins compared to high- frequency treatments (40 and 50 kHz). Jin *et al*., (2015) demonstrated that ultrasonic treatment conditions significantly alter the secondary structure of proteins,

impacting functionality. Huang *et al*., (2019) observed that prolonged ultrasonic treatment induces distinct structural changes, such as increasing α-helix content in soy protein isolate. Xue *et al.,* (2017) emphasized the importance of optimizing ultrasonic parameters, including sound pressure and sound field uniformity, for efficient protein extraction. Jin *et al*., (2015) highlighted that the specific ultrasonic frequency and treatment duration must be tailored to the protein type to maximize bioactive functionality. Huang *et al*., (2019) concluded that understanding protein-specific responses to ultrasonic waves is critical for leveraging UAE in bioactive component extraction.

2.4 RESPONSE SURFACE METHODOLOGY

The response surface methodology (RSM) is a commonly used mathematical and statistical approach for modelling and analysing processes where the response of interest is influenced by multiple variables. The main goal of RSM is to optimize this response. The variables influencing the process are referred to as independent variables, while the outcomes are termed dependent variables (Aydar, 2018). In general, the UAE process is influenced by several factors, including ultrasonic power and frequency, temperature, ultrasonication time, solvent properties and composition, particle size, and the solid-to-solvent ratio. Optimizing the extraction process is essential to obtain antioxidant compounds with high bioactivity. Numerous researchers have applied RSM to optimize the extraction of bioactive compounds from various biomass sources, such as olive tree leaves (Martínez-Patiño *et al*., 2019).

Bas and Boyai (2007) described response surface methodology (RSM) as a collection of mathematical and statistical techniques used to establish the relationship between independent variables and dependent variables (responses). It helps identify the effects of independent variables, either individually or in combination, on various processes. RSM is a powerful statistical tool widely employed in engineering applications to develop accurate models for optimization design (Aghbashlo *et al*., 2012). It is primarily used for enhancing and optimizing process parameters. RSM is extensively applied across various stages, including experimental design selection, generation of response surfaces, creation of contour plots, prediction and validation of model equations, identification of multi-response parameters and their optimal levels,

and determination of optimal conditions. This methodology allows process optimization to be achieved with minimal cost and time. Hossain *et al*., (2012) optimized ultrasound assisted extraction (UAE) conditions to maximize antioxidant activity, total phenol content, and polyphenol content of marjoram extracts. They identified optimal conditions using response surface methodology, and found that optimal UAE yields were significantly higher than solid/liquid extracts.

Tomsik *et al*., (2016) investigated ultrasound-assisted extraction for obtaining bioactive compounds from *Allium ursinum*. Using a three-level, four-variable face-centered cubic design combined with response surface methodology (RSM), the effects of temperature (40–80 °C), ethanol concentration (30–70%), extraction time (40–80 min), and ultrasonic power (19.2–38.4 W/L) were optimized. The second-order polynomial model indicated optimal conditions at 80°C, 70% ethanol, 79.8 min, and 20.06 W/L. The experimental data closely aligned with predicted values, validating the model. Ghasemzadeh *et al*., (2014) used response surface methodology to optimize ultrasound-assisted extraction of catechin, myricetin, and quercetin from curry leaves. The optimal conditions yielded these compounds with enhanced antioxidant activity (83%), significant anticancer effects against HeLa cells (67.2 μg/mL), and no toxicity to normal cells.

2.5 DRYING METHODS

Adnan *et al*. (2018) reported that drying was a well-established and ancient technique for preserving fruits, vegetables, and their derivatives by lowering their water activity (aw) and moisture content. There were several methods for drying, including sun drying, microwave drying, forced or natural convection drying, and most importantly, spray drying. The final one is a specialised and widely used method for turning liquid feedstocks into concentrated products or powders.

According to Sultana *et al*. (2024), the main goal of drying is to increase the shelf life of solid, liquid, or semisolid foods by removing water from them. By preventing microbial development and lowering chemical & enzymatic reactions, drying stops food from spoiling and degrading in quality while being stored. Furthermore, drying increases the availability of foods during off-season, improves transportation facilities, lowers handling costs, increases the applicability for producing

functional foods, nutraceuticals and many other value-added food products, including encapsulated fish oil.

Liquid extracts can be dried using a variety of techniques, such as spray drying, lyophilization, drum drying, hot air drying, vacuum drying, refractance window drying, and others (Fan *et al*., 2019). Freeze-drying and spray drying are commonly used drying techniques that have been engineered up to date in the food industry whereby diverse dehydration principles are employed in each technique specifically (Verma and Singh, 2015). The fourth generation of drying techniques includes the recently developed idea of refractive window (RW) drying (Shende and Datta, 2019). RW drying is typically used for drying products like heat-sensitive purees and pieces of fruits and vegetables (Karam *et al*., 2016). Khatri *et al*. (2024) stated different drying techniques contrast to conventional drying techniques like sun drying or hot air drying. Novel techniques like freeze-drying and vacuum drying use lower temperatures, which decreases heat-induced damage to the food's nutritional value and sensory qualities and helps preserve the vital nutrients, like vitamins and antioxidants, in food by reducing the exposure to oxygen and high heat, which can break down these substances. Innovative drying techniques, such as freeze drying, produce goods that are simpler to rehydrate, increasing consumer convenience while maintaining texture and flavour. A new method of dehydration called foam-mat drying is utilised to turn liquid solutions into powdered goods.

2.5.1 SPRAY DRYING

Tonon *et al.* (2008) stated that spray drying was commonly employed to create fruit juice powders. It produces high-quality powders with less water activity that were simpler to carry and store. The physico-chemical qualities of powders made by spray drying were dependent on a number of process variables, including the kind of atomiser and the parameters of the drying air (temperature, pressure), as well as the liquid feed (viscosity, particle size, flow rate). In order to produce goods with improved sensory and nutritional qualities as well as improved process yield, it is crucial to optimise the drying process. Verma and Singh (2015) reported that spray drying was a single processing step that used a hot gas to quickly dry a liquid or slurry into a dry powder. Spray drying was the recommended technique for products that are sensitive to heat, such as foods and medications. To distribute the liquid or slurry into a controlled drop size spray, all spray dryers used some kind of atomiser or spray nozzle.

Moisture evaporated when the spray and drying medium come into contact. Both co-current and counter-current flows of the heated drying gas can be directed towards the atomiser. These operational parameters are used into the spray drying process design to improve product recovery and yield final products that meet exact quality standards. The economical, quick, and one-stage drying technique known as spray drying was first used to make fruit powders but is now being used to make concentrated fruit juice products. The spray-drying method's unique selling point is the comparatively greater surface area achieved via atomisation, which results in the development of spherically shaped, regular droplets in the drying chamber (Adnan *et al*., 2018). The operation of a standard spray-drying evaporator (Fig 2.2), which typically applies hot air at high drying temperatures (150–220ºC) to dry the feed droplets within 50–80ºC at the outlet, involves a short drying contact time. Spray drying of thermosensitive components including vitamin C, β-carotene, lycopene, anthocyanins, and others is recommended.

Sobulska and Zbicinski (2020) suggested that the spray drying method was frequently used to produce food items in powder form by drastically lowering the moisture content and lowering the chance of spoiling, thereby extending shelf life. Spray dried powder also had the added benefit of lowering packing, storage, and shipping costs. The advantages of the powder form, like improved flowability and ease of mixing with other components, create opportunities for the creation of novel products with intricate compositions that might be used in the pharmaceutical, cosmetic, and food industries.

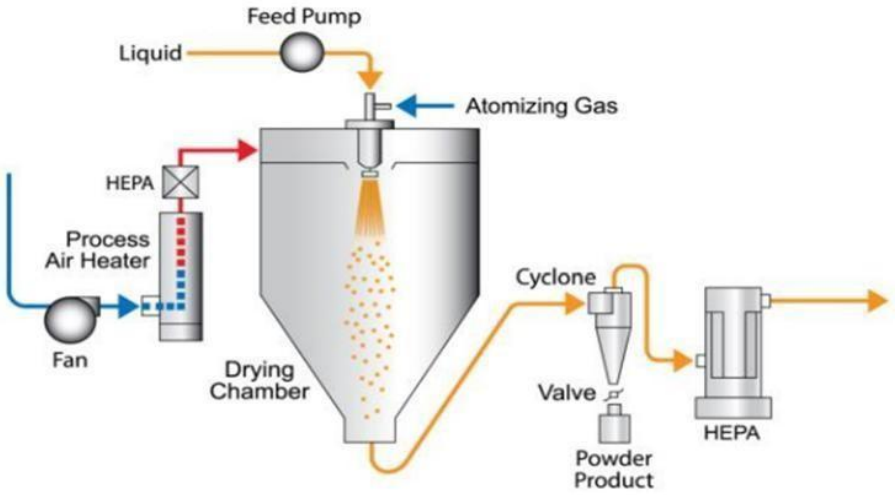


Fig. 2.2 Parts of spray dryer (Source: Adnan *et al*., 2018).

2.6 MICROENCAPSULATION BY SPRAY DRYING

"A process to entrap an active compound within a stable, protective substance to produce encapsulates of varied size and functional properties" is a definition of encapsulation. Typically, the enclosing composition is referred to as the "wall," and the active element as the "core." The structural components of encapsulation are the wall and core. For foods, encapsulation can be done for an ingredient of food composition (such as flavours), as well as for the food substance as a whole (e.g. chocolate). This divides encapsulation technology into three categories: macro, micro, and nanoencapsulation. The resulting capsule size indicates the category. When the particle size is more than 5000 µm, the technique is called macro encapsulation; when it produces particles that are between 0.2 and 5000 µm and between 2000A^{o} and 0.2 µm, it is called micro and nano encapsulation, respectively (Anandharamakrishnan and Ishwarya, 2015).

Ephrem *et al*., (2018) examined the process of encapsulating probiotics, enzymes, and bioactive components. Liquid extracts such as fruit juices are nutrient-dense and lose shelf life due to microbial, enzymatic, and chemical degradation. The bioactive compounds have poor water solubility and physicochemical instability. Therefore, to improve stability, the creation of encapsulating systems around the bioactive chemicals should be taken into consideration.

One of the most popular methods for encapsulating bioactive compounds for use in the food sector is spray drying (Assadpour and Jafari, 2019). It is distinguished by being a quick, inexpensive, and scalable process that atomises a liquid (solution, suspension, emulsion, etc.) to produce dry particles in a continuous operational stage. The product is sprayed using an atomiser and concurrently encounters a hot air stream that dries the ground material. The particles fall by gravity and dry in the drying chamber before eventually reaching the cyclone and being gathered as powder (Gómez-Gaete *et al*., 2024).

According to Jafari *et al*. (2023), the process of encapsulating solid, liquid, and gaseous components in sealed capsules with diameters ranging from nanometres to millimetres in order to preserve and prolong their shelf life is known as microencapsulation. It is a practical technique for enhancing the dispersion of bio actives in food and facilitating their passage into the gastrointestinal system. The

combination of the encapsulating material and the nucleus, which might be physical, chemical, or physicochemical in character, is the primary difference between the various methods of encapsulation. The most popular encapsulation method for bio actives is spray drying encapsulation due to its low cost, ease of usage, and reputation for producing high quality microcapsules.

2.6.1 CARRIER MATERIALS

Phisut (2012) reported that the physicochemical characteristics of powders vary depending on the drying conditions and carrier agents used. Understanding food characteristics is crucial for streamlining operations and lowering expenses, particularly when it comes to powders made or utilised in the food and pharmaceutical sectors. The low glass transition temperature (Tg) of the low molecular weight sugars basically sucrose, glucose, and fructose found in these products is the primary cause of the powder sticking issue. The temperature at which the polymer's amorphous phase changes from rubbery to glassy states is known as the glass transition temperature (Tg). By adding certain carrier agents, such as gums and polymers, to the product prior to atomisation, these issues can be resolved. Additionally, microencapsulation also makes use of a carrier agent. It can shield delicate food ingredients from adverse environmental factors, conceal or maintain tastes and scents, lessen volatility and reactivity, and enhance appeal to food product merchandising. Du *et al*. (2014) examined two distinct ratios of five distinct carrier materials: egg albumen, whey protein concentrate, gum Arabic, starch sodium octenyl succinate, and maltodextrin. The best yield was obtained from gum Arabic and starch sodium octenyl succinate among the investigated carrier materials. Maltodextrin, whey protein concentrate, and egg albumen came next. It was asserted that these carrier materials were more effective since whey protein concentrate and egg albumen were utilised in smaller ratios than other carrier materials. Furthermore, because gum Arabic and starch sodium octenyl succinate have larger molecules than maltodextrin, their higher glass transition temperature value explains their better yield compared to maltodextrin. Anandharamakrishnan and Ishwarya, (2015) stated that the first step in the spray drying encapsulation process was selecting the wall material. The kind of wall material affected the flowability, mechanical stability, and ultimate shelf life of the encapsulated product after drying, in addition to the feed emulsion stability prior to drying.

2.6.1.1 Maltodextrin

Kha *et al*. (2010) conducted the study on spray drying of gac fruit. Maltodextrin has been referred to as an appropriate drying aid to maintain its antioxidant qualities and colour. Maltodextrin concentration and drying temperature generally had a substantial effect on the colour properties of spray-dried powders. Maltodextrin concentration had a substantial impact on product colour and brightness ($p < 0.01$). Increasing the concentration of maltodextrin from 10% to 20% significantly increased the lightness of the products. Higher encapsulation efficiency (EE) was the result of increasing the concentration of maltodextrin; nevertheless, there was not a significant difference in EE between 20% and 30%. Anandharamakrishnan and Ishwarya (2015) mentioned that maltodextrin was a creamy white powdered hygroscopic polysaccharide with a taste that was either bland or only mildly sweet. Even in concentrated solutions, it had a low viscosity and was very water soluble. This made it possible for emulsions to have a higher solid content, which was beneficial for core retention during spray drying. Because it's inexpensive, it could be a good substitute for gum Arabic (GA), although it could also be combined with GA, whey protein, or modified starches to get optimal encapsulation outcomes. This is due to MD's weak emulsification ability, which could result in inadequate lipophilic core retention.

Ciechanowska *et al*. (2020) suggested that maltodextrin was one of the most widely utilised carriers in the manufacturing of powder. Among other things, it was previously used to make powdered watermelon, mango, acai, and cranberries. In terms of technology, maltodextrin was superior to other carrier agents, such as inulin, because it had a reduced propensity to condense the solution prior to the spray-drying procedure. There are various kinds of maltodextrin that can be separated based on the kind of starch that is hydrolysed, such as rice, corn, oats, or potatoes; alternatively, barley and wheat are examples of less popular starch types.

2.6.1.2 Corn starch

Vanzela *et al*. (2013) attempted to preserve carotenoids in dehydrated pumpkin by using the starches from cassava and maize to create an edible covering. Results shown that trans-a- carotene and trans-b-carotene were well-retained by the maize starch coatings, and the better colour qualities were displayed by dehydrated

goods. The prolonged digestion of regular maize starch in encapsulated form was investigated by Xu & Zhang, (2014).

Together with the quantity of slowly digesting starch, starch is the primary carbohydrate with the highest glycaemic index and nutritional value that helps with glycaemic management. Starch capsules demonstrated a significant increase in resistant and poorly digested starch. It is the perfect ingredient for glycaemic control and the development of speciality foods due to its pleasing sensory qualities. Chandralekha *et al*. (2016) used a spray drying approach with various wall materials to investigate the survivability of encapsulated yeast cells. A known amount of carrier agents, such as maltodextrin, maize starch, acacia gum, polyethylene glycol 8000, β-cyclodextrin, and skimmed milk powder, were combined with yeast slurry and supplied to the feed separately. Out of the six drying aids, corn starch and maltodextrin produced the best results in terms of cell survival (80.5%) and powder production (59%, w/w), respectively. When considering both powder yield and survivability, maize starch was the most practically appropriate carrier material.

2.6.1.3 Gum arabic

The most widely used gum additive is gum arabic, also known as acacia gum, which is a complex branching heteropolysaccharide made up of 1, 3-linked β-Dgalactopyranosyl groups and is extracted from *Acacia senegal* as well as *Acacia seyal* trees. It's the sole gum. utilised in the food sector due to its low water viscosity and great solubility. The gum's emulsification qualities are high. It has a pale white hue and is made up of D-glucuronic acid, L-rhamnose, D- galatose, and L-arabinose in the ratio 4:2:2:1 (Patel and Goyal, 2015). Tontul & Topuz, (2017) reported that one of the oldest and most well-known natural gums was gum arabic, which was made from the fluids of acacia trees. In contrast to the other gums, it was very soluble (up to 50%) in both hot and cold water and has a low viscosity. It was made up of a complex heteropolysaccharide with a 2% protein content and a highly ramified structure. The structure's functional characteristics were attributed to the proteins within it, whereas the arabinogalactan fraction possesses the ability to form films.

Sarabandi *et al.* (2018) investigated the effects of pectin and whey protein concentrate (WPC) as supplementary drying aids, as well as maltodextrin (MD) and gum arabic, on the powder yield, physical, functional, and microstructural

characteristics of spray-dried apple juice concentrate. The bulk and tapped densities of the samples had MD and GA which were the greatest and lowest, respectively, however the particle density was significantly reduced when WPC was employed. Considering all the factors, including solubility, wettability, and hygroscopicity, 10% WPC combined with MD produced the most economical powder with the greatest yield (60.85%) and suitable flowability, functionality, and physical characteristics. The impact of the carrier choice on the dry-material, density, colour, hygroscopicity, and anthocyanin content of powders following spray drying was investigated by Turak *et al*. (2019). As carriers, low-crystallized maltodextrin, arabic gum, maltodextrin plus arabic gum combinations (1:1; 2:1; 3:1), and rice starch were employed. The high dry matter content (96–99%) and low hygroscopicity (0.136–0.2 g H_2O g $^{-1}$ d.m.) of all the powders suggested a fair chance of keeping the microencapsulated anthocyanins safe throughout storage. High concentrations of gum arabic in powder form were characterised by decreased apparent density and water activity, although at the expense of a decline in colour parameter values. The carrier mixes that contained maltodextrin and arabic gum seemed to have a lot of promise to guarantee chokeberry powders of a higher calibre.

CHAPTER III

MATERIALS AND METHODS

This chapter covers the physicochemical properties of bael leaf powder, ultrasound-assisted extraction and microencapsulation of bioactive compounds from Indian bael leaves. It details the materials used for the extraction process and the subsequent optimization of process parameters to achieve maximum yield, antioxidant activity, and phenolic content and spray drying process.

3.1 MATERIAL AND SAMPLE PREPARATION

Indian bael leaves were sourced from the KCAEFT campus in Tavanur. The leaves were cleaned, washed, dried, and ground into a fine powder. The resulting powder was then stored in a glass container under dark, dry conditions to prevent oxidation of its components.

Plate 3.1. Shade drying

Plate 3.2 Heat pump drying

Plate 3.3 Bael leaf powder

3.2 PHYSICOCHEMICAL PROPERTIES OF BAEL LEAF POWDER

Before starting the extraction process, the physicochemical properties of the Indian bael leaf powder were analysed. Parameters like moisture content, bulk density, porosity, and color were measured using standard methods, as described in the following sections.

3.2.1 BULK DENSITY

Bulk density is defined as the ratio of the mass of powder sample to its total volume, including the void spaces between particles. It is typically expressed in kilograms per cubic meter. For this analysis, the bulk density of Indian bael powder was measured using a 10 ml cylinder. The powder was carefully filled into the cylinder, and its mass was recorded.

$$Bulk\ density\ (g/cm^3) = \frac{weight\ of\ powder\ (g)}{volume\ of\ beaker\ (cm^3)} \quad \ldots(1)$$

3.2.2 TRUE DENSITY

The Indian bael leaf powder was placed into a measuring cylinder, and toluene was gradually added to fill the void spaces. The volume of toluene used was then recorded, and the true density of the powder was calculated using the following equation.

$$True\ density(g/cm^3) = \frac{weight\ of\ sample\ (g)}{(bulk\ volume - volume\ of\ toluene)\ cm^3} \quad \ldots(2)$$

3.2.3 POROSITY

The porosity of the Indian bael leaf powder was calculated using the bulk and true density values, following the specified formula. The results represent the average of 10 replicates.

$$\textbf{Porosity} = \frac{True\ density - Bulk\ density}{True\ density} \quad \ldots(3)$$

3.2.4 MOISTURE CONTENT

The moisture content of Indian bael leaf powder was measured using an infrared moisture analyser. This instrument operates on the thermogravimetric principle, rapidly drying the sample with infrared heating elements and water evaporation channels while continuously monitoring the weight loss. The moisture loss (% wb) is displayed in real-time during the drying process, and the final moisture content is locked once the process is complete. Additional data, including moisture value, initial weight, starting value, and measurement time, can be viewed by pressing the display button.

Plate 3.4 Infrared moisture analyzer

3.2.5 WATER ACTIVITY

Water activity (a*w*) indicates the proportion of free moisture in a sample that is available for chemical and biological reactions. It is measured using a water activity meter, which assesses the relative humidity in the headspace when the liquid water in the sample reaches equilibrium with the vapor phase. For Indian bael leaves powder, water activity was measured using a water activity meter (Model: Aqua Lab, Decagon Devices Inc., Pullman, USA), as shown in Plate 3.5. The process involved placing the sample in a specialized cup provided with the device. The drawer knob was then turned to the OPEN position, exposing the sample port by pulling the handle. After inserting the sample, the chamber was sealed, and the knob was switched to the READ position. The meter displayed the water activity of the sample, adjusted to ambient temperature, on its screen (Kha *et al*., 2010).

Plate 3.5 Water activity meter

3.2.6 COLOUR MEASUREMENT

The Lovibond colorimeter, a high-precision instrument, was used to assess the color of Indian bael leaf powder by measuring its surface reflectance. It is calibrated using standard reference materials, such as white and black glass, to ensure accurate and consistent readings. The device provides color measurements in the CIELAB color space, capturing L (lightness), a* (red to green spectrum), and b* (yellow to blue spectrum) values. It also calculates chroma (C*), which reflects color intensity, and hue angle (h°), representing the dominant color tone, based on the a* and b* coordinates. These measurements offer a comprehensive view of the powder's color properties, supporting quality evaluation in terms of uniformity and overall appearance.

Plate 3.6 Lovibond colorimeter

3.2.7 WETTABILITY

According to Hogekamp *et al*. 2003, wettability can be defined as the ability of a powder bulk to be penetrated by a liquid for the reason that of capillary forces. The unit of wettability is seconds. Wall material of sample 1.5g was weighed in a weighing balance and taken using a spatula. Then the powder sample gently placed over the surface of 100 ml water in a beaker at 30ºC. With the help of a stop watch the time taken for complete wetting of powder was noted.

3.2.8 pH

pH is a measure of how acidic or basic (alkaline) a solution is, ranging from 0 to 14. A pH of 7 is neutral (like pure water), values below 7 indicate acidity, and values above 7 indicate alkalinity. pH paper is a strip of paper treated with pH sensitive dyes. To measure pH, simply dip the strip into the solution and compare the resulting color to a standard color chart. The matching color indicates the pH value of the solution.

Plate 3.7 pH paper

3.4 EXPERIMENTAL PROCEDURE

Preliminary studies were conducted based on assumptions and knowledge gained from previous literatures in the field of spray drying of juices and extracts. There were several tasks including selection of extraction methods, find out better combination of carrier material for spray drying, choose a suitable temperature range, come across appropriate feed flow rates, and select proper concentrations of wall material. These are the major factors which influence the quality characteristics of product, powder yield, storage stability and cost economy.

Preliminary studies were conducted with individual and binary blends of carrier materials viz. maltodextrin, corn starch and gum arabic at different concentrations, varying inlet temperature, and different feed flow rate in order to set appropriate independent variables. Based on the detailed review of literature and the preliminary studies conducted, the process parameters which form the independent variables were chosen. The quality characteristics which have keen influence on the efficiency of spray drying, stability and shelf life of the bael leaves powder were chosen as dependent variables.

3.4.1 ULTRASOUND-ASSISTED EXTRACTION (UAE)

The sample was combined with 80% ethanol as the extraction solvent at a fixed ratio. Ultrasound- assisted extraction (UAE) was performed using an ultrasonicator (Athena Technology, 33 kHz, 250 W, 250 VAC) under pre-set temperature and time conditions. The resulting mixture was filtered using Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary vacuum evaporator set to 70°C (Superfit digital bath) to remove the majority of the solvent. Any remaining ethanol was removed through open evaporation. The final extract was collected from the flask using ethanol, adjusted to a final volume of 10 mL, and stored at 4°C for further analysis.

3.4.2 ULTRASOUND WATER BATH

Ultrasound refers to sound waves with frequencies above the human hearing range (>20 kHz) and is increasingly being adopted in the food industry due to its numerous benefits. High-power ultrasound (US) at lower frequencies (20-100 kHz) can produce cavitation, a process that disrupts microbial cell membranes, leading to effective microbial inactivation. This technology preserves the nutritional and sensory properties of food, promotes uniform treatment, and reduces energy consumption. To enhance its effectiveness, ultrasound is often combined with other treatments like heat, pressure, or antimicrobial agents. An ultrasound production system typically includes three key components: a generator, a transducer, and an application system. The generator produces electrical or mechanical energy, which the transducer converts into ultrasound waves at the desired frequency.

In this study, ultrasound treatment was carried out using an ultrasound bath with a chiller (Athena Technology, Mumbai, Model ATSC-10), operating at a frequency of 33 kHz and 250W output power. The bath, constructed from stainless steel (SS 304)

and with dimensions of 445 mm × 420 mm × 545 mm, has a 10-liter capacity. It is equipped with a high-efficiency MOSFET-based SMPS generator and five piezoelectric sandwich-type (PZT) transducers fixed at the tank's base. These transducers convert high- frequency electrical energy into ultrasound waves, generating microscopic vacuum bubbles that rapidly form and collapse, a phenomenon known as cavitation. This intense cavitation ensures effective microbial inactivation. The generator's digital tuning system prevents frequency drift during operation, ensuring consistent performance

To maintain precise control over the sonication environment, the ultrasound bath features an integrated cooling system capable of maintaining temperatures between 10°C and 30°C. This cooling system consists of copper coils surrounding the tank, connected to a condenser and compressor for efficient heat regulation. Temperature is monitored using a PT-100 simplicon sensor, while a digital temperature controller (adjustable from 10°C to 30°C) and a timer (adjustable from 0 to 99 minutes) provide precise control over the processing conditions.

Plate 3.8 Ultra-sonicator Bath

3.4.3 OPTIMIZATION OF PROCESS PARAMETERS FOR ULTRASOUND PRETREATMENT

Preliminary studies were conducted for the extraction of bael leaves using ultrasound equipment based on the previous literature and research works in leaves extraction. The factors affecting the ultrasound assisted extraction are temperature, time and substrate ratio. These are the major factors which will influence the yield and quality parameters of the extraction. The time, temperature and substrate ratio were selected as per the equipment design and previous literature reviews and standardized based on the preliminary trials conducted. The temperature range and time duration were selected as 10 to 30℃ and 20 to 60 minutes, respectively based on the previous research.

Optimisation of extraction parameters was done using Response surface methodology (RSM). The process parameters which would influence the yield, antioxidant activity and phenolics were chosen as independent variables. Yield, antioxidant activity and phenolics were taken as dependent variables.

Independent Variables:

1. Temperature (°C): 10, 20, 30
2. Substrate ratio: 10, 15, 20
3. Time (min): 20, 40, 60

Response surface methodology was made use of for optimising the process variables. Nine dependent variables (responses) were employed for the purpose of optimisation. The formula $N = 2\ k\ (k - 1) + Co$ (where k is the number of factors and Co is the number of central points) gives the number of experiments or runs in the Box-Behnken design, N. There were 13 experiments with three components and five central points in this study. Design-Expert (version 12.0.0) was used as the statistical analysis software.

1. Yield
2. Antioxidant activity
3. Total phenolic content

Table 3.1 Experimental design with the actual values of process variables for the extraction of bael leaves

INDEPENDENT VARIABLES			
Run	Temperature (^oC)	Substrate ratio	Time (min)
1	20	15	40
2	30	20	40
3	30	10	40
4	30	15	20
5	10	10	40
6	20	20	60
7	10	15	60
8	20	10	20
9	10	20	40
10	30	15	60
11	20	20	20
12	20	10	60
13	10	15	20

3.4.3.1 Rotary vacuum evaporator

The rotary vacuum evaporator (Superfit Digital Bath) is a laboratory apparatus designed for the efficient and controlled evaporation of solvents under reduced pressure. This system works by rotating a sample flask within a heated water bath, which enhances evaporation by increasing the liquid's surface area and lowering the boiling point of the solvent. It includes a digital control panel for precise regulation of bath temperature, rotation speed, and vacuum pressure, ensuring optimal evaporation conditions.

Common applications include concentrating solutions, removing solvents, and conducting distillation processes. The device is equipped with high-quality glassware and a vacuum pump that maintains low pressure, enabling effective solvent removal. The Superfit model is specifically designed for ease of operation, delivering reliable performance and improved efficiency in small-scale laboratory settings.

Plate 3.9 Rotary evaporator

3.5 EXPERIMENTAL PROCEDURE FOR RESPONSE VARIABLES

The optimization of the process variables was conducted based on the response parameters such as yield, antioxidant activity and total phenolic content.

3.5.1 YIELD OF THE BAEL LEAF EXTRACT

The extraction yield was calculated using the following formula:

$$Yield = Weight\ of\ the\ flask\ after\ evaporation - Empty\ weight\ of\ flask \quad ..(4)$$

3.5.2 ANTIOXIDANT ACTIVITY OF BAEL LEAF EXTRACT

The antioxidant activity of Indian bael leaf extracts was evaluated using the DPPH radical scavenging assay, following the method described by Kumar *et al.*, (2020) with slight modifications. This assay relies on the ability of antioxidants to donate hydrogen atoms, stabilizing the DPPH radical by converting it into a non-radical molecule (Chan *et al*., 2014). To perform the assay, 1 mL of freshly prepared 2 mM methanolic DPPH solution was mixed with 1 mL of the extract in test tubes. The resulting reaction mixtures were thoroughly mixed and incubated in the dark for 30 minutes at room temperature. The absorbance was then measured at 517 nm using a UV-Vis spectrophotometer, with methanol serving as the blank. A control was prepared

by mixing an equal volume of methanol and DPPH without the extract. All samples were analyzed in duplicate. The radical scavenging activity (RSA) was calculated using the following equation:

$$\textbf{RSA (\%)} = \frac{C-S}{C} \times 100 \qquad \ldots(5)$$

where C is the absorbance of the control and S is the absorbance of the sample.

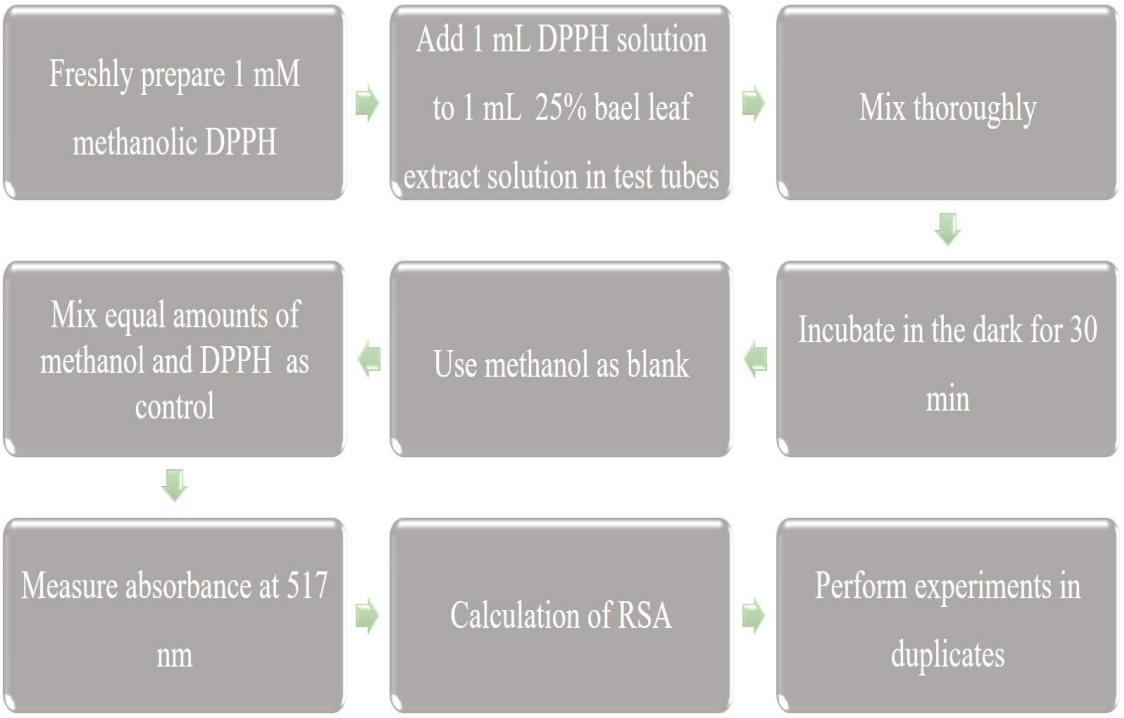


Fig 3.1 Procedure to determine antioxidant activity

3.5.3 TOTAL PHENOLIC CONTENT (TPC) OF BAEL LEAF EXTRACT

The total phenolic content (TPC) of Indian bael leaf extracts was measured following the method outlined by Kumar *et al.,* (2020), with minor modifications. This procedure begins by taking a very small aliquot (0.0125 ml) of the sample extract and diluting it to a total volume of 1.0 ml using distilled water. To this diluted sample, 2.5 ml of 10% Folin-Ciocalteu reagent is added. This reagent reacts with phenolic compounds present in the sample. The mixture is then incubated for 5 minutes at room temperature, allowing the initial reduction reaction to occur. Following this, 2 ml of a 7.5% sodium carbonate solution is added. The sodium carbonate creates the alkaline

conditions necessary for the development of the characteristic blue color complex. After adding the sodium carbonate, the reaction mixture is incubated in the dark for a critical period of 2 hours. This extended incubation in darkness allows the blue chromophore to fully develop and stabilizes it against light degradation. Before measurement, the tube is vortexed to ensure homogeneity. The absorbance of the resulting blue solution is then measured at a wavelength of 750 nm using a spectrophotometer. The phenolic content is quantified by comparing the sample's absorbance to a calibration curve generated from gallic acid standards (ranging from 0 to 0.1 mg/mL) processed identically. Results are calculated and reported as milligrams of Gallic Acid Equivalent per gram of the original extract (mg GAE/100 g extract). All steps, including sample and standard preparation, must be performed in duplicates to ensure reliability.

The TPC was calculated using the following equation:

$$\textbf{TPC (mg GAE/g)} = \frac{\textit{Concentration of sample calculated using standard curve} \times \textit{Volume made up}}{\textit{Weight of sample taken} \times \textit{Volume of aliquot samples used for estimation}} \quad \ldots(6)$$

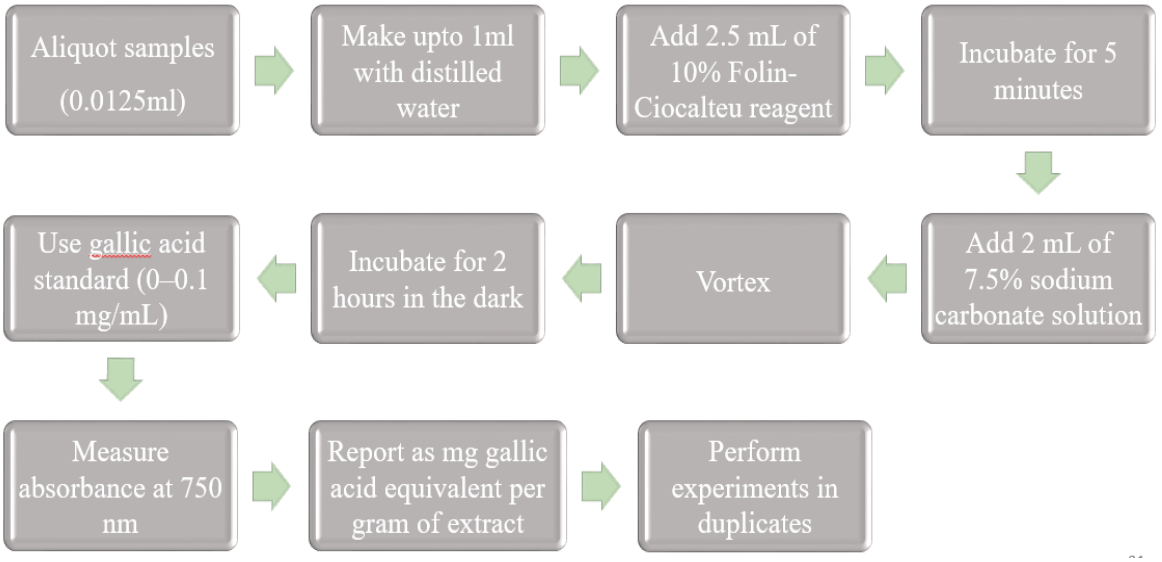


Fig. 3.2 Procedure to determine TPC

3.6 DEVELOPMENT OF BAEL LEAF POWDER BY SPRAY DRYING PROCESS

Spray drying is the technique used for conserving bael leaf extract in powdered form. The extract was blended with the proper carrier agent in order to produce spray dried powder. These carrier materials serve as the protective layer for the biochemical compounds in the leaf extract. The feed solution is the mixture of dilute extract and carrier agents. Using an atomiser, the solution is being sprayed into the drying chamber.

When atomised particles come into touch with a hot drying medium, instantaneous drying takes place. The spray dried powder is collected from the outlet of cyclone separator.

3.6.1 PARTS OF SPRAY DRYER

A spray dryer is the equipment used specifically for powder production. Spray drying is a unit operation that produces powder instantly from a liquid feed. The tall type spray drier with twin fluid pressure nozzle atomiser (M/s S.M. Scientech, Kolkata) was selected in this research work for microencapsulation which is depicted in Plate 3.8. This spray dryer uses a co-current fluid flow pattern with a 1000 ml/h evaporation rate. The air compressor, feed pump, atomiser, drying chamber, cyclone separator and control panel are the several components that make up the spray dryer.

3.6.1.1 Air compressor

The compressed atmospheric air required for the spray drying process is supplied by a compressor. After passing through a filter system, the air is preheated to the working temperature using a heater equipped with heating coils, which can raise the air temperature to as high as 350°C. The heated and compressed air is then introduced into the drying chamber using twin-fluid pressure atomizers. These atomizers help split the feed solution into a fine mist, facilitating efficient drying. This setup, consisting of an air compressor, air filter, and air heater, is collectively referred to as the hot air supply system.

3.6.1.2 Feed pump

The feed solution is pumped to the atomiser using a DC-operated peristaltic pump. A 500 ml beaker with the feed solution is placed promptly at the peristaltic pump's input. Five rollers on the pump compress hypalon, a 6mm-diameter natural

rubber tube. The tube containing the feed material can be compressed against the wall while these rollers rotate, assisting in the feed's forward motion and producing a vacuum behind each roller. The forward motion will resume as a result of this vacuum's assistance in sucking the feed solution once more. A rotary knob is kept to adjust the peristaltic pump's speed (rpm).

3.6.1.3 Atomizer

A twin fluid pressure nozzle atomiser is used to spray the feed solution into the drying chamber from the top in the form of tiny droplets. The most crucial step in the spray drying process is atomization, which allows for the dispersion of particles in the drying medium by increasing the surface area exponentially as a result of a significant decrease in particle size. Drying occurs in a matter of seconds without compromising the material's integrity because the feed and compressed air were fed into the nozzle simultaneously. The atomizer could produce a range of flow rates and droplet sizes depending on the compressor pressure that was chosen. In this equipment, the air compressor pressure is fixed at 2.5 kg/cm^2 which is 35 psi.

3.6.1.4 Drying chamber

The spray dryer's drying chamber is made up of SS 304 stainless steel, which is cylindrical in shape. To facilitate the effective flow of dried powder and air it is tapered towards the bottom end. The chamber's interior surface was polished to 180-200 grit fineness to avoid powder build up on the surface. Once the feed droplets make contact with the drying medium, heat and mass transfer occur in the drying chamber. Heat will be transferred by convection between hot air and feed droplets, and the heat will be transformed into latent heat as the moisture content evaporates. By exposing the particles below the wet bulb temperature, the resulting vapours envelop the particles and provide protection against heat damage.

3.6.1.5 Cyclone Separator

A cyclone outside the dryer is often employed to separate powder particles based on density differences. The conical part at the base of the drying chamber has a 65 mm duct attached to it. Depending on the blower's speed, the cyclone's spinning air movement will produce a pressure drop and sweep small powder particles away with the air. The blower rpm in this investigation was set at 1700 rpm. A Teflon gasket and a threaded flange at the bottom of the cyclone to collect the fine powder in glass bottles. By removing tiny particles from humid air, a powder recovery system with a cyclone is employed.

3.6.1.6 Control panel

An electrical control panel equipped with regulators, ON/OFF push buttons, and indicators is used to control operating parameters such as blower speed, inlet air temperature, and feed pump speed. While there is no direct outlet temperature control, the desired outlet temperature can be achieved by adjusting the blower speed and the input air temperature, both of which are displayed on the control panel. To address issues of atomizer blocking and clogging, the control panel also includes automated and manual buttons to avoid blockage of atomizer during working.

Plate 3.10 Spray dryer

3.7 MICROENCAPSULATION OF BIOACTIVE COMPOUNDS FROM BAEL LEAVES

3.7.1 SELECTION OF CARRIER MATERIALS

Preliminary studies from research shows that wide varieties of options can be employed from carbohydrate, proteins, gums & lipids. Carbohydrate are readily accepted in human diet and gums shows better emulsifying properties and prevents agglomeration of hydrophobic compounds. Proteins can be allergic to some people as they are mostly from animal source, less stable in acidic environments and is expensive compared to other carrier materials. Whereas lipids are not extensively used for encapsulation process as these have very less water dispersibility and prone to oxidation.

Maltodextrin was chosen due to its good water solubility and low viscosity and cornstarch due to its wide availability and biocompatibility. Moreover both these materials are inexpensive and considered as GRAS by FDA. Gum Arabic was chosen as it is excellent emulsifier stabilizing oil in water emulsions and usually paired with a carbohydrate source to enhance encapsulation efficiency.

3.7.2 PREPARATION OF FEED SOLUTION

The leaf extract and carrier agents was blended with water to make up to 500 millilitres of feed solution. The carrier material concentration was 15%. The carrier material was prepared in the ratio 3:1 and 2:1 (maltodextrin and gum arabic) and 2:1 (Maltodextrin and corn starch). Prior to processing, the extract was thawed to room temperature and gradually combined with the carrier material and water in the vessel. The mixture was thoroughly blended using a magnetic stirrer. To remove contaminants and clogs, the blended solution was sieved through a muslin cloth. The resulting solution, referred as the "feed solution," was used for spray drying.

3.7.3 EXPERIMENTAL PROCEDURE OF SPRAY DRYING

The spray dryer was set to the appropriate temperature and feed flow rate for the encapsulation process. The atomizer pressure was maintained at 2.5 kg/cm² and the blower speed was set to 1700 rpm. To stabilize the outlet air temperature, distilled water was pumped into the system prior to introducing the feed solution. Once a steady state was achieved, the water was replaced with the feed solution. The spray dryer inlet temperature was set to 160°C, while the feed flow rate was set at 10 rpm. The feed solution was then pumped into the drying chamber through the atomizer, where feed droplets lost their moisture during the drying process. Drying continued until the entire feed solution was processed, and the dried powder was collected at the outlet. After cooling, the extracted powder was carefully packed into aluminium-laminated pouches and metallized polyester pouches, which were then sealed airtight using a hand sealer. The samples were stored in airtight containers under atmospheric conditions for further examination. The process flow chart for producing spray-dried bael leaf powder is shown in figure 3.3

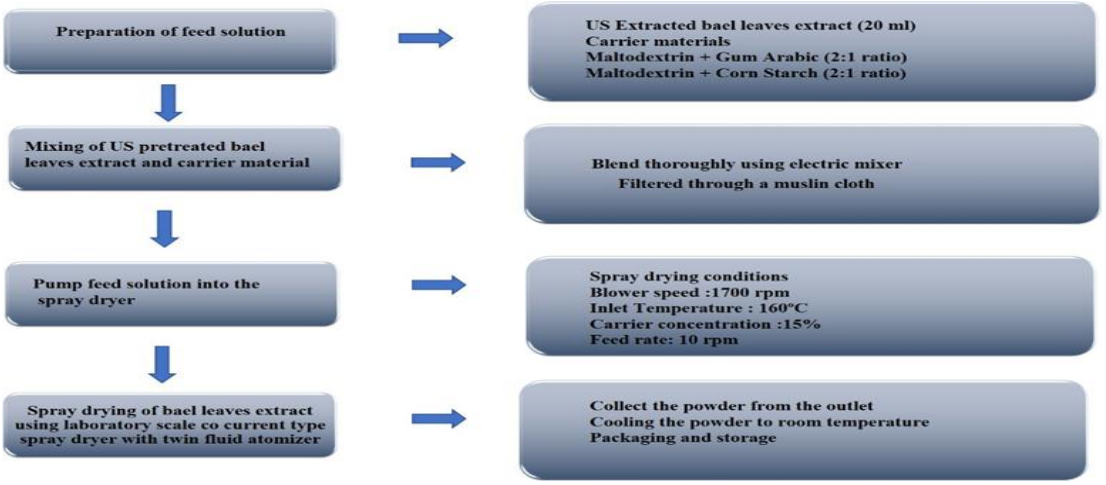


Fig 3.3 Procedure for spray drying

3.8 PHYSICAL PROPERTIES OF SPRAY DRIED BAEL LEAF EXTRACT

3.8.1 MOISTURE CONTENT

Moisture content (% wb) is the amount of water present in a product which influence the storage stability of a product. The materials with more moisture content will prone to faster deterioration during storage. According to Santana *et al*. 2017, moisture contents of powder produced by spray drying were generally lower than 5% and could be classified as microbiologically safe and can be stored for long term. These lower moisture contents of spray dried powders limit the ability of the water act as a plasticizer, therefore, affects the caking of the powder during storage (Tontul & Topuz 2017). Moisture content of spray dried bael leaf extract samples were quantified using the method described in section 3.2.4.

3.8.2 WATER ACTIVITY

Water activity is the amount of free moisture available for biological and chemical reactions. Water activity plays an important role in extension of shelf life. Powders with water activity less than 0.3 consider as microbiologically and chemically safe. Water activity of spray dried bael leaf extract was measured using Aqua lab water activity meter (Model: Aqua lab, Decagon Devices Inc., Pullman, USA) as explained under the section 3.2.5.

3.8.3 TOTAL COLOUR DIFFERENCES (ΔE)

Color properties of the food powders generally evaluated with the Hunter L*, a *, b* values. It is difficult to make conclusions only from these values. Therefore, these discussions were carried out on total color differences (ΔE). Total colour difference with respect to raw feed solution was calculated. Most of the pigments which are responsible for colour of a product were found to be heat sensitive in nature. The total colour difference should be minimum for the better spary dried product. The total color difference is calculated from equation 7. The colour of the spray dried bael extract was measured using Hunter lab colour flex meter (Hunter Association laboratory, Inc., Reston, Virgina, USA). Detailed procedure for colour determination was explained in the section 3.2.6.

$$\text{Total color difference} = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2} \quad \ldots(7)$$

Where;

L_0, a_0, and b_0 = Color parameters for the feed and

L, a, and b = Color parameters of the product

3.8.4 WETTABILITY

The Wettability is a measure for the capability of a powder to be wetted with water at given temperature. Wettability is the time in seconds expected to accomplish finish wetting of the spray dried passion fruit juice powder. For this, a glass funnel held on a stand and it was set over the beaker containing 100 ml of distilled water at room temperature. A glass rod was kept inside the funnel to obstruct its lower opening. To this setup, one gram sample was placed around the glass rod and after that the glass rod was lifted. The time taken for complete wetting of powder particles were noted using a stop watch. Determination of wettability was found out thrice for spray dried powder and the average value was considered as wettability of powder (Jinapong *et al*., 2008, Falade and Omojola, 2010). Powders wetted within 15 seconds termed as instant spray dried powder (GEA Niro, 1978).

3.8.5 BULK AND TAPPED BULK DENSITY

Bulk density of a product is the ratio of mass to the volume. The bulk density of the powder samples were determined according to the method described by Bhandari *et al*. (1992). In this method known weight of samples were loosely filled into 10 ml graduate cylinder. Note the volume filled by the powder. Bulk density can be calculated as per the equation given in section 3.2.7. Tapped density or true density of a product is the ratio of mass to the volume after specified compaction process of the material. Tapped bulk density density can be calculated by tapping method. The cylinder along with the powder was then tapped 100 times on a flat surface to reach constant volume. The final volume was noted and tapped bulk density was calculated by dividing the powder weight by the final volume of the powder in the cylinder as given below. The both densities were expressed in g/cm3.

$$\text{Tapped density } \left(\frac{g}{cm^3}\right) = \frac{\text{Weight of sample(g)}}{\text{Tapped volume occupied by sample } (cm^3)} \quad \ldots (8)$$

3.8.6 FLOWABILITY AND COHESIVENESS

3.8.6.1 Carr's index (CI)

The Carr's index or compressibility index was calculated from the loose bulk density and tapped bulk density (Jinapong *et al*., 2008).

$$CI = \frac{\rho_{tapped} - \rho_{bulk}}{\rho_{tapped}} \quad \ldots(9)$$

3.8.6.2 Hausner ratio (HR)

The cohesiveness was evaluated in terms of hausner ratio (Shishir *et al*., 2015). It was calculated from loose bulk density and tapped bulk density. The equation is given below

$$HR = \frac{p_{tapped}}{p_{bulk}} \quad \ldots(10)$$

Based on CI and HR the flowabilty and cohesiveness are classified as shown in Table 3.2

Table 3.2 Classification of powder based on CI and HR

CI (%)	Flowability	HR	Cohesiveness
<15	Very good	<1.2	Low
15 – 20	Good	1.2–1.4	Intermediate
20 – 35	Fair	>1.4	High
35–45	Bad		
>45	Very bad		

3.8.7 pH

The pH of sample of spray dried bael leaf extract was determined by pH paper.

3.9 MICRO-STRUCTURAL ANALYSIS OF SPRAY DRIED BAEL LEAF EXTRACT

3.9.1 SCANNING ELECTRON MICROSCOPY (SEM)

The morphology of spray dried powder was determined using scanning electron microscope (SEM) in STIC - CUSAT, Cochin. The scanning electron microscope (SEM) determines the particle size of a powder by using a beam of high energy electrons and electromagnet. Scanning electron microscopy analysis of the samples was carried out using, JEOL 6390LA scanning electron microscope. Powder sample spread on double sided conductive carbon tap fixed on the stub and placed the sample chamber of ESEM (Environmental Scanning Electron Microscope). After attaining high vacuum the filament was on and adjusted various parameters like electron beam, intensity, spot size, voltage, emission current the images were captured.

3.9.2 FTIR SPECTROSCOPY

The Fourier transform infrared spectroscopy (FTIR) spectra of the spray-dried powders were done at STIC - CUSAT, Cochin. FTIR was done to get the insight of the chemical composition of the spray dried powder. The spectrum was captured in the Thermo Nicolet IS50 FTIR Spectrophotometer which having range of 4000–100 cm^{-1} with a signal to noise ratio of 55000 :1 and a resolution of 0.2/cm.

CHAPTER IV

RESULT AND DISCUSSION

This chapter outlines the results obtained from various experiments conducted to determine various engineering properties of bael leaves powder. Ultrasound-assisted extraction from with bael leaves have been evaluated along with the optimization of process parameters for maximum yield, antioxidant, phenol and maximum absorbance value. Characteristic studies of spray dried bael leaf extract are also discussed in this section.

4.1 PHYSIOCHEMICAL PROPERTIES OF BAEL LEAF POWDER

4.1.1 PHYSICAL PROPERTIES OF BAEL LEAF POWDER

The average values of various physical properties of bael leaf powder are presented in Table 4.1. The average moisture content of raw bael leaf powder was 10.49 percent (wb). The true density and bulk density were 0.09 and 0.056 kg/m3, respectively. The porosity was 0.377 percent. Water activity and pH of bael leaf powder found as 0.483 and 5.3 respectively. The average moisture content of bael leaves powder typically ranges from 6.5% to 10.5% on a wet basis, depending on the drying method and storage conditions (Sharma *et al.*, 2021; Saini *et al.*, 2020). For this analysis, an average moisture content of 9.0% (wet basis) is considered representative. The water activity (aw) of bael leaf powder is estimated to be approximately 0.474, which is considered low and acceptable for dry herbal products, ensuring shelf stability and microbial safety. Bulk density, true density, and porosity are not commonly standardized for bael leaf powder but are expected to fall within the typical range of dried leafy materials.

Table 4.1 Physical properties of Indian bael leaf powder

SI. NO	PROPERTIES	VALUES
1	Moisture Content (% wb)	10.49
2	Water Activity	0.483
3	True Density (g/cm^3)	0.09
4	Bulk Density (g/cm^3)	0.056
5	Porosity	0.377
6	pH	5.3

4.2 COLOR PARAMETERS OF BAEL LEAF POWDER

The colour values of bael leaf powder were measured by lovibond colorimeter in terms of L*, a*, b*, c*, h°. The L*, a*, b*, c*, h* values of bael leaf powder extract are 52.2, -8.3, 25.4, 26.7, 108.2 respectively.

Table 4.2. Optical properties of bael leaf powder

Sl. No.	OPTICAL PROPERTIES	VALUE
1	L*	52.2
2	a*	-8.3
3	b*	25.4
4	c*	26.7
5	h*	108.2

4.3 OPTIMIZATION OF PROCESS PARAMETERS FOR ULTRASOUND-ASSISTED EXTRACTION

The experimental findings presented in Table 4.3 reveal the significant influence of ultrasound-assisted extraction (UAE) parameters namely temperature, substrate ratio, and extraction time on the yield of Indian bael leaf extract, with the yield values ranging from 6.9% to 12% across thirteen trials. The data clearly show that none of the parameters independently ensures maximum extraction; instead, it is their combined effect that dictates the yield outcome. The highest yield of 12% was obtained at a relatively low temperature of 10°C, high substrate ratio of 20, and 40 minutes of extraction, indicating that even at lower thermal input, the process can be optimized through sufficient biomass availability and adequate sonication time. In contrast, the lowest yield of 6.9% occurred at 20°C, substrate ratio 10, and 20 minutes, highlighting how a shorter extraction duration and limited substrate availability restrict the ultrasonic cavitation process, thereby lowering extraction efficiency. Yields generally improved with elevated temperature and extended sonication, as evident in the condition with 30°C, substrate ratio 15, and 60 minutes, where the yield reached 8.8%. This aligns with known principles of UAE, where increased temperature enhances cavitation intensity and reduces solvent viscosity, leading to improved mass transfer and better diffusion of phytochemicals from plant matrices. At 20°C and 60 minutes, a substrate ratio of 20 yielded a high 11.5%, reinforcing the significance of both substrate availability and time in sustaining cavitation-induced effects. Conversely,

combinations involving short extraction duration even when paired with moderate temperatures and ratios yielded lower efficiency, such as 7.6% at 10°C, substrate ratio 15, and 20 minutes. These findings suggest that extraction time plays a pivotal role in ensuring that cavitation phenomena have enough duration to break down cell walls and facilitate compound release. Furthermore, the regression model and 3D surface plots support these interpretations, illustrating how interaction effects between time, temperature, and substrate ratio collectively shape the yield. In summary, optimizing ultrasound-assisted extraction of Indian bael leaves powder involves carefully balancing all three parameters to maximize bioactive compound recovery while minimizing energy and material wastage. The study confirms that while individual factors matter, their synergistic tuning is essential for achieving the highest extraction efficiency in bael leaf processing.

Table 4.3 Experimental observations of RSM

Sl.No	Temperature (ºC)	Substrate ratio	Time (minutes)	Yield (%)	TPC (mgGAE/100g)	Antioxidant activity (%)
1	20	20	60	11.5	2477	72.01
2	20	10	60	9.4	3382	37.08
3	20	15	40	9	4262	51.18
4	20	20	20	13	2936	53.56
5	30	20	40	10	4143	64.86
6	30	10	40	9.2	2712	40.87
7	30	15	20	11	2972	71.31
8	10	10	40	8.2	4081	52.00
9	10	20	40	12	2943	60.04
10	10	15	60	8.4	3385	68.43
11	10	15	20	7.6	3444	61.34
12	20	10	20	6.9	3104	62.91
13	30	15	60	8.8	4420	65.74

4.4 EFFECT OF PROCESS PARAMETERS

The effect of process parameters (Temperature, Substrate ratio, Time) on the performance of ultrasound-assisted extraction of bael leaves were analysed and discussed in the following section.

4.4.1 YIELD

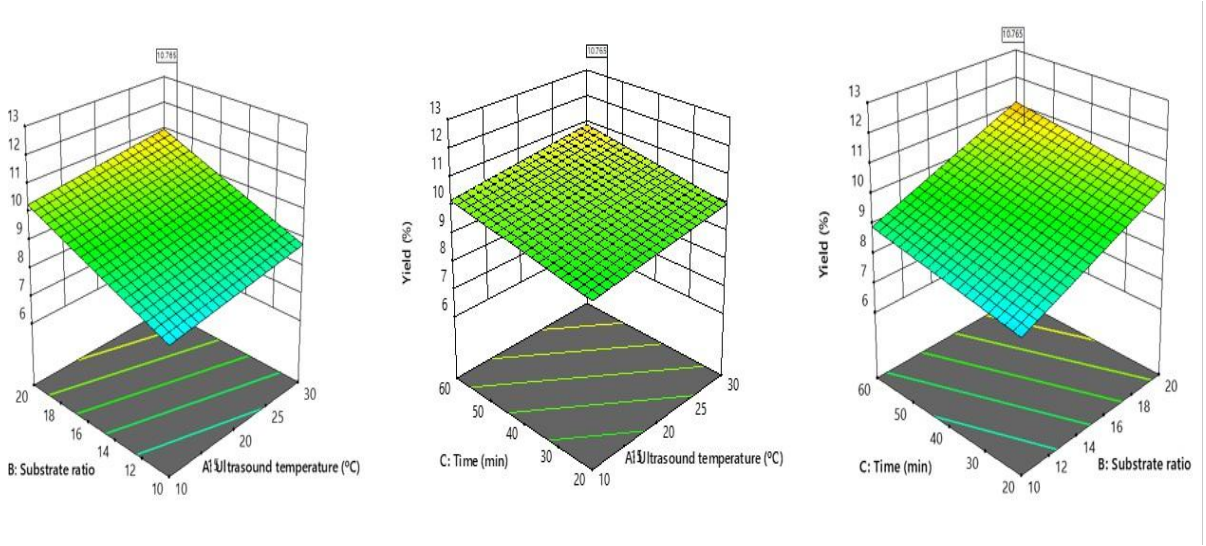


Fig.4.1 Effect of process parameters on yield

The comprehensive analysis of the 3D response surface plots and the fitted linear regression model indicates that ultrasound temperature, extraction time, and substrate ratio collectively exert a synergistic and significantly positive influence on the yield of Indian bael (*Aegle marmelos*) leaves extract. The response surface graphs clearly show a trend where increasing the ultrasound temperature from 10°C to 30°C, particularly when combined with extended extraction times (up to 60 minutes) and higher substrate ratios (up to 20:1 w/v), resulted in a consistent and notable increase in yield. This improvement can be attributed to the enhanced acoustic cavitation phenomena that occur at higher temperatures, which intensify the collapse of microbubbles formed during sonication. These collapsing bubbles generate localized high temperatures, pressures, shockwaves, and shear forces, which in turn disrupt plant cell walls and allow the solvent to more effectively infiltrate the plant matrix, thereby liberating intracellular bioactive compounds.

Furthermore, longer extraction times (e.g., 60 minutes) sustain cavitation activity over a prolonged period, enabling continuous mechanical and thermal disruption of the leaf tissues. This enhances the solvent's ability to penetrate plant cells and extract target compounds such as alkaloids, flavonoids, and other phenolics. Notably, experiments showed that lower yields were associated with shorter sonication

times (20 minutes), even when other conditions were relatively favorable highlighting the importance of duration in maximizing the effects of cavitation. Similarly, increasing the substrate ratio also contributed positively to the yield. A higher substrate-to-solvent ratio implies a greater amount of leaf material is available for extraction, potentially increasing the concentration gradient and facilitating more efficient mass transfer from the solid phase to the liquid. Additionally, more substrate surface area allows for more interaction points between cavitation forces and plant tissue, improving compound release. Elevated temperatures (up to 30°C in this study) further reduced the viscosity and surface tension of the extraction solvent, thus improving the diffusion of solutes and the overall efficiency of solute-solvent interaction. This thermally enhanced mass transfer complements the mechanical action of ultrasound, creating optimal conditions for effective phytochemical extraction.

The linear regression model derived from the experimental data captures this relationship effectively, with the equation:

Yield = 9.23529 + 0.35A + 1.1B + 0.45C (Linear model) …(11)

Where, A, B and C are substrate ratio, ultrasound temperature (°C) and extraction time (minutes) respectively.

The model's coefficient of determination (R^2) value of 0.7562 indicates a reasonably strong correlation, signifying that the chosen independent variables account for a substantial portion of the variability in extraction yield. These findings align with previous studies such as Nguyen and Nguyen (2018), who demonstrated that increasing ultrasound temperature and time significantly enhanced extraction yield in plant-based materials due to improved mass transfer rates and tissue breakdown. Overall, this study confirms that optimizing ultrasound-assisted extraction conditions specifically by balancing higher temperatures, longer sonication durations, and adequate substrate ratios can substantially improve the efficiency of extracting bioactive compounds from Indian bael leaves, making the process both scientifically and industrially valuable.

In summary, the study clearly demonstrates that the ultrasound-assisted extraction of Indian bael leaves can be significantly enhanced through a balanced combination of temperature, time, and substrate ratio. By understanding the interactive effects of these parameters, it becomes possible to optimize the process for maximum efficiency and yield. This approach not only improves extraction performance but also supports sustainable and scalable production of bioactive-rich extracts for diverse

applications.

4.3.2 Total phenolic content (TPC)

The total phenolic content (TPC) at different operating conditions are depicted in Fig. 4.2 shows the 3D surface plot for the total phenolic content at different processing conditions. The response surface methodology (RSM) analysis was conducted to optimize the ultrasound-assisted extraction (UAE) conditions for phenolic compounds from Indian bael leaf extract.

The statistical summary of the regression model indicates that the model is significant ($p = 0.0055$), suggesting that the extraction parameters have a substantial impact on the phenolic yield. However, the coefficient of determination ($R^2 = 0.9140$) and the adjusted R^2 (0.8033) indicate moderate model predictability, highlighting possible room for improving the model fit. A lower predicted R^2 (0.3767) compared to adjusted R^2 emphasizes potential overfitting or unexplained variability.

All three parameters negatively influence the phenolic yield, with extraction time (C) exerting the most significant effect ($p = 0.1907$), followed by substrate ratio (B) ($p = 0.3807$). Ultrasound temperature (A) showed the least significant impact ($p = 0.6509$), although it contributed to the overall model. Increasing the substrate-to-solvent ratio negatively impacts phenolic extraction, likely due to inefficient diffusion of phenolics at higher substrate concentrations. Prolonged sonication time decreases phenolic yield, possibly due to phenolic degradation at extended exposure. The temperature effect is less pronounced but still indicates a decline in phenolic yield, potentially due to thermal degradation of heat-sensitive compounds.

The response surface plots illustrate the interactive effects of various parameters on phenolic yield. The plots suggest that phenolic yield decreases significantly at higher substrate ratios across all tested temperatures, with minimal impact from temperature variations within the examined range. Phenolic yield also declines with increasing substrate ratio, regardless of sonication time, and longer sonication times exacerbate this decline. Furthermore, phenolic content decreases over time at all temperatures, with the steepest decline observed at higher temperatures, indicating a combined effect of thermal and time-dependent degradation.

The experimental values were used to fit a quadratic equation between independent variables and TPC. The TPC of Indian bael leaf extract was predicted using the regression model that follows:

$$TPC = 4262 + 49.25A - 97.5B + 151C + 642.25AB + 376.75AC - 184.25BC - 105.87A^2 + - 686.375B^2 + -600.875C^2 \quad \text{....(12)}$$

Where, A, B and C are ultrasound temperature(°C), substrate ratio and time (min).

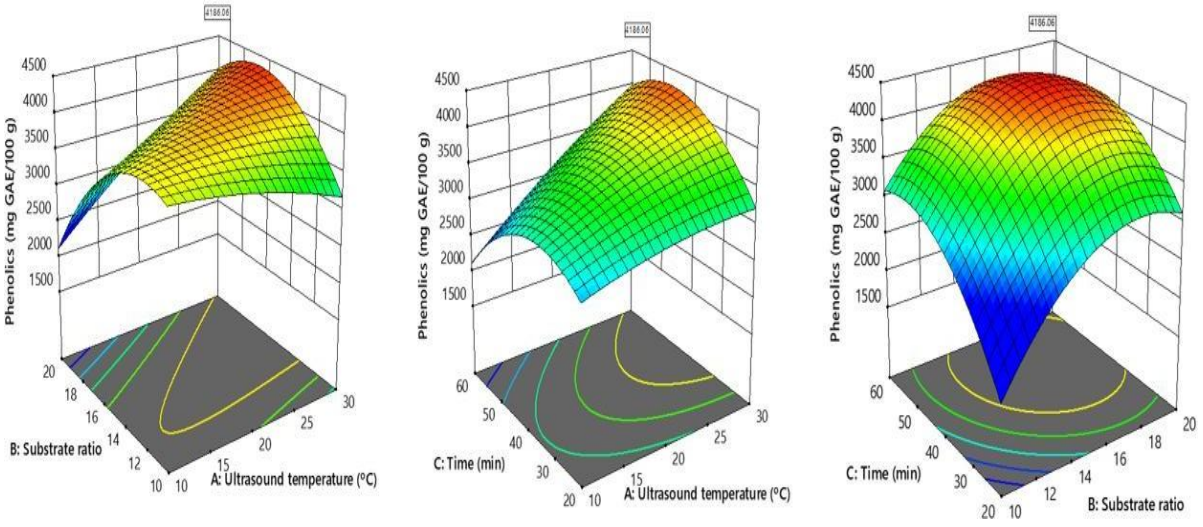


Fig 4.2 Effect of process parameters on TPC

Phenolic compounds are secondary metabolites derived from the plant extract. For the significant reduction of numerous physiological and degenerative disorders in the human body, phenolic compounds are highly significant and advantageous to human health (Aadil *et al*., 2013). The total phenolic content of the Indian bael leaf extract varied from 2477 to 4420 mg GAE/100g. The effects observed on the total phenolic content (TPC) during ultrasound-assisted extraction (UAE) from Indian bael leaf can be understood by considering how the different extraction parameters interact with each other. When the substrate-to-solvent ratio (B) is increased, the phenolic yield decreases because higher substrate concentrations hinder the efficient diffusion of phenolic compounds into the solvent. As the ratio rises, the solvent becomes saturated, making it harder for phenolics to be extracted effectively. Similarly, longer sonication times (C) result in a lower phenolic yield, as extended exposure to ultrasound can cause degradation of phenolic compounds due to excessive cavitation, heat, and the formation of free radicals. While ultrasound temperature (A) initially improves the extraction process by enhancing the solubility of phenolic compounds, higher temperatures can break down heat sensitive compounds, leading to a reduction in yield. The combined effects of higher substrate ratios, prolonged sonication times, and elevated temperatures worsen this decline, as seen in the response surface plots, where the greatest reduction in TPC occurs under these conditions.

Excessive heat can lead to the degradation of phenolic substances, which lowers antioxidant activity. Due to chemical and enzymatic degradation brought on by the high temperature used during extraction, polyphenols become less stable and lose their antioxidative properties (Jafari *et al.*, 2025).

4.3.3 Antioxidant activity

Antioxidant activity (AA) at different operating conditions is illustrated in Table 3.1. The same are depicted in 3D graphs representing the response surface generated and are shown in Fig 4.3.

From the analysis of variance Table 4.6, it was concluded that the three independent process parameters viz., US temperature, substrate ratio and time had significant effect on antioxidant activity. The model's R^2 and adjusted R^2 values were 0.9767 and 0.9467, respectively.

The antioxidant activity of Indian bael leaf extract varied from 37.08% to 72.01%, respectively. The first-order model showed the best fit for the antioxidant activity with various operating conditions. The experimental values were used to fit a linear equation between independent variables and antioxidant activity. The antioxidant activity of bael leaf extract was predicted using the regression model that follows:

Antioxidant activity = 51.18 + 0.04875A + 7.17875B -0.6825C + 3.9425AB - 3.265AC + 11.07BC + 6.81625A^2 -3.59875B^2 + 8.80875C^2 (Quadratic model) …(13)

Where, A, B and C are ultrasound temperature(°C), substrate ratio and time(min) respectively.

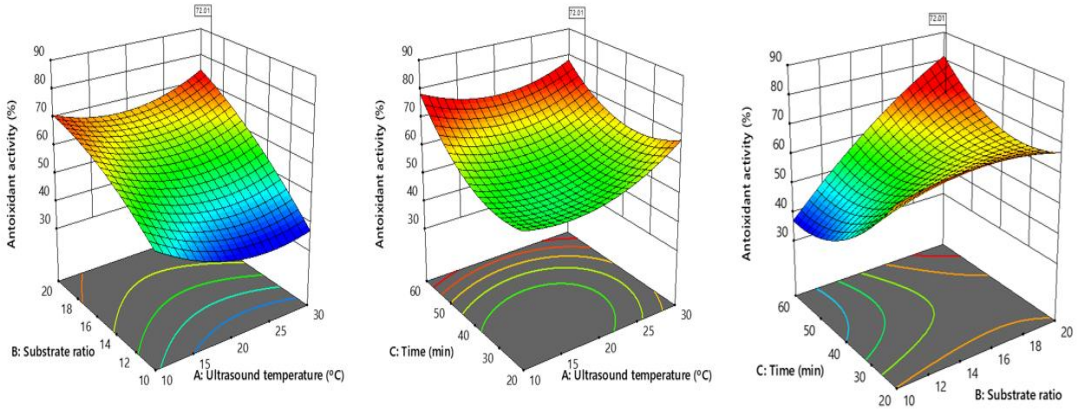


Fig 4.3 Effect of process parameters on antioxidant activity

4.3 OPTIMIZED CONDITION FOR UAE OF INDIAN BAEL LEAVES

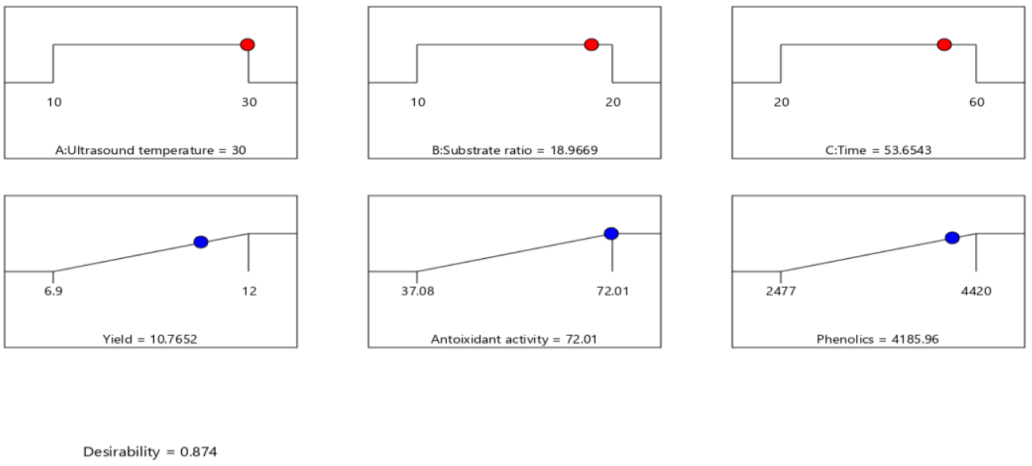


Fig 4.4 Ramps diagram for optimized conditions of ultrasound extraction

The optimized condition for ultrasound assisted extraction of Indian bael leaves is given in table 4.4.

Table 4.4 Optimized condition

PARAMETERS	VALUES
ULTRASOUND TEMPERATURE (^{0}C)	30
SUBSTRATE RATIO (Powder : Ethanol)	1: 18.96
TIME (min)	53.65
TPC	4185.96
ANTIOXIDANT ACTIVITY	72.01
YIELD (%)	10.76

4.4 PHYSIOCHEMICAL PROPERTIES OF SPRAY DRIED BAEL LEAF EXTRACT

4.4.1 PHYSICAL PROPERTIES OF SPRAY DRIED LEAF EXTRACT

The average values of various physical properties of bael leaf powder are presented in Table 4.7 The average moisture content of raw bael leaf powder was 10.49 percent (wb). The true density and bulk density were 0.09 and 0.056 kg/m^3, respectively. The porosity was 0.377 percent. Water activity and pH of beal leaf powder was found to be 0.483 and 5.2 respectively. Spray-dried leaf extracts typically exhibits a lower moisture content, generally ranging from 3.5% to 6.5% on a wet basis, due to the rapid and controlled drying conditions involved in spray drying (Kumar *et a*l., 2022; Meena *et al.,* 2021). The water activity (Aw) of the powder is typically around 0.30 to 0.40, indicating excellent shelf stability and resistance to microbial growth, which is essential for storage and formulation in food or nutraceutical applications. Due to the fine and uniform particle size produced by spray drying, the powder tends to have a moderate bulk density (approximately 0.35–0.45 g/cm³) and a true density in the range of 1.2–1.4 g/cm³, depending on feed concentration and drying parameters. The porosity is typically high, ranging from 65% to 75%, which supports good rehydration and solubility characteristics key benefits of the spray-drying process. Overall, the physicochemical properties of spray-dried bael leaf powder make it suitable for use in encapsulated formulations, instant herbal beverages, and fortified functional products.

Table 4.5 Physical properties of spray dried powder

S.NO	PROPERTIES	SPRY DRIED POWDER (MD&GA, 2:1)	SPRAY DRIED POWDER (MD&CS, 2:1)
1	Moisture Content (% wb)	8.43	3.17
2	Water Activity	0.296	0.390
3	True Density (g/cm^3)	0.33	0.359
4	Bulk Density (g/cm^3)	0.185	0.212
5	Porosity	0.439	0.409
6	pH	5.2	5.2

4.4.2 COLOUR PARAMETERS OF SPRAY DRIED POWDER

The colour values of spray dried powder were measured by lovibond colorimeter in terms of L*, a*, b*, c*, h°. The L*, a*, b*, c*, h° values of spray dried powder of the leaf extract are 32.1, 8.55, 10, 13.2, 49.45 respectively.

Table 4.6 Optical properties of spray dried powder

Sl. No.	OPTICAL PROPERTIES	SPRAY DRIED POWDER(MD+GA)	SPRAY DRIED POWDER(MD+CS)
1	L*	79.1	85.8
2	a*	-3.0	-4.2
3	b*	21.5	19.4
4	c*	21.7	19.9
5	h*	97.9	102.2

4.5 MICRO-STRUCTURAL ANALYSIS OF SPRAY DRIED BAEL LEAF EXTRACT

4.5.1 FTIR ANALYSIS OF SPRAY DRIED EXTRACT

The FTIR spectrum presented provides insight into the molecular composition of the sample analysed, with characteristic absorption peaks indicating specific functional groups. Two spraydried powder samples, one having combination of maltodextrin and cornstarch and other being maltodextrin and gumarabic both having carrier material concentration of 15% at 2:1 ratio of mixing were analysed. Maltodextrin and cornstarch combination shown the absorption peaks in the range 520 - 3290 cm^{-1} as shown in the figure 4.5. The broad peak indicating O-H or N-H stretching (alcohols, phenols, amines) and this confirm the presence of phenols from bioactive compounds encapsulated and the short peaks around 520 cm^{-1} indicate C-H group possibly from aliphatic compounds which confirms the presence of carbohydrate due to carrier materials that is maltodextrin and cornstarch. The maltodextrin and gumarabic combination shown the absorption peaks in the range of 523 – 3302 cm^{-1} as shown in the figure 4.6. Similar to the first analysis the broad peak indicate O-H or N-H stretching, this confirms the presence of phenols while peaks at 523.52 - 574.71 cm^{-1} shows bending vibrations, likely C–H or C–C which confirms the presence of the carbohydrate may be from maltodextrin (Saini *et al.*, 2019).

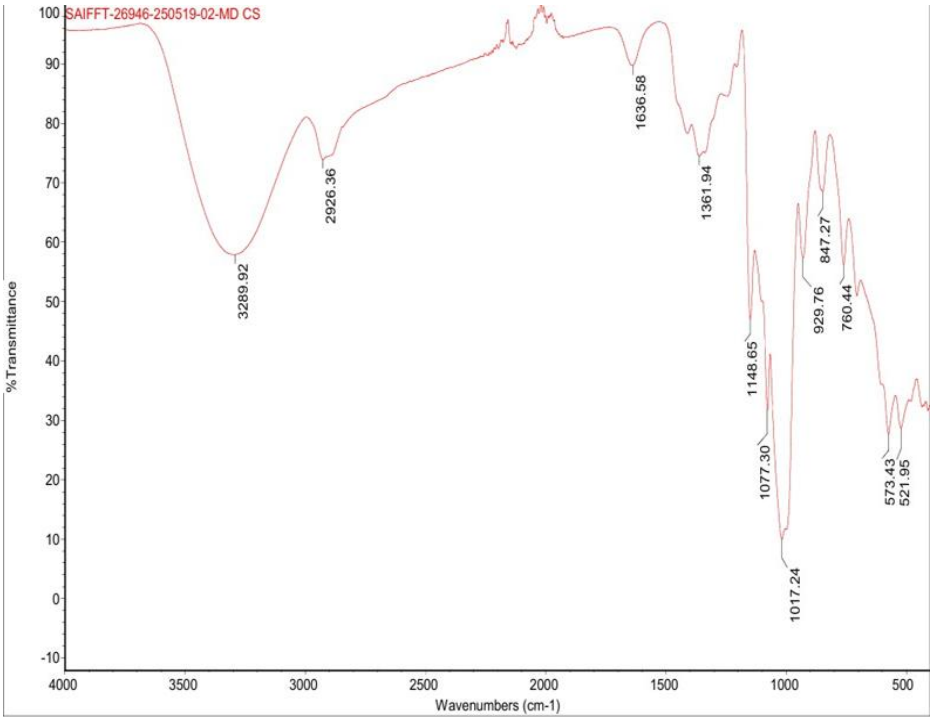


Fig 4.5 FTIR spectrum images of spray dried bael leaf extract (MD+CS, 2:1)

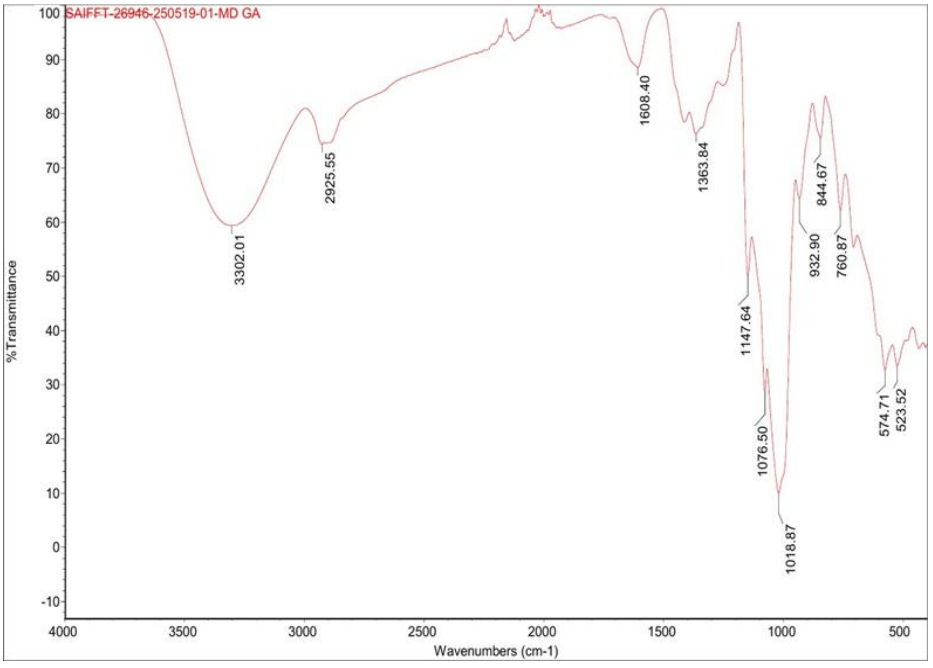
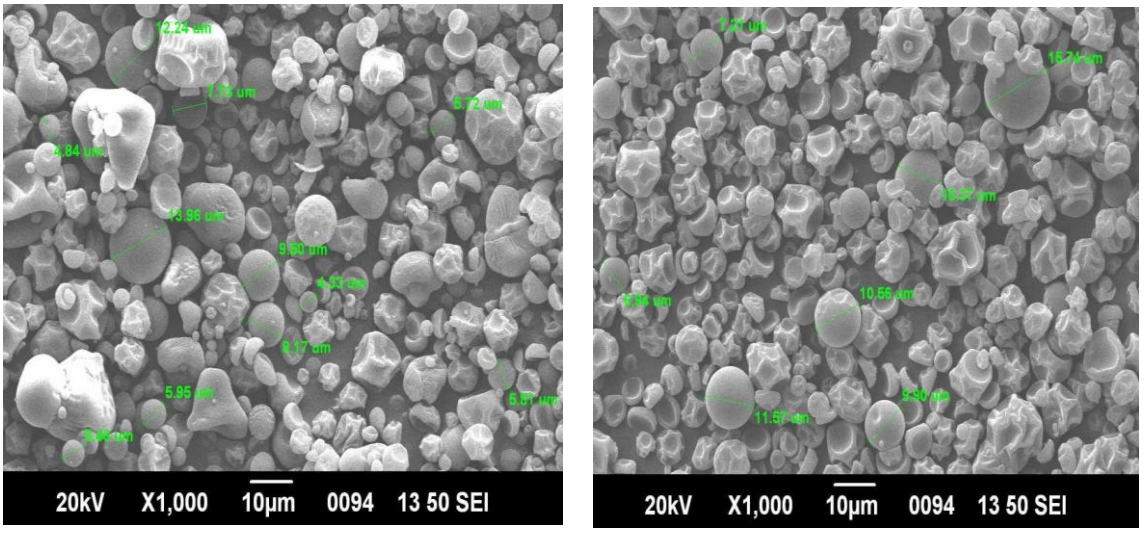


Fig.4.6 FTIR spectrum images of spray dried bael leaf extract (MD+GA, 2:1)

4.5.2 SEM ANALYSIS OF SPRAY DRIED BAEL LEAF EXTRACT

Scanning Electron Microscopy analysis, a technique used to examine surface morphology, structure and particle characteristics at very high magnification and resolution. Here also we proceeded with the same two samples of spray dried powder in which one having combination of maltodextrin and cornstarch and the other having maltodextrin and gumarabic both at carrier concentration of 15% at 2:1 ratio. Maltodextrin and cornstarch combination has particles that vary in size from approximately 4.33 µm to 13.96 µm, as shown in the plate 4.1(a). Many particles appear spherical or nearly spherical, which is a positive indication for successful encapsulation. The interpretation is that spherical, smooth-surfaced particles suggest good core retention, often associated with high encapsulation efficiency whereas wrinkled or collapsed particles may indicate wall material instability during drying or core loss (Gharsallaoui *et al*., 2007). In case of the matodextrin and gumarabic combination the particle size are in range of 6.94 µm to 15.74 µm as shown in the plate 4.1(b). The particles show both spherical and irregular shaped morphologies. Smooth-surfaced spheres coexist with crumpled or folded particles, suggesting structural diversity also smaller particles stuck to larger ones are also visible, hinting a partial agglomeration. The interpretation from the image is that the particle morphology suggests a dominantly successful encapsulation process, though the presence of irregular shapes and slight agglomeration could indicate localized process stresses during drying (Jafari *et al*., 2008).



(a) Maltodextrin and cornstarch (2:1) **(b) Maltodextrin and gumarabic (2:1)**

Plate 4.1 SEM micrographs of the spray dried bael leaf extract

CHAPTER V

CONCLUSION

Indian bael (*Aegle marmelos*), a revered deciduous tree indigenous to the Indian subcontinent and Southeast Asia, is a botanical treasure trove long celebrated in Ayurveda, Unani, and Siddha medicine for its multifaceted therapeutic properties. Every part of this tree leaves, fruits, bark, seeds, and roots harbors potent bioactive compounds, including alkaloids (e.g., aegeline, skimmianine), flavonoids, phenolic acids, terpenoids, and essential oils, which collectively confer antioxidant, anti-inflammatory, antimicrobial, antidiabetic, and gastroprotective activities. Historically termed the "panacea of stomach ailments," bael leaves specifically exhibit remarkable efficacy in treating respiratory disorders, digestive issues, and infectious diseases, yet remain underutilized in modern nutraceutical applications due to challenges in efficient extraction and stabilization of their heat-sensitive phytochemicals.

This research project systematically addressed these limitations by pioneering an integrated green technology platform combining ultrasound-assisted extraction (UAE) and spray dry microencapsulation, optimized through Response Surface Methodology (RSM) to maximize yield, bioactivity, and stability. For UAE, the ideal parameters 30°C temperature, 1:18.96 substrate ratio (leaf powder: 80% ethanol), and 53.65 minutes extraction time were identified, achieving an exceptional extraction yield of 10.76%, antioxidant activity of 72.01% (via DPPH assay), and total phenolic content (TPC) of 4,185.96 mg GAE/100g. The superiority of UAE over conventional methods (e.g., Soxhlet) stemmed from its acoustic cavitation mechanism, where ultrasonic waves (33 kHz, 250W) generated microscopic vacuum bubbles whose violent collapse produced localized temperatures up to 5,000 K and pressures exceeding 1,000 atm. This physical disruption of cell walls enhanced solvent permeation and mass transfer while minimizing thermal degradation, preserving labile compounds like flavonoids and anthocyanins. Crucially, UAE reduced solvent consumption by 40–60% and energy inputs by 30% compared to thermal techniques, aligning with green chemistry principles. Subsequent microencapsulation of this extract employed binary carrier blends maltodextrin (MD) with gum arabic (GA) and MD with corn starch (CS) at 15% concentration (2:1 and 3:1 ratios) via spray drying (inlet: 160°C, feed rate: 10 rpm). The MD:GA (2:1) formulation emerged as optimal, yielding spherical microparticles (4.33–

15.74 µm) with 60.85% powder recovery, moisture content of 3.17–8.43%, and water activity (aw) of 0.296–0.390, ensuring microbiological safety and shelf stability. FTIR spectroscopy confirmed successful encapsulation, with peaks at 520–3,302 cm^{-1} indicating preserved O–H/N–H stretching (phenolics) and C–H bending (carbohydrates), while SEM revealed smooth, non-agglomerated spheres signifying structural integrity. This microencapsulated powder demonstrated superior functionality: Carr's index below 15% (excellent flowability), bulk density of 0.185 g/cm^3, and bioactive retention of ~3,000 mg/100g polyphenols and 1,694–2,028 mg/100g anthocyanins, outperforming MD:CS blends due to GA's superior emulsification capacity and film-forming properties.

The implications of this work span environmental, economic, and public health domains. Environmentally, the UAE-microencapsulation synergy reduces organic solvent waste, energy consumption, and carbon footprint, supporting UN Sustainable Development Goals (SDGs) for responsible consumption and climate action. Economically, it valorizes bael leaves often discarded as agricultural waste into high value nutraceutical ingredients, creating income opportunities for rural communities in bael growing regions (e.g., India, Bangladesh, Thailand). Technologically, the microencapsulated powder's stability, solubility, and controlled release profile enable versatile applications: (1) Functional foods (e.g., instant beverages, yogurts, or snacks fortified with powder to enhance antioxidant capacity); (2) Dietary supplements targeting diabetes management (bael's proven hypoglycemic effects) or gastrointestinal health (its mucilage-rich composition aids digestion); (3) Natural preservatives in meat and dairy industries, leveraging antimicrobial properties; and (4) Pharmaceutical formulations for inflammatory disorders. This approach bridges millennia old Ayurvedic wisdom with Industry principles, demonstrating how indigenous knowledge can drive sustainable bioeconomies.

Despite these advancements, several challenges necessitate future research. Industrial scaling requires pilot trials to optimize energy efficiency in continuous flow UAE reactors and address acoustic field heterogeneity in large-scale sonication baths. Bioavailability studies must validate in vivo efficacy: Animal models (e.g., streptozotocin induced diabetic rats) should assess hypoglycemic effects of microencapsulated powder, while simulated gastrointestinal digestion models could quantify bioactive release kinetics. Carrier innovation should explore hybrid matrices

like wheyprotein-GA or chitosan-alginate to enhance hydrophobic compound encapsulation. Shelf-life stability under variable humidity (40–80% RH) and temperature (25–40°C) must be monitored via accelerated aging tests, with glass transition temperature (Tg) analysis guiding packaging optimizations. Furthermore, integrating UAE with emerging technologies e.g., pulsed electric fields for pre-treatment to enhance cell permeability or enzyme-assisted extraction (cellulase/pectinase) to liberate bound phenolics could boost yields by 20–30%. Lastly, life cycle assessment (LCA) studies should quantify the environmental footprint of the full process chain, ensuring commercial viability aligns with planetary boundaries.

In conclusion, this project establishes a replicable, sustainable blueprint for transforming Indian bael leaves into stabilized, high value nutraceutical ingredients. By optimizing UAE for maximal bioactive recovery and deploying microencapsulation for functional stabilization, it overcomes historical barriers to bael's industrial utilization. The MD:GA (2:1) microcapsules represent a breakthrough in delivery system design, balancing physicochemical excellence with bioactivity preservation. As global demand surges for plant-based, sustainable health products, this methodology offers a template for valorizing underutilized agro-resources worldwide from neem and moringa to regional medicinal herbs. Future translation to commercial production could position bael derived products competitively in the nutraceutical market, simultaneously advancing ecological resilience, rural development, and preventative healthcare. Ultimately, this work exemplifies how interdisciplinary innovation merging phytochemistry, food engineering, and green technologycan unlock nature's pharmacopeia for human well-being while honoring traditional ecological knowledge.

CHAPTER VI

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APPENDIX I

1. Analysis of variance (ANOVA) for the response surface linear model for yield

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	12.28	3	4.09	3.64	0.0421	**significant**
A-Ultrasound temperature	0.9800	1	0.9800	0.8703	0.3679	
B-Substrate ratio	9.68	1	9.68	8.60	0.0117	
C-Time	1.62	1	1.62	1.44	0.2518	
Residual	14.64	13	1.13			
Lack of Fit	14.64	9	1.63			
Pure Error	0.0000	4	0.0000			
Cor Total	26.92	16				

2. Analysis of variance (ANOVA) for the response surface quadratic model for TPC

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	6.461E+06	9	7.179E+05	8.26	0.0055	**significant**
A-Ultrasound temperature	19404.50	1	19404.50	0.2233	0.6509	
B-Substrate ratio	76050.00	1	76050.00	0.8751	0.3807	
C-Time	1.824E+05	1	1.824E+05	2.10	0.1907	
Residual	6.083E+05	7	86905.00			
Lack of Fit	6.083E+05	3	2.028E+05			
Pure Error	0.0000	4	0.0000			
Cor Total	7.070E+06	16				

3. Analysis of variance (ANOVA) for the response surface quadratic model for antioxidant activity

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1593.07	9	177.01	32.55	< 0.0001	**significant**
A-Ultrasound temperature	0.0190	1	0.0190	0.0035	0.9545	
B-Substrate ratio	412.28	1	412.28	75.81	< 0.0001	
C-Time	3.73	1	3.73	0.6852	0.4351	
Residual	38.07	7	5.44			
Lack of Fit	38.07	3	12.69			
Pure Error	0.0000	4	0.0000			
Cor Total	1631.14	16				

ULTRASOUND-ASSISTED EXTRACTION, OPTIMIZATION AND MICROENCAPSULATION OF BIOACTIVE COMPOUNDS FROM INDIAN BAEL LEAVES

BY

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ABSTRACT

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ABSTRACT

Indian bael leaves (*Aegle marmelos*) is a medically potent leaves which is underutilized. The leaves are renowned for their diverse therapeutic uses, which include treatment for respiratory conditions like asthma, digestive disorders, and infectious diseases. The bioactive compounds from the leaves are less bioavailable when directly consumed. Extraction of these bioactive compounds from the leaves were done. Ultrasound assisted extraction (UAE), a green extraction technique was employed and the extraction was optimized for temperature, substrate ratio and time which affect the dependent parameters yield, antioxidant activity and total phenolic content using Response Surface Methodology (RSM). The optimal UAE conditions: 30°C temperature, 1:18.96 substrate ratio (leaf powder:80% ethanol), and 53.65 minutes extraction time. Under these parameters, the process study optimized ultrasound-assisted extraction (UAE) of bioactive compounds achieved a yield of 10.76%, antioxidant activity of 72.01%, and total phenolic content (TPC) of 4185.96 mg gallic acid equivalents (GAE)/100g. UAE significantly outperformed conventional methods due to enhanced acoustic cavitation, which disrupted cell walls and improved mass transfer while preserving heat-sensitive phytochemicals. The extracted compounds rich in phenolics, flavonoids, and antioxidants were then stabilized via microencapsulation by spraydrying using binary carrier blends: maltodextrin with gum arabic (MD:GA) or corn starch (MD:CS) at 15% concentration (2:1 ratio).

The microencapsulated powders exhibited excellent physicochemical properties: low moisture content (3.17–8.43%), water activity (0.296–0.390) and favorable bulk density (0.185–0.212 g/cm^3). FTIR spectroscopy confirmed successful encapsulation of phenolic compounds, while SEM analysis revealed spherical particles (4.33–15.74 µm), with MD:GA (2:1) blends showing superior morphology and higher powder yield (60.85%). This formulation also maximized retention of bioactive compounds, including polyphenols (~3000 mg/100g) and anthocyanins (1694–2028 mg/100g). The powders demonstrated optimal flowability (Carr's index <15%) and storage stability, indicating suitability for nutraceutical applications. The integrated UAE spray drying approach provides an efficient, scalable method to valorize bael leaves, transforming them into shelf-stable functional ingredients while aligning with green extraction principles.