IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

# A Natural Solution to Food Preservation: The Multifaceted Benefits of Betel Leaf

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## Abstract

The quest for safe and effective food preservation methods has led to a resurgence of interest in natural preservatives. Betel leaf, a traditional herb in many Asian cultures, has been found to possess remarkable antimicrobial and antioxidant properties, making it an attractive alternative to artificial preservatives. This review aims to unlock the potential of betel leaf as a natural preservative, exploring its bioactive compounds, mechanisms of action, and applications in food preservation. We also discuss the benefits of natural preservation over artificial methods, including enhanced food safety, reduced chemical contamination, and improved nutritional quality. Our analysis highlights the promising prospects of betel leaf as a sustainable and effective natural preservative, paving the way for further research and development in this area.

KEYWORDS: Piper Betle, Betel leaf Natural preservatives, Antimicrobial, Betel leaf essential oil, Eugenol, Extraction

## Introduction

The need for plant-based natural preservation is being driven by wellness challenges, consumer demands, and the need for sustainable and suitable substitutes to synthetic preservatives. Natural preservation involves using nature-based materials to extend the food freshness restricting degradation brought on by pathogenic proliferation, rancidity and biocatalytic action [1]. Natural preservatives are used to compensate synthetic chemicals, which may have adverse health effects such as hypersensitivity, allergy, hyperactivity, neurological damage, and cancer [1, 2, 3]. Plant extracts, such as oils, phenolic compounds, terpenoids and flavonoids, are the most common holistic additive used due to their ability to combat microbes and oxidative stress [4]. Natural antimicrobials and antioxidants are crucial instruments for securing foods from bacteria and decay processes [5].

Natural preservatives can be administered to a wide range of food items, covering poultry, dairy, fruits, vegetables, and beverages. For example, plant extracts have been used to safeguard milk, fatty foods, fruit juices, and even fermented delicacy like idli batter [1, 6, 7, 8]. They can be incorporated into the product formulation, coated on the surface, or embedded within packaging material [9]. They are regarded more eco-friendly and align with sustainability ideas [1]. They are often obtained from regenerative resources such as plants, animals, and microorganisms [8]. This can minimise the environmental load associated with the generation and disposal of synthetic chemicals. Furthermore, natural preservatives play a crucial role in minimizing food waste by prolonging shelf life.

Artificial preservation technique encompasses the use of synthetic chemicals to protract the freshness of food products by controlling microbial growth and decay [8]. There is escalating scientific evidence depicting that synthetic chemicals used as food additives may have adverse health effects, including obesity, cancer, asthma, and heart problems [10]. Consuming foods containing chemical preservatives like nitrates and benzoates may unveil individuals to potential health hazards, including hypersensitivity, hyperactivity, and cancer. Plant extracts offer a way to avoid these risks [8]. Consumers are looking for products that are free from artificial additives, leading to more demand for natural alternatives. Traditional preservation methods often involve high energy consumption and can produce greenhouse gases, raising environmental concerns. Combination of plant extracts and advanced packaging technologies provides a pathway towards reducing synthetic chemical use and promoting sustainable food preservation practices [1].

Betel leaf, scientifically known as *Piper betle L.*, is a plant with deep cultural roots and a history of traditional use, particularly in Southeast Asia. The origin of paan (betel) is most likely Malaysia [4, 8]. The Piperaceae family, to which betel leaf belongs, has a long history dating back 5500-7000 BC. Betel leaves are widely consumed as a condiment in Africa and Asia, particularly in India and Taiwan [11]. In India, these heart-shaped, dark green leaves are commonly called "paan" and are used to freshen breath, clean the mouth after meals. [8, 11, 12]. Among the natives of the Cordilleras, betel leaf is combined with betel nut and used as an alternative to cigarettes [13]. Betel leaf is significant in other Southeast Asian nations, Taiwan, Malaysia, Thailand, and Sri Lanka. There are approximately 100 species that have been identified and cultivated in different countries around the world, with 40 found only in India and 30 in Bangladesh and West Bengal. Spanning various regions of India and beyond its borders, betel leaf goes by various names depending on its characteristics such as structure, color, fragrance, flavor, and dimension [8]. Because of its qualities presenting a distinctive, aromatic, and pungent flavor, it is used as a masticator by most Asian people [4].

Traditionally, betel leaves have been used for medicinal purposes for centuries, treating wounds, indigestion, and inflammations. They are also used for their antiseptic properties. In some cultures, boiled betel leaves are used as a mouthwash, and as a remedy for various ailments like coughs, colds, asthma and constipation [4, 8]. The plant's popularity extends beyond its cultural and traditional uses, as it has a range of beneficial properties. Leaves exhibit antimicrobial activity, which helps protect food material from harmful microorganisms, making it useful as a food preservative [4, 8, 10, 14]. Betel leaves harbour a variety of bioactive substances, including polyphenols and terpenes. These components contribute to the aroma, taste, and medicinal properties of betel leaves [4].

## Types of natural preservatives

Natural preservatives can be sourced from different natural sources which comprises of plant, animal and microbial origin. Plant origin includes plant extracts, such as those from spices, herbs, fruits and vegetables [5], for example betel leaf extract, rosemary oil, oregano, thyme, clove, olive leaves, green tea, citrus extracts, tamarind and lemon [4, 12]. Essential oils (EOs) extracted from discrete regions of aromatic plants like petals, leaflets, petioles as well as seeds [5, 6], contain bioactive compounds like acid, aldehydes, aliphatic alcohols, iso-flavonoids, ketones, phenolic compounds and terpenes [5]. Few enzymes from animals can act as natural preservatives. Enzymes like lysozyme and lactoperoxidase found in milk and other animal tissues [ 1, 2, 4, 5, 13], derived from different animals can act as a natural preservative. Microorganisms produce variety of enzymes and substances which can serve as natural preservative [1]. Fermentative bacteria, yields germicidal substances like biological acids (e.g., lactic acid), bacteriocins (e.g., nisin, pediocin, reuterin), hydrogen peroxide and diacetyl [3, 4, 5, 9] during fermentation, which aims to preserve food and improve shelf life of a product.

Table 1 Types of natural preservatives [1].

| PRESERVATIVE | ORIGIN | FUNCTION | UTILIZATION | MERITS | OBSTACLES | SHELF-LIFE STABILITY |
|---|---|---|---|---|---|---|
| Plant extracts | Essential oils, phenolics, flavonoids, alkaloids, saponins. | Disrupting microbial membranes, modifying intracellular pH, antimicrobial and antioxidant actions. | Dairy, beverages, fruits, vegetables, meats. | Natural, multi-functional, User-friendly and highly effective in small amounts | Intense taste, efficacy can be influenced by external factors | Prolongs freshness by 3-14 days for fruits and vegetables, and up to 7 days for meats and dairy. |
| Microbial preservatives | Lactic acid bacteria, organic acid, bacteriocins. | Production of antimicrobial substances (e.g., organic acids, peptides) during fermentation. | Dairy products meat and poultry, beverages, bakery products. | Naturally sourced, minimal ingredients, and functional benefits. | Effectiveness varies based on microbial strains and surrounding conditions. | Adds 1-4 weeks to the shelf life of dairy products and 7-14 days for meats and baked goods. |
| Enzymatic preservatives | Lysozyme, lactoperoxidase, glucose oxidase. | Degrade or modify components used by spoilage organisms (e.g., cell wall hydrolysis, oxidation). | Dairy, meat, beverages, bakery | Organic and efficient at low concentrations. | May cause allergic reactions, stability influenced by acidic/basic and thermal conditions | Increases the stability by 7–14 days for dairy and beverages, up to 10 days for meats and bakery items. |

Natural preservatives can also be familiarised based on their primary ability of slowing down the food spoilage [1]. Antimicrobial agents are the compounds that prevent or kill microorganisms such as bacteria, yeasts, and moulds, which are accountable for food deterioration and foodborne illnesses [3, 4, 5, 9]. Many plant extracts, essential oils, and microbial metabolites (like organic acids and bacteriocins) fall within this category [1, 4, 5, 9]. Betel leaf extract, with its phenolic compounds and eugenol, exhibits high antibacterial, antioxidant and antifungal properties [8, 15]. Few agents prevent or delays the oxidation of food components, particularly lipids, which can lead to oxidative spoilage, off-flavours, and nutrient depletion [4, 5, 16]. Plant extracts enriched with phenolic compounds, flavonoids, and other antioxidants are important antioxidant agents [1, 3, 4, 10]. Some natural compounds can delay the phenolic browning of foods, especially fruits and vegetables [4, 9]. Examples of anti-browning agents are erythorbic acid and calcium lactate [2, 4]. Some natural agents can inhibit enzymatic activity that promotes food degradation beyond microbial spoilage and oxidation.

## *Piper betle*

In India, the heart-shaped, dark green leaves are generally known as "paan" and are mainly used to refresh breath and soothe the mouth after meals. [8, 11, 12]. India expends 15–20 million betel leaves yearly and grows them on approximately 50,000 acres, with an entire worth of 9 billion rupees [8]. There are around 100 betel leaf species uncovered globally, with 40 traced only in India and 30 in Bangladesh and West Bengal. The leaves have different names across different regions based on features like structure, colour, aroma, flavor, and dimension [11]. Names include Venmony, Magadhi, Salem, Kauri, Banarasi, Mysore, and Bangla [8].

The betel plant is a climbing plant that lives for many years. It has glossy leaves and produces white flower clusters. The plant grows by attaching itself to a nearby tree or support using roots that grow from each joint of the stem. [15, 16]. Dimorphic traits exist in betel plants, guiding biomass release and constituent diversity [4]. The plant's leaves are alternate and simple, with colors ranging from a soft yellowish-green to a more vibrant bright green [6]. Fresh betel leaves are generally deep green and heart-shaped [11]. Their texture is springy and chewable, while their flavor is bold, pungent, and sometimes appetizing [14]. Male plants significantly have narrowly ovate leaves, whereas female plants hold cordate leaves. The range of leaf colors is quite wide, encompassing many shades of green, yellow, red, and black in certain cultivated varieties, which can be used as a visual aid for recognizing specific cultivars [6]. The aroma and taste of betel leaves are due to essential oils, phenols, and terpenes [4, 15]. These components add to the medicinal characteristics of the leaves.

## Chemical composition of betel leaf

Healthy betel leaves primarily comprise water (85-90%), proteins (3-3.5%), minerals (2.3-3.3%), fats (0.4-1.0%), and fibres (2.3%) [12-16]. They also have carbohydrates (0.5-6.1%), and have a nutritional value of 44 kcal per 100g [4, 8, 11, 13]. Vitamins present embodies Vitamin C (0.005-0.01%), Vitamin A (1.9-2.9 mg per 100g), nicotinic acid (0.63-0.89 mg per 100g), Vitamin B1 (10-70 µg per100g) and Vitamin B2 (1.9-30 µg per100g) [8, 13, 15].

Table 2 Chemical composition of Betel leaf [11]

| PROXIMATE PRINCIPLES AND DIETARY FIBRE (per 100g edible portion) | |
|---|---|
| Moisture (g) | 85.92 ± 0.16 |
| Protein (g) | 2.62 ± 0.28 |
| Ash (g) | 2.59 ± 0.18 |
| Total Fat (g) | 0.75 ± 0.04 |
| Carbohydrate (g) | 6.16 ± 0.33 |
| FAT SOLUBLE VITAMINS (per 100g edible portion; blank space in the table indicates value below detectable limit) | |
| Ergocalciferol(D2) (µg) | 2.27 ± 0.25 |
| Tocopherols | |
| Alpha(mg) | 0.02 ± 0.01 |
| Beta (mg) | |
| Gamma (mg) | 0.05 ± 0.00 |
| Delta (mg) | |
| Tocotrienols | |
| Alpha (mg) | 0.02 ± 0.00 |
| Beta (mg) | |
| Gamma (mg) | |
| Delta (mg) | |
| Phylloquinones (µg) | 204 ± 4.9 |
| MINERAL AND TRACE ELEMENT CAROTENOIDS (per 100g edible portion; blank spaces in the table indicate values below the detection limit) | |
| Aluminium (mg) | 1.85 ± 0.43 |
| Arsenic (µg) | 1.68 ± 0.00 |
| Cadmium (mg) | |
| Calcium (mg) | 196 ± 13.7 |
| Chromium (mg) | 0.019 ± 0.005 |
| Cobalt (mg) | 0.002 ± 0.001 |
| Copper (mg) | 0.29 ± 0.02 |
| Iron (mg) | 2.87 ± 0.29 |
| Lead (mg) | 0.004 ± 0.003 |
| Lithium (mg) | 0.005 ± 0.006 |
| Magnesium (mg) | 89.94 ± 14.98 |
| Manganese (mg) | 1.79 ± 0.62 |
| Mercury (µg) | |
| Molybdenum (mg) | 0.002± 0.002 |
| Nickel (mg) | 0.043 ± 0.017 |
| Phosphorus (mg) | 55.72 ± 3.36 |
| Potassium (mg) | 5.40 ± 5.2 |
| Selenium (µg) | 14.04 ± 1.71 |
| Sodium (mg) | 0.39 ± 2.32 |

Betel leaves are rich in a wide array of bioactive compounds, including polyphenols, terpenes, alkaloids, tannic acid, sterolic compounds, phyto-glycosides, saponins. Specific bio-active compounds identified in betel leaves includes anethole, estragol, eugenol, methyl eugenol, hydroxy catechol, caryophyllene, 1,8-cineole, chavibetol, chavicol, safrole and hydroxychavicol. Other compounds found in betel leaf extracts include α-pinene, cis-sabinene, β-farnesene, β-salenene, eugenyl acetate, garmacerene-B, spathulenol, globulol, and allylpyrocatechol diacetate. Different extraction methods uncovered compounds like eugenic, isoeugenic acid, 4-allyl12-diacetoxybenzene, hydroxychavicol, dis-methylene squalene deoxy-para-Dione-A, and 1-n-dodecanyloxy resorcinol [4, 8].

The essential oil content scales from 0.08% to 0.2%. Sixty-five chemical ingredients can be isolated in the essential oil of betel leaves using distillation, including 5-(2-propenyl)-1,3 benzodioxol (25.67%), eugenic acid (18.27%), and acetophenol (8.0%). Other major compounds are chavibetol acetate (15.5%) and chavicol (53.1%). Betel oil also has terpenes and terpenoids which exhibit antimicrobial property. The essential oils also include phenylpropanoids, aldehydes, and sesquiterpenes [4, 7, 8, 15].

Table 3 Molecular composition of betel leaf with its attributes [8]

| Compound | Biological properties | Organoleptic properties | Application |
|---|---|---|---|
| Eugenol | Insecticidal, pain reliever, antioxidant, anti-mutagenic, analgesic, malignancy-preventing, antiseptic, thymoleptic, fungicide | Pungent aroma with clove-like flavor | Oral pain reliever |
| Hydroxychavicol | Anti-exudative, antioxidant, antimutagenic, bactericidal, tumor-suppressing, fibrinolytic enhancer, ulcerative healing properties | Slight creosote smell | Used in mouthwash for curing and treating oral abscesses. |
| Methyl eugenol | Anti-edematous, toxic to human cell line, insecticidal activity | Clove-like fragrance | Utilized in the production of fragrances, perfumes, personal care products, and cleaning agents. |
| Chavibetol | Topical pain reliever, antineoplastic, anti-thermic, immunomodulatory, bacteriostatic | Sharp, spicy smell | Used as an essential oil. |
| β- caryophyllene | Pain relief, anti-inflammatory, carcinostatic, main treatment for atheromatous and osteoporosis | Clove like aroma with the aromatic warmth of black pepper | Employed as a preventive agent against multiple diseases, such as diabetes, endometriosis, cerebral ischemia, anxiety disorders, depression, liver fibrosis, and neurodegenerative diseases similar to Alzheimer's. |
| Chavicol | Antiseptic, antimicrobial, antioxidant | Phenolic scent | Used as a flavor |
| Safrole | Antimutagenic, anti-phlogistic, purifying mediator, antimicrobial, immunosuppressive | Fiery fragrance | Used in candy and beverage preparation |
| Estragole | Antifungal against some bacteria | Odor similar of anise, rich sweetness | Used as a food additive and essential oil |
| Anethole | Anthelmintic, antimicrobial, gastroprotective, insecticidal, antifungal and anti-inflammatory | 13 times sweeter than sugar | Incorporated as a flavoring additive in food formulations. |
| Iso-eugenol | Anti-inflammatory antimicrobial, antioxidant | Carnation like fragrance | Utilized to add flavor to chewing gums, baked goods, and soft drinks. |

Bioactive components are obtained through various extraction methods, such as maceration, soxhlet extraction, ultrasound-assisted extraction, microwave-assisted extraction, supercritical fluid extraction, and steam distillation, which help to isolate and purify these components [4, 8, 15]. Solvents with varying polarities, including acetone, methanol, ethanol, ethyl acetate, hexane, and chloroform, are commonly used to purify various components from betel leaf.

## Extraction of essential oil from *piper betle*

Essential oil extract can be obtained using many methods, each guiding the oil's formulation and properties. The key methods for extraction include maceration, soxhlet extraction, solvent extraction, steam distillation, hydro-diffusion, ultrasound extraction, microwave accelerated extraction [4, 8]

*Maceration* method involves immersing betel leaves in solvents like methanol, ethanol, hexane, or chloroform. Hydroxychavicol is the main compound that can be extracted through this method, typically around 66–80% yield can be obtained. The leaves are cleaned, dried and grinded into powder, and placed inside the maceration chamber. A defined quantity of the solvent is added from the top of the chamber until the powder is completely covered with the solvent. Rate of stirring and period of contact are autonomous factors that impacts the extraction yield. Regular solvent changes at definite intervals boosts the mass transfer efficiency, improving extract recovery and bio-active compound extraction. In a study, 1 kg of betel leaf powder was exposed to maceration in a chamber with 21 litres of 70% ethanol for 3 days, with the solvent being swapped daily. Following maceration, the extract was obtained through filtration or decantation. The resulting filtrate was then concentrated using a rotary evaporator or water bath to remove excess solvent [4, 15, 17].

*Soxhlet extraction* involves placing ground plant extract in a porous thimble within the extractor. A solvent, chosen for its proficiency to dissolve target compounds, is heated in a round-bottom flask, causing it to evaporate and ascends into a condenser. The condensed solvent drips into the thimble, dissolving the desired compounds. The solvent, now containing the extracted compounds, is redirected back into the flask, and the cycle repeats. This process enhances extraction efficiency and separates the solute from insoluble impurities. After extraction, the solvent is removed using a rotary evaporator or other methods to acquire the concentrated extract [8,18].

*In Solvent Extraction* efficient solvents like ethanol, methanol, hexane, chloroform, ethyl acetate, and water which isolates antibacterial components like chavicol, eugenol, caryophyllene, cylene and chalarene are used. The oil output depends on the extracting agent, elution time, and temperature. Ethanol extracts of betel leaves have robust bactericidal activity against microorganisms like E. coli and Staphylococcus aureus. To extract the essential compounds from betel leaves, they are first ground into a fine powder to increase their surface area for absorption. The powdered leaves are then mixed with an ideal solvent in a container. The extraction process can be carried out using either maceration or a Soxhlet apparatus. After extraction, the mixture is filtered to remove any solid particles. The eluent is then separated from the extract using a rotavapor under vacuum, resulting in a concentrated extract [4, 17].

*Steam Distillation* is majorly used to extract essential oils, optimizing the volatile compounds' peculiar aroma and antimicrobial properties. Steam distillation isolates compounds such as p-allylphenol, eugenol, eugenol acetate, and terpinol [8, 19].

*Hydro-distillation* is widely used due to its simplicity, budget friendly, and sustainable nature [15]. Begin by rinsing the betel leaves with distilled water to remove any sort of impurities. Next, dry the leaves either by air-drying them in the sun for 3-5 days or using a hot air re-circulator to achieve a dampness percentage of 12-15%. Finally, chop the leaves into small fragments or processed into a powdered form to optimize interface area, facilitating effective isolation [7, 16]. Place the betel leaves in a round-bottom flask. Pour distilled water to the flask at a ratio of 1:5 (leaves to water ratio) [12]. Unite the Clevenger apparatus to the flask. Place the setup on a heating unit. Heat the suspension to 80-100°C using the heating mantle. Ensure the mixture boils smoothly without any hassle to allow the steam to carry the essential oils. Carry on the hydro-distillation process for around 3-4 hours. As the water reaches boiling point, the steam infused with essential oils ascends and is then tempered by the condenser. The condensed steam, now containing the extracted essential oils, accumulates in the Clevenger arm. Collect the essential oil from the Clevenger's arm. Incorporate anhydrous sodium sulphate to remove any remaining water droplets. Store the oil in vials at a set temperature of around 4°C for future analysis [4, 7, 12, 16].

*Ultrasound-Assisted Extraction* can enhance essential oil yield compared to traditional hydro-distillation. The betel leaves are typically dehydrated and pulverized to enhance interfacial area for better interaction with the solvent and ultrasound waves. The powdered betel leaves are mixed with the chosen solvent in the extraction vessel at a specific solid-to-solvent ratio (e.g., 1:20 g/ml). The extraction vessel is placed in an ultrasonic bath or an ultrasound probe is immersed in the mixture. The ultrasound apparatus is then operated at a predetermined frequency and power for a specific duration (e.g., 30 minutes or 90 minutes pre-treatment). This process facilitates the rupture of plant cell walls, resulting in the release of intracellular components into the solvent [15]. After the ultrasound treatment, the mixture is usually subjected to maceration for a further period with controlled parameters like temperature, solvent concentration, and solute-to-solvent ratio to maximise yield [15]. The extract is then filtered to remove solid particles. The extractant is typically extracted from the filtrate using a rotavapor to obtain a concentrated extract containing the desired compounds [4, 8, 15].

*Microwave-Assisted Hydro-distillation* offers the edge over conventional hydro-distillation for essential oil extraction. Leaves are washed to remove any foreign particles dirt or contaminants, followed by dry heat treatment in a hot air oven at 80 to 90°C for 30 to 40 minutes. Shredded betel leaves are positioned into a round bottom flask (RBF), followed by incorporation of distilled water to the flask at a ratio of 1:5 (leaves to water). The entire apparatus is placed over a heating mantle. Sample is irradiated with microwave energy. A typical microwave oven works at a frequency of 2.45 GHz with a power output of 500W. The ratio is generally 0.33 L/W. Mixture is heated, the microwave energy infiltrates biological materials, inducing rapid thermal effects on polar compounds [4].

Table 4 Methods of extraction [8, 15, 19]

| Extraction technique | Isolated bio-actives | Advantages | Disadvantages |
|---|---|---|---|
| Maceration | Hydroxychavicol | 1. Simplest method<br>2. Suitable for heat sensitive compounds | 1. Lengthy process<br>2. Susceptibility to Microbial Growth and Rancidity |
| Soxhlet extraction | Hydroxychavicol chromanol, eugenol, 1-phenyl propene-3,3-diol diacetate | 1. No filtration required.<br>2. Can separate a solute from insoluble residue.<br>3. Utilizes heat generated by distillation flask. | 1. Slow and tedious process.<br>2. Good amount of solvent needed to carry out this process.<br>3. Thermolabile substances should not be processed. |
| Solvent extraction | Chavicol, chalarene, caryophyllene, cylene, eugenol | 1. Highly efficient.<br>2.The choice of solvent can be altered according to the properties of bioactive compound. | Difficult to maintain uniform activity levels due to the unpredictable nature of active molecule concentrations. |
| Steam distillation | p-allylphenol, eugenol, eugenol acetate, terpineol | 1. Cost effective and environment friendly<br>2. Oil quality is more reproducible. | 1. Essential oils are volatile compounds and may be evaporated along with steam.<br>2. Heat control can be difficult |
| Hydro-distillation | Eugenol, estragole, linalool, α-copaene, anethole | 1. Higher oil yield.<br>2. No organic solvent needed<br>3. Is cheap and environment friendly. | 1. Heat control is difficult.<br>2. Complete extraction is not possible. |
| Ultrasound-Assisted Extraction | 2-hydrazinopyridine, morphinan, imidazole, acetamide, 2,8-dimethyl quinoline | 1. Efficient extraction<br>2. Low investment cost.<br>3. Minimal amount of solvent required. | 1. Repeated extraction needed.<br>2. Ultrasound energy can create negative effect on active constituents.<br>3. Filtration step crucial. |
| Microwave-assisted hydro-distillation | Indole, imidazole, 2-ethylacridine, pyridine | 1. Less solvent required.<br>2 Short extraction duration.<br>3. Fast and continuous extraction. | 1. High capital cost.<br>2. Filtration required after extraction. |

## Piper betle bioactive profile

Betel leaf possesses a wide array of bioactive properties, making it valuable in various applications. These properties are attributed to the presence of numerous bioactive substances, including polyphenols, terpenes, and other phytochemicals [4, 18].

Betel leaf shows antimicrobial activity, preserving food items from harmful microorganisms and toxicants, showcasing its potential as a great natural food preservative [4, 8, 15]. It suppresses the augmentation of various microbes, involving di-derm bacteria and fungi [4]. Few significant antibacterial compounds include α-pinene, cis-sabinene, eugenol and β-farnesene [4, 8, 13]. Antimicrobial action of leaf essential extract is owing to the presence of various prominent bioactive compounds comprising hydroxychavicol, chavibetol, estragole, eugenol and chavicol which modifies cytoplasm of the microorganism effecting its cell wall. These compounds hinder DNA replication, alters electron transport chain and inhibit ATP synthesis, promoting to microbial cell death [4].

Betel leaves have antioxidants, including bioflavonoids, isoprenoids, tannins, and glycosidic compounds [4]. Antioxidants help ameliorate radiation-triggered lipid damage. Alcoholic extracts of betel leaf are great sources of natural antioxidants [8]. Phenolic compounds like allyl pyrocatechol and chavicol promote to antioxidant effects [8, 15].

Betel leaves exhibit anti-inflammatory characteristics [4, 6, 8]. The bio-active components responsible for the anti-inflammatory features of betel leaf include phenolic compounds, flavonoids, and terpenoids [4, 6, 8, 15]. Eugenol, found in betel leaves, has influential anti-inflammatory and pain-relieving effects. Betel leaf extract has been found to help minimize inflammatory responses in multiple ailments such as rheumatism, respiratory disease, and dermatitis [4, 8].

Betel leaf is proven to combat fungal infections, with the essential oil showing strong antifungal properties against Trichophyton mentagrophytes, Candida albicans and Aspergillus niger [4, 8, 12, 19]. The antifungal activity is contributed by compounds like eugenol, chavicol, and terpenes. Betel leaf extract may have complimentary effects alongside other antifungal drugs, such as fluconazole, potentially enhancing the performance. Ethyl acetate extracts of betel leaves have shown greater restrictive action on *Aspergillus niger*, *Aspergillus* sp., and *Rhizopus* sp., indicating it could play a role in controlled pest management and food preservation strategies [4, 8, 12, 15].

The presence of polyphenol compounds gives betel leaf anti-carcinogenic features Flavonoids present in betel leaf not only provide anti-inflammatory action but also shows allergy prevention, antioxidant, antimicrobial, anticancer, and anti-diarrheal [8, 11, 15, 19]. Flavonoids can improve programmed cell death activity in various cancer cells. Bioactive compounds like chlorogenic and hydroxychavicol encounters carcinogenic effects of tobacco [8, 15, 20].

Betel leaf demonstrates anti-diabetic traits. Betel leaf extracts can influence main enzyme related to diabetes [20], it has shown to reduce liver glucose-6-phosphatase and fructose-1,6-bisphosphatase enzymes action, also exhibits improved liver hexokinase levels [8]. Methanolic extracts of Kapoori (KM) and Sanchi (SM) cultivars show prominent α-amylase and α-glucosidase enzyme inhibition, showcasing anti-diabetic activity [20].

Betel leaf has stomach protecting characters and can help in gastric mucosal protection. Ingestion of betel leaf orally produces substantial effects, improves rat intestinal mucosal gut lining and bile secretion, as well as pancreatic juices production. It has been shown to enhance mucin production, rather than inhibit acid release. It is also used to cure dysentery. It enhances digestion and promotes salivary gland production [8, 20]

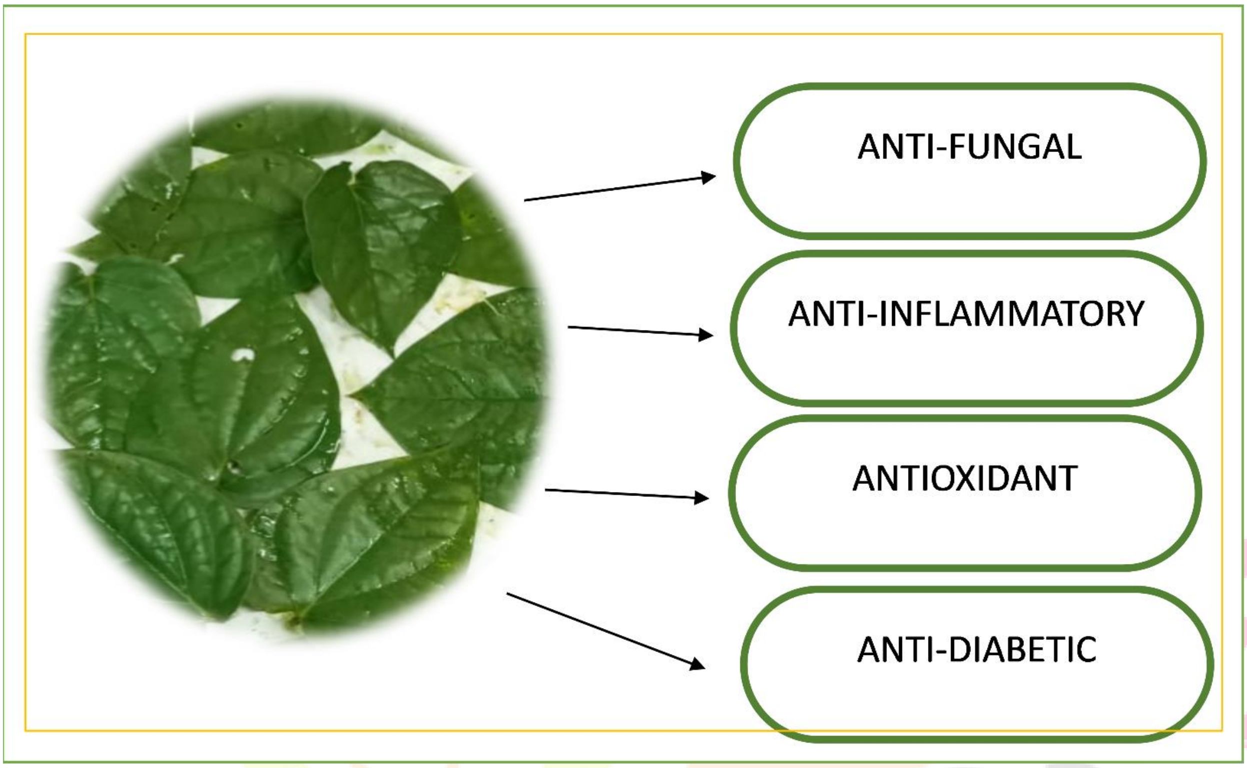


*Figure1- Bioactive Properties of Piper betle*

*Betel leaves are known for their impressive bioactive properties, particularly their antifungal, antioxidant, antidiabetic, and anti-inflammatory activities. The leaves have shown to effectively combat fungal infections, scavenge free radicals, and regulate blood sugar levels. Their anti-inflammatory properties have been found to alleviate symptoms associated with various inflammatory conditions.*

## Factors affecting betel leaf essential oil production and recovery

The extraction process depends on region of cultivation, precipitation, soil type and plant varieties [4]. Geographical demarcations result in different gradations of secondary metabolites, leading to contrasting performances in different regions [4, 6, 15]. The bio-chemical composition of the essential oil also varies among cultivars, with different regions exhibiting different major compounds. Epigenetic variations can cause swings in seasonal patterns, anatomical features, and chemical composition, impacting oil output. [6, 7]. Leaf colour can also act as a useful morphological marker for cultivar recognition, linked to particular oil compositions [6]. The output of essential oil from betel leaves is impacted by factors such as seasonal fluctuations, ecological conditions, and climate [6]. The quantity of essential oil yield differs from variant to variant of Piper betle L. For instance, Mitha betel leaves contain around 2.0% essential oil, Bangla leaves around 1.7%, and Sanchi leaves approximately 0.8% when calculated on a dry weight basis. Other cultivars like Sada Bangla have shown variable recovery rates with enhanced extraction methods [6, 8, 15].

The procedure of extraction significantly impacts the yield and composition of BLEO. Studies have stated that ultrasound pre-treatment can yield better results than traditional hydro-distillation method. Alterations to steam distillation, such as using a salt incorporated brine, can also increase oil yield [4]. The choice of solvent in solvent extraction methods also influences the yield and antimicrobial activity of the extract [4, 15]. Drying methods and conditions such as time and temperature affect the water percentage of the raw material, which in turn has an impact on essential oil production [19]. Fresh samples with more moisture content can sometimes yield more oil. Curing of betel leaves has been shown to improve essential oil yield, possibly due to the creation of more open structure that allows for easier movement of substances [15]. Measures such as time, temperature, particle size, solid-solvent ratio, and the addition of substances like NaCl concentration can impact the productivity of essential oil extraction [15, 19]. Maximizing these factors is essential for enhancing oil yield. For instance, adding a considerable amount of NaCl can enhance essential oil content by potentially contracting the outer skin and enabling oil to diffuse, while very high concentrations may create hinderance [19].

Betel leaves are highly ephemeral, resulting in major post-harvest losses. Spoiled leaves can mitigate microbial infections [6]. Proper storage and maintenance are important to maintain leaf quality before extracting oil. Extracting oil from excess or unsold leaves can be used to reduce losses and manage farm waste [6, 7]. While leaves are the main source, non-leafy portions like stalk, rhizomes, and roots also contain prime oils and other valuable components [4, 6]. The petiole, for instance, have agents with prospect in the flavour and cosmetics industry [4].

## Modern applications of betel leaf in food technology

Betel leaf extract can be used to preserve sapota juice for about a month under refrigerated conditions without affecting its quality. Studies on CLEO-enriched sapota juice have shown that pH, total soluble solids (TSS), and acidity drastically escalated, while acidity and ascorbic acid (AA) significantly reduced under refrigerated conditions at 4°C for a 30-day storage period. Spike in acidity is likely due to several reasons, comprising the deterioration of lactose and the alteration of sugars into acids by microbial contamination, leading to the formation of lactic acid from hexose sugars [21]. The reason that leaf extract treated samples showcased a higher titratable acidity could be because the oil prevented the metabolism of citric acid which might have decomposed in the control samples. Betel leaf essential oil enriched sapota juice exhibits strong antifungal and antibacterial activity compared to control samples, ascribed to the presence of bioactive compounds like eugenol, estragole, and linalool, which are present in higher percentages [12, 21]. Amount of 1.75 mg/mL of betel leaf extract was found to minimize oxygen loving microorganisms counts and slowdown yeast and mold growth [17]. Basak (2018) found that betel leaf essential oil (BLEO) can preserve apple juice, with a concentration of 0.19 µL/mL being palatably approvable and extending the juice's shelf life [12, 21].

Betel leaf extract can safeguard tilapia slices for up to 12 days. It can be applied to tilapia slices, with liposomes incorporated with betel leaf extract acting as an antimicrobial agent [4, 15].

Betel leaf essential oil microemulsions have illustrated exceptional antifungal potency against *Aspergillus flavus* in tomato paste [1]. *Aspergillus flavus* is a common spoilage causative agent in tomato paste that releases mycotoxin [1]. Betel leaf essential oil microemulsion can serve as a natural preservative, enhancing the shelf life of tomato products [1]. Researchers found that adding a small amount (0.25 mg/g) of leaf extract to tomato paste was acceptable to consumers, based on taste tests using fuzzy logic. This incorporation also helped lengthen the shelf stability of tomato paste by weeks [1].

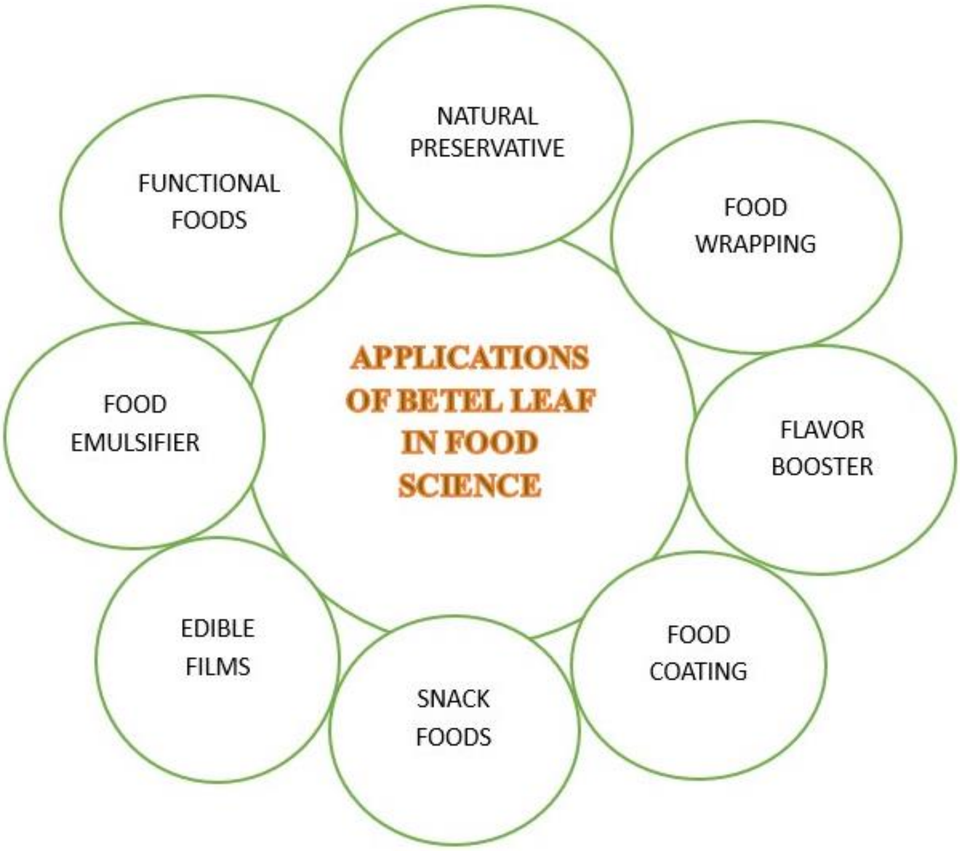


*Figure 2 Applications of betel leaf in food industry*

*Betel leaves have versatile applications in the food industry, leveraging their unique flavor, aroma, and medicinal properties. They are used as a wrapping material for various snacks, can be used as food emulsifier and also as a functional food. Betel leaves are also employed as a natural food preservative, due to their antimicrobial properties, and as a flavoring agent in beverages, like tea and juice.*

Betel extract can preserve khoa for up to 9 days and prolong the stability period of raw milk by 5 hours [15]. Leaf infusion can be used to improve the shelf life of raw milk. Incorporating 0.5% (v/v) betel leaf extract as an additive to raw milk has been said to provide safety for up to 11 hours at 37°C [8, 15]. Incorporating betel leaf extract into Dahi helps in preservation due to the antimicrobial and antioxidant compounds present in them. Dahi with 0.5% betel leaf extract demonstrated improved performance compared to traditional Dahi after seven days of refrigeration [8]. Betel leaf oil can be adjusted with sapota pulp in the preparation of goat milk Shrikhand. Research has focused on enhancing the sensory qualities of Shrikhand, including its taste, texture, appearance, flavor, and color, to achieve optimal levels [8, 15].

Betel leaf flour can reduce cholesterol in broiler meat [13]. A study on pork found that meat treated with betel leaf extract had elevated water activity compared to salt-cured meat, which initially led to a higher bacterial count [13, 18]. The extracts have antimicrobial action hostile to infectious agents, such as *E. coli*, *Shigella dysenterae-1*, and *S. aureus* [4].

## Regulatory framework for natural preservatives

As consumers become wary of the potential health hazards linked to synthetic preservatives, demand for natural alternatives is on the rise. This demands clear regulatory measures for their safe and effective use [4, 10].

The European Food Safety Authority (EFSA) and the Food and Drug Administration (FDA) are the main regulatory bodies dutiful for overseeing the approval of food additives, including natural preservatives, in the European Union and the United States, respectively. The Codex Alimentarius, a joint FAO/WHO expert committee, also plays a major role in assessing potential hazards and setting worldwide benchmarks for food safety and regulation [9].

The European Union has ratified stringent guidelines for the clearance of natural additives in food products, including scientific authentication, product specification, manufacturing and storage information, safety assessments, GMP compliance, labelling, and notification provisions. Regulation EC 1333/2008 created a registry of authorized food additives, which covers some natural preservatives like rosemary (E 392), tocopherol-rich extracts (E 306), γ-tocopherol (E 308), δ-tocopherol (E 309), carotenes (E 160a), and annatto, bixin and norbixin (E 160b) [1, 4, 18].

Significant discrepancies in regulations exist between regulatory bodies. Regulatory agencies have differing opinions on certain substances. Estragole, for example, is deemed safe by the FDA, but the EU prohibits its use as a flavoring due to genetic toxicity concerns. Similarly, Codex Alimentarius has recommended optimal level for carotenoids in certain meats that is not adopted by the US legislation. These differences can create barriers to the global use of certain natural preservatives [9, 15].

## Future prospects and emerging trends

Studies should further examine traditional and modern methods for extracting beneficial bioactive compounds from betel leaves, such as essential oils, to harness their potential as natural preservatives [4, 8]. The inclusion of betel leaf extracts can greatly enhance the durability and barrier performance of film. For instance, edible films created with sago starch and 20% betel leaf extract show improved properties [8]. Betel leaf extracts can be added to eco-friendly films to produce active packaging that protects food, prolongs its freshness further preventing its deterioration [1, 4]. Leaf essential can be conjugated with modified atmospheric packaging and non-thermal plasma to enhance the durability of food products [11, 12]. This approach, known as hurdle technology, uses many preservation techniques simultaneously to achieve better results than any single method alone [1]. Further studies should be done to determine permissible organoleptic concentrations for extracts infused with edible coatings [6, 7].

Administering nanotechnology to capsulate and deliver betel leaf extracts could improve their antifungal and antioxidant properties [4, 7]. Nano-emulsions, like BLEONE (betel leaf essential oil nanoemulsion), have shown to be efficient in maintaining freshness and prolonging the stability of fermented foods, like Idli batter [7]. Merging betel leaf extracts with processing technologies like cold plasma, pulsed electric fields (PEFs), and high-pressure processing (HPP) can offer effective control of microorganisms while maintaining the food's organoleptic properties and nutritional value [1]. These technologies have shown remarkable sink in energy usage and greenhouse gas emissions compared to standard thermal procedures.

Investigating and formulating multifunctional betel leaf-based additives, such as colorants and preservatives, can cater to the increasing need for safer food additives [9, 10, 17]. Natural compounds like anthocyanins, carotenoids, and betalains, found in betel leaves, possess pigmentation, antioxidant capacity, and antimicrobial activity, making them potential applicants for dual-purpose additives [9, 11, 15]. Finding new methods to extract valuable compounds from unused or waste betel leaves can minimize crop residues and generate alternate revenue sources [6, 7, 15]. Creating eco-friendly and bio-degradable packaging materials using natural fibres and bioplastics can reduce ecological footprint and provide functional and economical benefit for protecting food quality and safety. These extracts and essential oils can be used in many other industries, such as cosmetics, tonics, air fresheners, food additives, and pharmaceuticals [4, 8, 15].

## Conclusion

The quest for safe and effective food preservation methods has led to a renewed interest in natural preservatives. Among the various natural preservatives, the betel leaf has emerged as a promising candidate due to its remarkable antimicrobial and antioxidant properties. This review has provided a comprehensive overview of the betel leaf's potential as a natural preservative, highlighting its bioactive compounds, preservation mechanisms, and applications in food preservation. The benefits of using betel leaf as a natural preservative are multifaceted. Firstly, it offers a safer alternative to artificial preservatives, which have been linked to various health problems. Secondly, the betel leaf's antimicrobial and antioxidant properties make it an effective preservative, capable of prolonging the freshness of food products. Thirdly, the use of plant derived source as a natural preservative promotes sustainability, as it reduces the reliance on synthetic chemicals and supports the use of renewable resources. Despite the promising potential of betel leaf as a natural preservative, there are still several challenges that need to be addressed. Further research is required to fully understand the preservation mechanisms of betel leaf and to optimize its use in various food systems. Additionally, the adaptability of betel leaf-based preservation methods is needed to be evaluated to ensure their viability for industrial applications.

In conclusion, economic viability of natural preservatives depends on factors such as production costs, required concentrations, impact on sensory properties, regulatory approval processes, and ongoing research to optimise their efficacy and cost-effectiveness. The increasing consumer preference for natural products and the growing market for sustainable food preservation suggest a positive future for the economic viability and wider adoption of natural preservatives, provided that the associated challenges are effectively addressed through innovation and research. The betel leaf has shown great promise as a natural preservative, offering a range of benefits over artificial preservatives. As the food industry continues to seek safer and more sustainable preservation methods, the betel leaf is certainly worth further investigation. With continued research and development, the betel leaf has the potential to become a valuable resource for natural preservation, supporting the production of safer, healthier, and more sustainable food products.

## Acknowledgment

We would like to express our sincere gratitude to Babasaheb Bhimrao Ambedkar University for providing us with the necessary resources and support to complete this review. We also extend our appreciation to the teaching faculty for their valuable insights and expertise, which greatly contributed to the quality of this manuscript.

## References

1. Hugo Miguel Lisboa, M. B. (2024). Innovative and Sustainable Food Preservation Techniques: Enhancing Food Quality, Safety, and Environmental Sustainability. Sustainability, 1-43.
2. Hardik Sharma, R. R. (2023). The science of food preservation: A comprehensive review of synthetic preservatives. Journal of current research in food science, 1-3.
3. Z.I.M. Sharif, F. M. (2017). Review on methods for preservation and natural preservatives for extending the food lonevity. Chemical Engineering Research Bulletin, 2-8.
4. Viet Thanh Tran, T. B. (2023). Recent applications of natural bioactive compounds from Piper betle (L.) leaves in food preservation. Elsevier, 1-8.
5. Ejigayehu Teshome, S. F. (2022). Potential of natural preservatives to enhance food safety and shelf life: a review. The Scientific World Journal, 1-10.
6. Mondal, B. (2024). Epigenetics of Betel Leaf Essential Oil: A Determinant of Industrial Niche of the Cultivar. Indian Journal of Pharmaceutical Sciences, 1-10.
7. Sangeetha Karunanithi, P. G. (2024). Innovation in Idli batter preservation through betel leaf essential oil-based nanoemulsion: Evaluating fermentation kinetics and physicochemical analysis. Elsevier, 5(8), 1-9.
8. Tripti Singh, P. S. (2023). A literature review on bioactive properties of betel leaf (Piper betel L.) and its applications in food industry. ELSEVIER, 61(10), 1-11.
9. Claudia Novais, A. K.-B. (2022). Natural Food Colorants and Preservatives: A Review, a Demand, and a Challenge. Journal of agriculture and food chemistry, 1- 15.

10. J. S. (2024). Evaluation of some food preservatives and natural plant extracts as antimicrobial agents for safety. Discover Food, 1-15.

11. M Sindhuja, R. A. (2020). DEVELOPMENT OF NOVEL FOODS USING BETEL LEAVES. Annals. Food Science and Technology, 2- 9.

12. Fathima Beevi Abdul Kareem, A. E. (2024). Exploring the preservation efficiency of cured betel leaf essential oil in augmenting the quality of fruit juice: a comprehensive evaluation of physicochemical, microbial, and sensory parameters. J Food Sci Technol, 61(10), 1-12.

13. Corpuz, A. V. (2020). Potential of Piper betel Leaf Extract as Meat Preservative. European Journal of Molecular & Clinical Medicine, 7(11), 1-8.

14. Nayaka, N. M., Sasadara, M. M., & D. A. (2021). Piper betle (L): Recent Review of Antibacterial and Antifungal Properties, Safety Profiles, and Commercial Applications. NATIONAL LIBRARY OF MEDICINE, 26(8), 14.

15. Sangeetha Karunanithi*, G. M. (2022). A Review on Piper betle L.: Antioxidant, Antimicrobial, Extraction and Application in Food Product Development. Acta Scientific NUTRITIONAL HEALTH, 1-13.

16. Rajapaksa, L. A. (2006). Piper betle: a potential natural antioxidant. International Journal of Food Science and Technology, 1-4.

17. Voon, W., & W. Y., G. N. (2014). Application of betel leaves (Piper betle L.) extract for preservation of homemade chili bo. International Food Research Journal, 21(6), 67.

18. Michel M. Beya, M. E. (2021). Plant-Based Phenolic Molecules as Natural Preservatives in Comminuted Meats: A Review. Antioxidants, 1-15.

19. Mai Huỳnh Cang, P. T. (2020). Betel (Piper betle L.) leaf essential oil extraction. International Journal of Advanced Engineering Research and Science (IJAERS), 7(6), 1-4.

20. Subramaniam Yogeswaria, K. H. (2020). Antidiabetic, Antithrombin and Cytotoxic bioactive compounds in five cultivars of Piper betle L. Elsevier, 1-5.

21. Mitali Madhumita, P. G. (2021). Natural preservative efficacy of cured betel leaf essential oil for sapota juice: Effect on physicochemical, microbial, and sensory properties. Journal of food processing and preservation, 2-8.

22. Suryasnata Das 1, A. R. (2019). Effect of different extraction techniques on total phenolic and flavonoid contents, and antioxidant activity of betelvine and quantification of its phenolic constituents by validated HPTLC method. 3 Biotech., 9(1), 9.

23. Das, S., Ray, A., Nasim, N., Nayak, S., & Mohanty, S. (2019). Effect of different extraction techniques on total phenolic and flavonoid contents, and antioxidant activity of betelvine and quantification of its phenolic constituents by validated HPTLC method. NATIONAL LIBRARY OF MEDICINE, 9(1), 56.

24. Basak, S. (2018). The use of fuzzy logic to determine the concentration of betel leaf essential oil and its potency as a juice preservative. Elsevier, 2-4.

## Conflict of interest

The authors have declared no conflicts of interest for this article.