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COMPREHENSIVE REVIEW ON GREEN (SUSTAINABLE) EXTRACTION TECHNIQUES AND THERAPEUTIC OVERVIEW OF BIOACTIVE COMPOUNDS FROM *MYRICA ESCULENTA* (KAPHAL)

Suprabha Nishad[1], Kalpana Kushwaha[1], Pratima Katiyar*

[1]Research Scholar, School of Pharmaceutical Sciences, C.S.J.M University, Kanpur.

*Faculty, School of Pharmaceutical Sciences, C.S.J.M University, Kanpur.

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***Corresponding Author**

Pratima Katiyar

Faculty, School of Pharmaceutical Sciences, C.S.J.M University, Kanpur.

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ABSTRACT

The rising interest in green and sustainable methods during the separation of active phytochemical have contributed to the progress of eco-friendly innovation extraction method that minimize environmental impact while enhancing the efficiency of the process. This comprehensive review explores various green extraction techniques, including microwave-energy extraction, biocatalyst aided extraction, sonication based extraction, mechanical cold pressing, supercritical fluid based separation and high pressure liquid extraction for the isolation of phytochemical from *Myrica esculenta* (Kaphal), a medicinal plant known for its rich therapeutic potential. These advanced methods, when combined with safe solvents for example GRAS (generally recognized as safe) solvents or natural deep eutectic solvents (NADES), offer several benefits over traditional approaches, including minimized solvent use, lower energy consumption and improved selectivity for target compounds. The review further highlights the therapeutic properties of bioactive compounds derived from *M. esculenta*, including antioxidants, anti-inflammatory, antimicrobial, antipsychotic, antidiabetic, anthelminthic, hypotensive, anti-cancer and hepatoprotective activities, demonstrating the plant's promising role across the pharma and nutraceutical field. By focusing on sustainable and efficient extraction strategies over conventional extraction procedures challenges are currently overcome. All relevant information related to *Myrica*

esculenta including phytochemistry, green extraction method and therapeutic potential provides valuable insights into the future of green chemistry in natural product research and its potential to enhance both human health and environmental sustainability. This information is acquired by searching Scopus, google scholor, pubmed and web science for English language papers.

KEYWORDS: *Himalayan bayberry*, Phytoconstituents, Medicinal plant, traditional use, Green extraction, extraction technique, ethanopharmacology and sustainable Utilization.

INTRODUCTION

Myrica esculenta (bayberry) *Myrica esculenta* Buch.- Ham. ex D. Don, Synonyms. *Myrica nagi* Hook.f., commonly referred to as Katphala and also Bayberry is a dioecious evergreen tree that ranges in the height from 3-15m and is found in the subtropical region of Himalayas from Ravi eastward to the Assam, Khasi, Jaintia, Naga and Lushai hills.[1] The genus *Myrica, a member of the* family *Myricaceae*, comprises approximately 97 species of tiny trees and fragrant shrubs. These species are distributed globally, occurring in both temperature and subtropical climates.[2] Ayurveda has traditionally been used to treat asthma, bronchitis, anti-allergic and anti-inflammatory properties. This plant's barks has been used an antiseptic, cough, chronic dysentery and fish poison.[3] Numerous tribal groups in rural Orissa, India, have long utilize the bark of *M. nagi* to cure mental disease. The hydrolysable tannin castalagin, two prodelphinidin dimers-namely, epigallocatechin-(4βeta → 8)-epigallocatechin 3-O-gallate and 3-O-galloyl epigallocatechin-(4β → 8)-epigallocatechin 3-O-gallated counterpart- as well as other notable compound such as gallic acid, myricanol, myricanone and epigallocatechin 3-O-gallate are all found in the bark of *M. nagi*.[4] Myrica plants thrive in agricultural and marginal regions, mixed woods, and soils that are low in nitrogen.[5] Originally, its bark, roots, and leaves were used to treat a variety of diseases and disorders.[6] Aside from in addition to folk medicine applications, the bark is also utilize to production of paper and ropes[7] Recently, researchers have begun to investigate *M. esculenta* phytochemistry in depth due to its multiple ethno medicinal applications. For example, tannins derived from the bark are utilized as natural dye or coloring agent.[8] The existence of distinctive bioactive substances, include alkaloids, flavonoids, glycosides, tannins, terpenoids, saponins and volatile oils.[9] It was initially separated as light-yellow crystals from the bark of *Myrica nagi Thunb*., which was gathered from India during the late 1700s.[10]

Classification: Scientific classification of ***Myrica esculenta*** (kafal)

Kingdom : *plantae*

Phylum : *tracheophyta*

Class : *tagnoliopsida*

Order : *fagales*

Family : *myricaceae*

Genus : *Morella*

Species : *esculenta*

BOTANICAL DISCRIPTION

Morphological evaluation of *M. esculenta* plant & its components (**Figure 1a–d**) describe as small to modium size woody, evergreen plant, typically growing between 3 to 15 meters in height. Its lanceolate, obovate leaves are primarily concentrated located at the tips of branches exhibiting either entire or serrated margins. The leave exhibit a dark green coloration on the upper surface and a lighter, pale green shade underneath typically measuring about 9–12 cm long and 3-3.5 cm wide[11] In *Myrica esculenta*, the pistillate (female) Small, sessile blooms (without stalks), solitary and accompanied by bracts. There are either no sepals or petals or they are not clearly apparent. The pistillate (female) flowers of *Myrica esculenta* are small, sessile (lacking stalks) and occur singly, each accompanied by a bract. Sepals and petals may be absent or morphologically indistinct from one another. Inflorescence arising from the leaf axil, in the form of a catkin, is approximately 4.2 cm lenght and bears around 25 blooms distributed along a thin, thread-like structure while each one of the stamens (male) flowers is arranged in a compound raceme[12] flowering begins in late October and continues through December. Fruit setting begins in November, with ripe fruits becoming accessible between April and June.[13]



Figure 1 ***Myrica esculenta:*** **(a) Entire plant (b) foliage (c) bark and (d) fruits.**

The exterior bark is rough, vertically wrinkled and exhibit a greyish-dark coloration while the inner bark is smooth and dark brown in appearance. It has a hard fracture and a distinctly bitter taste, and unpleasant fragrance. In fresh samples, the bark is made up of flat or curved pieces of varied widths and thickness, most of which are coated in green lichens. The outside surface is greyish dark and rough, with longitudinal fissures. A rough inner surface with a yellowish brown hue is observed, gradually turning brown on drying; fracture is short and brittle; taste - bitter, astringent; scent - nauseating (dried and powdered bark).[14]

Chemicals found in dark yellow bark include myricanol, myricanone, myricetin, myricitrin, and glycosides. The fruit resembles rich red raspberries. They have minimal pulp and a large spherical seed at the center. Also Seeds from fully developed *Myrica nagi* fruit can be utilized to germinate in the spring. Saplings about 10-15 cm tall are planted in late spring. According to ayurvedic literature, bark is caustic, bitter and pungent. It is said to help with fever, asthma, bronchitis, and other respiratory problems, urine discharges, constipation, tumours, anaemia, depression, chronic dysentery, and ulcers. Fruit is edible, and the region's residents use it to make pickles, preserves and cool drinks.[15, 16, 17]

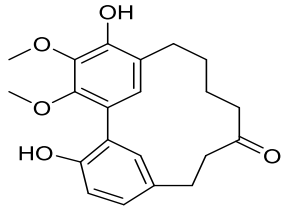
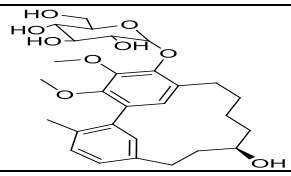
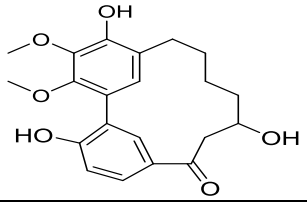
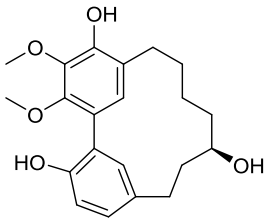
PHYTOCHEMISTRY

Numerous preliminary phytochemical studies carried out on the fruit[18] leaves[19] and bark[14] *M. esculenta* has a number of active phytoconstituents that have a wide range of biological effects. This plant was discovered to be a rich source of phenolic chemicals, flavonoids, and flavonols. Other bioactive substances reported in the plant include alkaloids, glycosides, diarylheptanoids, flavonoids, steroids, saponins, triterpenoids and volatile compounds, which have been classified and summarized below.

Diarylheptanoids

Diarylheptanoids are a biologically active class of chemicals found in practically all species of Myrica, including *M. esculenta*. Diarylheptanoids reported from this plant are summarised in the table.

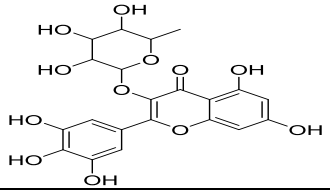
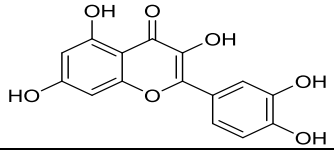
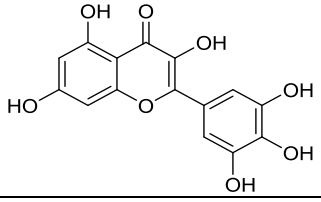
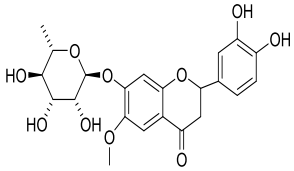
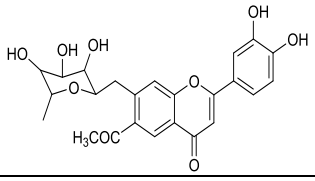
Table 1: Diarylheptanoids isolated from *M. esculenta.*

S. No.	Name of compound	Structure	Plant Part	Reference
1	Myricanone		Bark, Leaf	[8,20,21, 22, 23]
2	5-O-β-D-glucopyranosyl myricanol		Leaf	[22]
3	13-oxomyricanol		Root	[24]
4	Myricanol		Bark, Leaf	[8, 20, 21,22,23]

Flavonoids

Flavonoids are another biologically potent class of compounds and are particularly effective against diseases induced by free radicle damage. Flavonoids reported from *M. esculenta* are summarized in table 2.

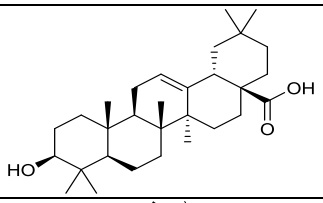
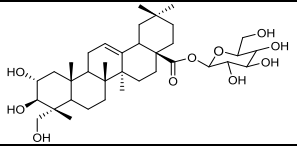
Table 2: Flavonoids isolated from *M. esculenta.*

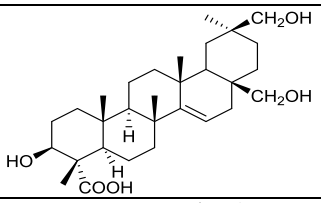
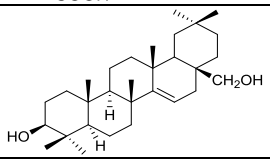
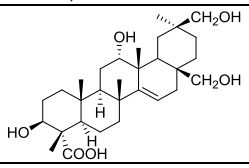
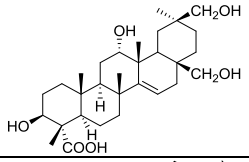
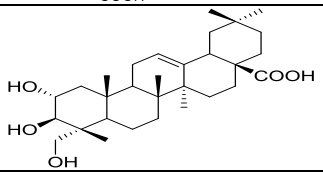
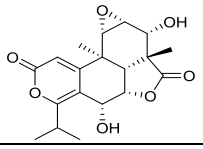
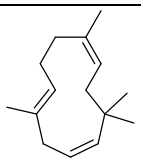
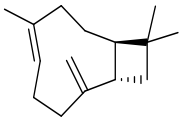
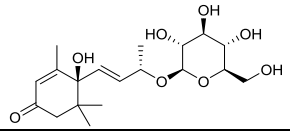
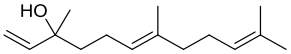
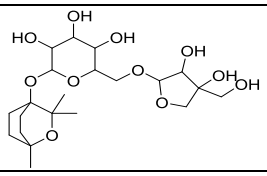
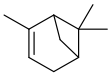
S. No	Name of compound	structure	Plant part	Reference
1	Myricitrin		Bark, Leaf	[21,22,23]
2	Quercetin		Leaf	[20,21]
3	Myricetin		Leaf	[21,22]
4	Flavone 3',4'-dihydroxy-6-methoxy-7-O-α-L-rhamnopyranoside		Leaf	[25]
5	Flavone 3,4- dihydroxy-6-methyl 7-O-α-L-rhamnopyranoside		Bark	[26]

Terpenes

A number of terpenes which includes pentacyclic triterpenes, sesquiterpenes, monoterpenes and one nor-diterpene have been reported from *M. esculenta* are summarized in Table 3.

Table 3: Terpenes isolated from *M. esculenta.*

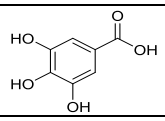
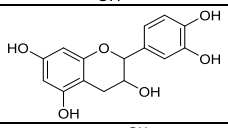
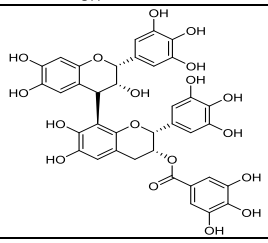
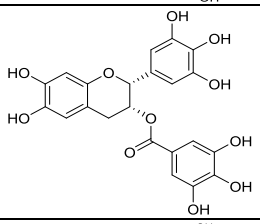
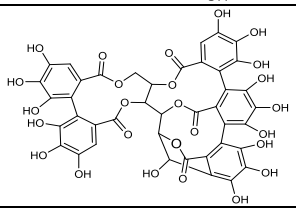
S. No	Name of compound	Structure	Plant part	Reference
Pentacyclic triterpenes				
1	Oleanolic acid		Leaf	[20]
2	Arjunglucoside		Leaf	[21,22]

3	3β, 28, 30-trihydroxytaraxara-23-oic acid		Bark	[27]
4	3β, 28-dihydroxytaraxerane		Bark	[27]
5	3β, 12α, 28, 30-tetrahydroxytaraxeran-23-oic acid		Bark	[27]
6	3β,30-dihydroxy- taraxerane-23-oic acid		Bark	[27]
7	Arjunolic acid		Leaf	[21,22]
Diterpene				
8	Nagilactone C		Leaf	[21]
Sesquiterpenes				
9	α-Caryophyllene		Leaf	[21]
10	β-Caryophyllene		Leaf	[1,20,21]
11	Corchoionoside C		Leaf	[21,22]
12	Nerolidol		Leaf	[21]
Monoterpenes				
13	Myresculoside		Leaf	[28, 22]
14	α-Pinene		Leaf	[21]

Tannins

Tannins generally contain repeated units of phenolic (gallic acid) and flavonols and have been reported for several biological activities. Tannins reported from *M. esculenta are* summarized in Table 4.

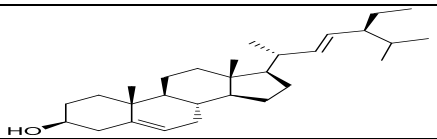
Table 4: Tannins isolated from *M. esculenta.*

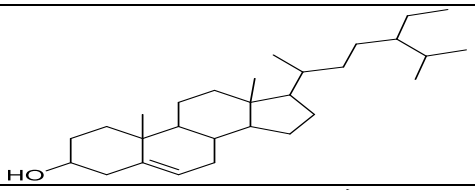
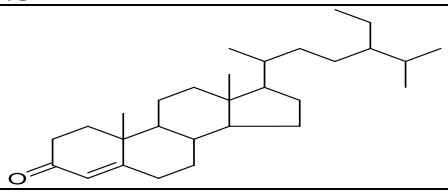
S. No.	Name of compound	Structure	Plant part	Reference
1	Gallic acid		Bark, leaf	[8,20,21,22,23
2	Catechin		leaf	[20]
3	Epigallocatechin-(4βeta→8)-epigallocatechin 3-O-gallate		Bark	[8]
4	Epigallocatechin 3-O-gallate		Bark, leaf	[8,20]
5	Castalagin		Bark	[8]

Steroids

Steroids are known for their diverse biological activity which particularly depends on number of carbon atoms. Some steroidal molecules have also been reported from *M. esculenta*. These are summarized in Table 5.

Table 5: Steroids isolated from *M. esculenta.*

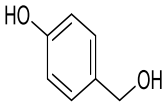
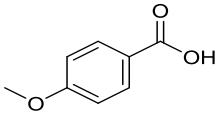
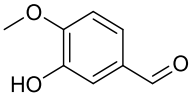
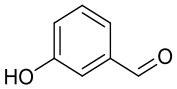
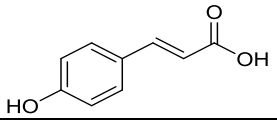
S. No	Name of compound	Structure	Plant part	Reference
1	**Stigmasterol**		Leaf	[20]

2	**β- sitosterol**		Bark, leaf	[20,21,22,23]
3	**β- rosasterol**		Bark	[23]

Simple Aromatic compounds

Some aromatic compounds with hydroxyl, carbonyl, and carboxyl functionalities have also been reported in *M. esculenta.* These are summarized in table 6.

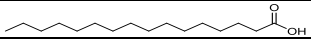
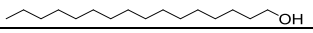
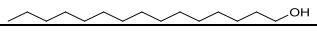
Table 6: Simple aromatic compounds isolated from *M. esculenta.*

S. No	Name of compound	Structure	Plant part	Reference
1	**4-Hydroxy methyl phenol**		**Bark, leaf**	[21,23]
2	**4-Methoxy benzoic acid**		**Bark, leaf**	[21,23]
3	**Isovanillin**		**Bark, leaf**	[21,23]
4	**3-Hydroxybenzaldehyde**		**Bark, leaf**	[21,23]
5	**p-Coumaric acid**		**Leaf**	[20]

Aliphatic compounds

Some long chain aliphatic alcohols and a long chain aliphatic acid have been reported from *M. esculenta* which are summarized in Table 7.

Table 7: Aliphatic compounds isolated from *M. esculenta.*

S. No.	Name of compound	Structure	Plant part	Reference
1	**Palmitic acid**		**leaf**	[20]
2	**n-Hexadecanol**		**leaf**	[1,20]
3	**n-pentadecanol**		**leaf**	[1,20]

Summary of extraction techniques

Effective extraction of bioactive constituents is essential for producing high-quality extracts with significant pharmacological activity. The extraction procedure typically entails the use of solvents, temperature control, and time to achieve the desired yield of active constituents. These are a few popular conventional extraction techniques.

Conventional Extraction Techniques

The conventional, tried-and-true methods, frequently based on solvents, that leverage the concepts of mass transfer and solvency to separate desired molecules from a matrix (such plants). Maceration, percolation, and Soxhlet extraction are typical examples.

1. **Solvent extraction (liquid-liquid extraction):** Among the most techniques for removing bioactive substances from *Myrica esculenta* is solvent extraction. Based on the molecules' polarity of interest, different solvents like ethanol, methanol, chloroform, water and hexane are utilized. The ability of ethanol and methanol to extract a wide range of phytochemicals, for example flavonoids and phenolic compounds which have antioxidant properties, makes them popular.[29] The nature of the sample to be extracted determine whether the solvent extraction involve partitioning between two immiscible liquids or extraction from a solid using a liquid solvent. One common method for performing solvent extraction involves the following steps: A frozen matter undergoes crushing and uniform mixing. After adding anhydrous sodium sulfate to the powder, it is dissolved in a solvent such as diethyl ether and allowed to mix at room temperature for a number of hours. Filtration is performed to separate the extract, while the undissolved fraction undergoes further solvent extraction until no more material can be extracted. In the final step, different extract fractions are combined, the mixture is concentrated via evaporation and vacuum distillation is applied.[30]

2. **Maceration:** This approach extracts active components from rough-pulverized plant matterial, such as leaves, stem bark or root bark, by placing it in a container and immersing it in a solvent (menstruum) until completely saturated. After being sealed, the container is let to stand for a minimum of three days. During this time, the mixture is stirred occasionally; If stored in a container, it should be shaken regularly to enhance the extraction process. Once extraction is complete, the liquid extract (micelle) is isolated from the solid residue (marc) through filtration or decantation. Solvent from the micelle is evaporated-often in a water bath or oven to concentrate the extract. This approach works well for isolating constituents from heat-sensitive botanical sources.[31] [32]

3. Infusion: This is an isolation method comparable to maceration. The plant material is first pounded into a fine powder and stored in a clean container. An extraction solvent, either hot or cold, is then added to soak the powdered material and the combination is allowed to stand for a brief time. This approach is effective for isolating bioactive compounds that are easily soluble in the chosen solvent. It is also well-suited for preparing fresh extracts intended for immediate application. A solvent-to-sample ratio in the range of 4:1 to 16:1 is commonly used, depending on the particular requirements.[33]

4. Digestion- This extraction technique uses moderate heat throughout the procedure. First, pour the extraction solvent into a clean vessel, then introduce the powdered plant sample. The mixture is then heated in a water bath or oven at approximately 50°C. Applying heat during extraction lowers the solvent's viscosity, enhancing the recovery of secondary metabolites. The approach is ideal for plants rich in compounds that are quickly solubilized.[34]

5. Decoction- This method necessitates ongoing continuous hot extraction with a predetermine volume using as the liquid phase. The dried, powdered plant sample is transferred to a clean vessel, then combined with water and thoroughly mixed. Consistent heat application throughout the procedure improves the efficiency of extraction. Extraction generally takes about 15 minutes, with the solvent-to-sample ratio usually set between 4:1 and 16:1. This method proves particularly effective for recovering water-soluble and thermally stable compounds from plant matter.[35]

6. Percolation- This technique is carried out using a percolator. This is a glass vase with a narrow cone form with holes on either end. To begin, wet the dried and the ground plant material is added to the extraction solvent in a clean vessel. An extra amount of solvent is added, and the preparation is allowed to rest for nearly 4 hours. Subsequently, the mixture is added to the percolator, the lower outlet is capped, and it is kept at rest for 24 hours. The top of the percolator is filled with solvent to ensure the plant material is entirely moistened. Next, the bottom outlet of the percolator is unsealed to let the liquid gradually flow out. As extraction proceeds, the solvent is steadily added, and gravity guides within the botanical sample. Solvent is added gradually until it reaches roughly 75% of the desired final volume. The liquid extract is subsequently removed by decantation & filtration. Finally, squeezing the marc & additional solvent is added to meet the needed final volume of the extract.[36]

7. Soxhlet extraction: The Soxhlet method is a well-known technique in which a solvent is continually cycled over a sample, ensuring that chemicals are extracted over a prolonged period. This approach works particularly well for extracting essential oils, fatty acids, and other non-polar chemicals from *Myrica esculenta*. However, it requires a big amount of solvent and takes longer to complete. The bark of *Myrica esculenta* was carefully washed and left to dry in the shade for 15 days. The shade-dried bark was roughly ground using a mortar and pestle, along with grinding tool. Using Soxhlet extraction (continuous hot percolation), 250 g of the plant powder was first treated with petroleum ether (60-80°C) to defatting, and subsequently with methanol. The methanolic extract was evaporated under reduced pressure at 50-55 °C following complete extraction. Using a rotary evaporator (Heidolph), the solvent was evaporated under reduced pressure at 40°C. The obtained dried extracts were stored at 2-4°C in sealed vials for subsequent applications. This extract was employed in nanoparticle fabrication during this investigation.[37]

8. Hydro distillation and steam distillation method: Essential oils can be extracted using two primary methods: hydro distillation (HD) and steam distillation (SD), but they differ in their approach to heating the plant material. In HD, the plant material is immersed directly in boiling water, whereas in SD, steam is passed through the plant material that is suspended above the boiling water. Steam distillation is commonly used to extract heat-sensitive oils since it allows for lower extraction temperatures. The root is used to produce Katphala oil, which can be extracted using either HD or SD procedures. While both procedures are effective, HD is more frequently employed for Katphala oil due to its cost-efficiency.[38]

9. Reflux:- Reflux extraction is a method for extracting chemicals from *Myrica esculenta* (also known as Himalayan *bayberry*) that involves continually heating and condensing a solvent over the plant material. This process uses volatile organic solvents to extract components, which are subsequently heated and distilled. The solvent condenses and returns to the extraction vessel for repeated soaking.[39]

Table 8: Advantages and limitation of conventional Extraction methods.

Extraction Techniques	Benefits	Limitation	References
Maceration	Easy and reasonably priced. little energy consumption. Perfect for substances that are sensitive to heat. Adaptable solvent scalable	Time-consuming and less productive High volumes of solvents Risk of microbial growth: Manual management	[40]

Infusion	Easy to use, inexpensive, and suitable for heat-sensitive compounds Adaptable and scalable solvent selection	Spending less time effectively High need for a solvent Hazard of contamination from manual work	[40]
Decoction	Beneficial for hard plant substances Easy and cheap Rapid extraction Traditional and proven methods without the need for organic solvents	Unsuitable for compounds that are sensitive to heat Limited solubility could Change the chemical composition Short shelf life Potential volatile loss	[41]
Percolation	Effective extraction method Easy to use and scalable Adaptability to solvents ongoing procedure Beneficial for heat-sensitive substances Minimal use of solvents (in certain situations) Excellent for coarse and fine materials	Large volumes of solvent are needed for this time-consuming process (in some cases) Problems with solubility and selectivity Restricted extraction rate control Unsuitable for extremely fine particulates Extraction efficiency may decline with time due to labour-intensive setup potential solvent Losses Laboratory to Industrial Transition	[41]
Soxhlet Extraction	High efficiency of extraction ideal for non-volatile substances quite easy to use for solvent recovery good for solid samples Large quantities can be handled by standardised procedures	Able to manage enormous amounts excessive solvent usage Not recommended for substances that are heat-sensitive Issues with solvent recovery restricted ability to pick Over-extraction risk energy-intensive Unsuitable for extremely delicate materials	[42]
Hydrodistillation	Easy and affordable technique for preserving volatile compounds without the use of organic solvents Extraction of volatile Components with selectivity The essential oil Industry widely uses scalability to preserve aromatic properties	Energy-intensive extraction process with a long extraction time Some volatile compounds are lost Some plants have low yields unsuitable for compounds that are not volatile Possibility of contamination of water and solvents limited Control over extract composition needs significant plant material	[43]
Steam Distillation	Good for fabrics that are sensitive to heat Use of solvents is minimal. High extracted oil purity that is scalable Ideal for a variety of materials Maintains aromatic qualities	Need a large amount of water. Some plants have lower yields. Possible depletion of volatile substances Not suitable for some plant species Use of energy Heat damage risk	[44]

Green extraction techniques

Sustainable extraction techniques are environmently friendly, energy-efficient, and cost-effective extraction technologies that reduce solvent consumption while protecting both consumers and the environment. These are a few popular sustainable extraction techniques:

1. **Ultrasound-assisted extraction:** Ultrasound-assisted extraction (UAE) is an advanced and efficient method that employs ultrasonic waves to isolate bioactive compounds. The process disrupts cell walls, allowing chemicals to be released into the solvent.[45] This method is advantageous in that it decreases extraction time, minimizes solvent usage, and can yield higher concentrations of bioactive chemicals than standard procedures. An ultrasound-assisted method was employed to extract proanthocyanidins from the bark of *Myrica esculenta* using water as the solvent. Optimal conditions included an ultrasonic frequency of 20 kHz, power of 800 W, a solid-to-liquid ratio of 1:10 (g/mL), temperature of 60°C, an extraction time of 75 minutes, and two extraction cycles. involved lower thermal conditions, shorter extraction duration, and decreased solvent consumption. The extract comprised 60% proanthocyanidins, which was identified as prodelphinidin through a qualitative evaluation.[46, 47, 48]

2. **Supercritical-fluid extraction:** Supercritical fluid extraction, which relies on CO_2 like a solvent, is a green extraction process with excellent yields and minimum environmental impact. SFE is selective, enabling for the precise extraction of non-polar molecules like essential oils. This approach is popular because it preserves the integrity of thermolabile compounds while reducing the usage of hazardous solvents. Supercritical Fluid Extraction (SFE) is a sophisticated technology renowned for its high yield, purity, and time efficiency.[49] It outperforms existing extraction procedures and allows for the selective extraction of chemicals. However, it can be expensive due to the specialised equipment and working conditions necessary. Despite its high initial cost, SFE is frequently preferred for its efficiency in producing high-quality extracts in less time.[50, 51]

3. **Microwave based extraction:** An electromagnetic wave with a frequency between 300 MHz and 300 GHz is called a microwave. Percy Lebaron Spencer, an engineer, unintentionally discovered the heating action of microwaves.[39] Microwave-assisted extraction heats the solvent and plant material with microwave energy. This makes the extraction procedure more efficient by increasing the permeability of the plant cells and

boosting the solubility of bioactive chemicals. MAE is time-efficient and requires lesser amounts of solvents than standard procedures.[52, 53]

4. Enzyme-Assisted Extraction (EAE): Enzyme-assisted extraction of *Myrica esculenta* uses biocatalyst to dissolve plant cell walls and release bioactive chemicals, increasing extraction efficiency while lowering the need for harsh solvents and high temperatures when compared to conventional procedures. Enzyme-assisted extraction of *Myrica esculenta* is a technique that uses biocatalyst to break down the plant's cell walls, allowing more efficient isolation of bioactive compounds.[54, 55]

5. Pressurized Liquid Extraction (PLE): Pressurised Liquid Extraction also known as Accelerated Solvent Extraction (ASE), is a process for extracting chemicals from solid matrices at high pressures and temperatures. It has various advantages over traditional extraction procedures, such as quicker extraction periods and lower solvent use. For example, a research of strawberry tree fruits utilizing PLE with various solvents and parameters like as temperature, static extraction time, and extraction cycles confirmed the technique's efficiency. Similarly, PLE has proven effective in isolating bioactive compounds from various botanical sources like passion fruit leaves and laurel leaves.[56]

6. Pressure-Enhanced Fluid Extraction (PEFE) Pressure-Enhanced Fluid Extraction (PEFE) is a modern extraction technique that involves using elevated pressure-sometimes in conjunction with specific solvents or supercritical fluids-to efficiently extract bioactive compounds from plant materials. When applied to *Myrica esculenta*, a medicinal plant often known as box myrtle or kaphal, this method can dramatically elevate the yield and quality of extracted phytochemicals compared a typical extraction processes.[57,58]

Current trends and progress in innovative extraction technologies

The traditional extraction methods, which are commonly employed for the extraction of botanicals, such as Soxhlet extraction, maceration, decoction, infusion, and percolation methods, have a number of drawbacks, including low selectivity, lengthy extraction times, and the use of large amounts of organic solvents, which can endanger operator and environmental safety.[59, 60] In order to lessen the extraction process's negative environmental effects while also making the finished product safer for everyday use by humans, new green extraction techniques are being used more and more to extract natural chemicals and phytocomplexes. The selection of appropriate extraction methods is predicated on an initial

assessment of the benefits or drawbacks associated with the functionality of the extraction instruments and the chemical makeup of the target substances.[61]

In an attempt to satisfy the growing demand for ecologically friendly and sustainable extraction processes, researchers and industries are now concentrating more on creating and implementing green extraction techniques. Green extraction methods such as microwave-assisted extraction, supercritical fluid extraction, pressurised liquid extraction, ultrasonic-assisted extraction, enzyme-assisted extraction and pulsed electric field extraction (PEFE) are increasingly adopted in scientific research related to food and plant analysis, with a clear upward trend in the literature (**Figure 2)** Green extraction techniques are procedures created to minimize environmental effect by decreasing or eliminating Isolating bioactive substances from natural materials frequently requires the use of toxic solvents and reagents. Compared to conventional extraction methods, these techniques offer multiple benefits, including shorter extraction times, reduced consumption of toxic organic solvents, higher extraction yields, and the ability to automate processes, thereby improving reproducibility and lowering overall energy usage.[62] To choose the most appropriate extraction methodology, the next section gives a full description of the advantages and disadvantages associated with each method **Table 8**

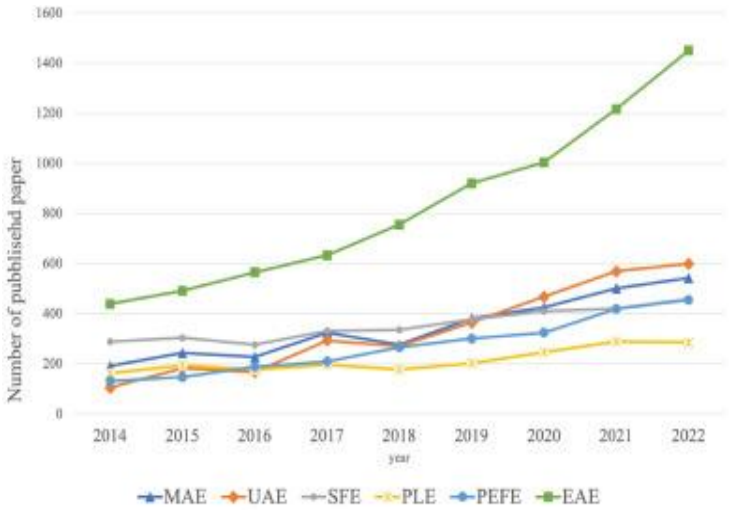


Figure 3: Trend of advanced extraction methods from 2014 to 2022.

Table 9: Advantages and limitation of Green (sustainable) extraction methods.

Extraction Techniques	Advantages	Disadvantages	References
Supercritical Fluid Extraction (SFE)	Quicker extraction time Effective use of energy Reduced use of solvents high yield of extraction mild circumstances for substances that are heat-sensitive Precision and selectivity Potential for environmental friendliness	High starting price Compound degradation risk Some samples have limited penetration. Challenges of scaling up Needs specific equipment. Accumulation of pressure	[63]
Ultrasound-Assisted Extraction (UAE)	Reduced extraction time: Reduced use of solvents Increased yield of extraction Mild extraction circumstances Eco-friendly Easy setup of the equipment Ideal for a variety of substances	Compound degradation risk Minimal wear on industrial scale-up equipment Optimization is necessary. Not suitable for every material	[64] [65]
Microwave-Assisted Extraction (MAE)	Degradation risk of compounds Wear on industrial scale-up equipment is minimal. Optimization is needed. Not the best for every material	Expensive equipment Overheating risk Scaling up difficulties Limited solvent compatibility necessitate optimization Material Limitations	[66]
Enzyme-Assisted Extraction (EAE)	Eco-friendly Increased production Elevated selectivity Improved bioavailability and less solvent use in mild operating conditions Suitable for other green methods	Expensive enzymes Optimisation is necessary for longer extraction times. Limited substrate specificity enzyme sensitivity Potential product Contamination	[67]
Pressurized Liquid Extraction (PLE)	Quick recovery Reduced use of solvents High yield & efficiency Ideal for compounds that are thermally stable Eco-friendly (when employing green solvents) Friendly to automation Numerous uses	Expensive equipment risk of deterioration Compatibility of colvents Complex scale-up may be necessary Matrix interference optimization may be necessary.	[68,69]
Pulsed Eelctric Field Extraction (PEFE)	Brief extraction time Minimal heat requirement Adequacy for separating heat-sensitive	Increased expense of upkeep. Accurate control of the variable.	[70]
Green Solvent Extraction (GSE)	Eco-friendly Safe for use in Pharmaceutical and food applications Renewable and sustainable Reduced regulatory overload	Reduced extraction efficiency (occasionally) Limited choice of solvents Difficult solvent behavior scalability issues	[71]

	Gentle extraction conditions Adaptable solvent choices Simple integration	Some green solvents' costs need to be optimized	
Solvent-Free Microwave Extraction (SFME)	Solvent-free and environmentally friendly Quick extraction times High-quality extracts and water and energy efficiency Ideal for compounds that are heat-sensitive Sustainable and scalable Cleaner product (lab to small industry)	Required moisture content Equipment cost scale-up restrictions: only Volatile Compounds; overheating risk needs optimization	[72]
Cold Press Extraction	Heat-sensitive chemicals are preserved by the absence of heat. No chemicals or solvents Superior unadulterated oils sustainable and environmentally friendly maintains nutritional value, flavor, and fragrance. Easy-to-use and inexpensive equipment	Low yield in contrast to heated techniques Restricted to nuts, fruits, and seeds high in oil Requie for a lot of raw materials. Slow process and might not be appropriate for production on a large scale. Slow extraction duration may be less economical because of the low yield.	[73]

Additionally, emerging (supercritical fluid, subcritical fluid, microwave-assisted, ultrasonic-assisted, enzyme-assisted, and pulsed electric field-assisted) and traditional (Soxhlet, maceration, and hydrodistillation) techniques, as well as separation, isolation, and identification by various analytical methods and quantification using various chromatographic and spectrophotometric methods[74] are included in the flow chart depicted in **Figure**

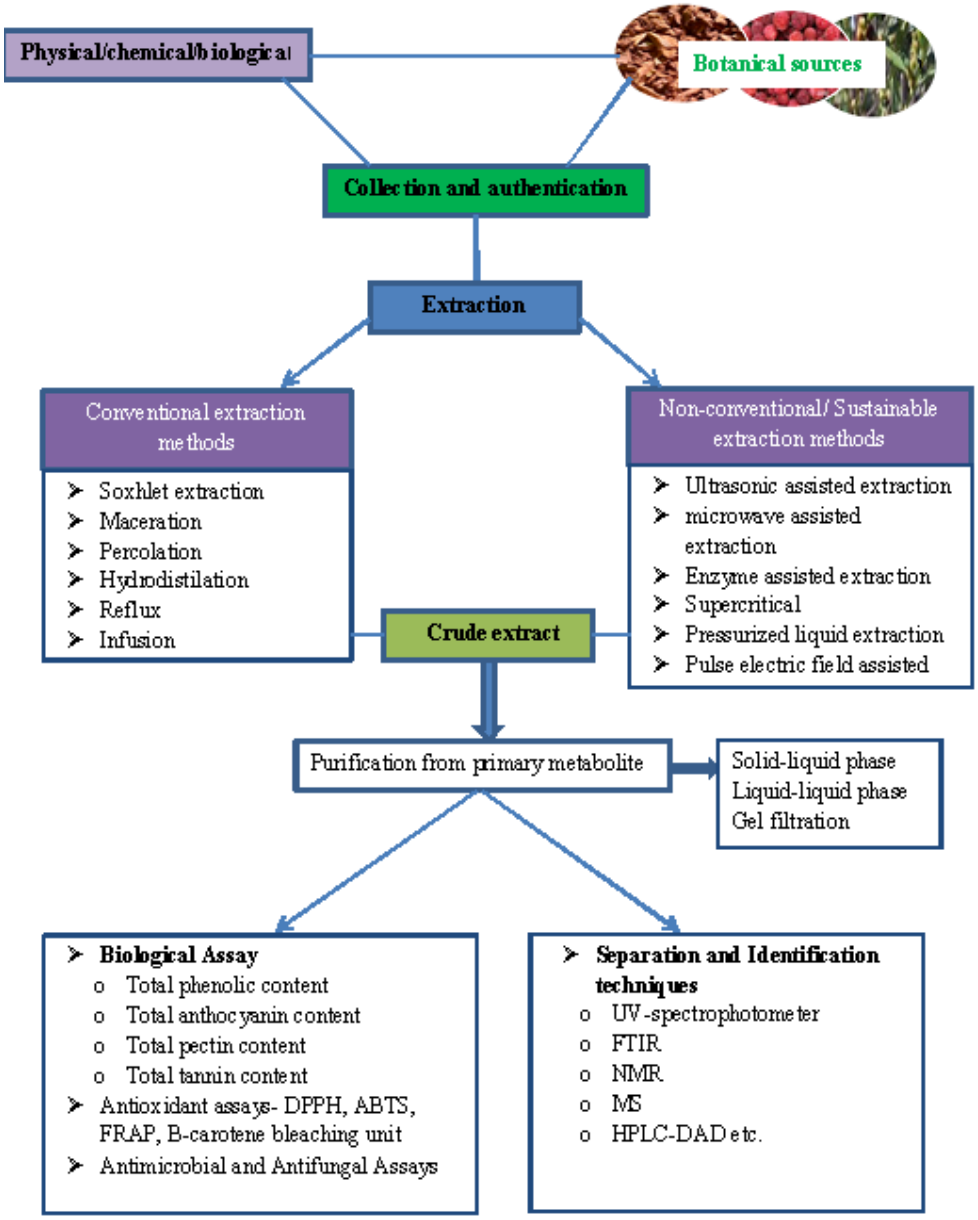


Figure 3: Flow chart for the extraction and characterization of bioactive compounds extracted from botanical sources.

Reported Pharmacological Activities of *Myrica esculenta* Extracts

Extracts of *Myrica esculenta* obtained using various methods exhibit diverse pharmacological properties including:

Antioxidant activity: Free radical scavenging tests such as ABTS, FRAP, and DPPH demonstrated that crude extracts from M. esculenta fruit could scavenge free radicals. The investigation also revealed the presence of total flavonoids (1.59 mg quercetin equivalent/g) and total phenolic (2.51 mg GAE/g) Additionally, it was observed that the maximum levels

of flavonoids (810.5±23.4 mg Catechin/100g of fruit weight) and phenolic content (2,603.0±20.6 mg GAE/100g f.w.) were found in the acidic acetone extract for M. esculenta, which was followed by acetone, acid methanolic, and methanolic extract. Gallic acid, myricetin, caffeic acid, catechin, chlorogenic acid (traces), trans-cinnamic acid, p-coumaric acid, and ellagic acid were among the chemicals with antioxidant qualities found in the extract. According to DPPH (1390.0±29.6 mg CE/100 g f.w.), ABTS (1252.0±23.7 mg BHAE/100 g f.w.), ferric reducing activity (1070.0±30.7 mg AAE/100 g f.w.), and superoxide anion scavenging activity (1611.6±48.2 mg AAE/100 g f.w.), the acidic acetone extract exhibited the highest radical scavenging activity. Numerous investigations have shown that *Myrica esculenta* extracts contain antioxidants, particularly phenolic and flavonoid components. These compounds aid in the neutralisation of free radicals, thereby preventing Oxidative damage is associated with a range of long term diseases, including cancer and heart-related ailments. The conventional DPPH method outlined by[75] was adopted for this investigation. A solution containing 25mL of 400 mM DPPH was combined with 25mL of 0.2 M MES buffer (pH 6.0 adjusted using NaOH) and 25mL of 20% (v/v) ethanol. A volume of 2.7 ml DPPH radical cation solution was combined with 0.9 mL of sample extract and kept at room temperature for 20 minutes. The reduction in absorbance at 520nm was measured using a UV-VIS spectrophotometer. Results were reported as millimoles (mM) of ascorbic acid equivalent (AAE) per 100g fresh fruit weight.[76]

Anti-inflammatory activity and analgesic activity: *Myrica esculenta* extracts, particularly those derived from the fruits and leaves, have demonstrated considerable anti-inflammatory properties. The observed effects result from down regulating pro-inflammatory mediators and enzymatic activity, with COX-2 being notably affected. The plant exhibits confirmed anti-inflammatory properties, attributed to the presence of bioactive constituents such as flavonoids and phenolic compounds. The anti-inflammatory potential of an aqueous extract of *Myrica esculenta* has been evaluated through both in vitro and in silico methods[77] The findings revealed that the extract markedly suppresses the activity of 15-lipoxygenase (15-LOX), an enzyme implicated in inflammation. In silico docking analysis demonstrated that bioactive compounds present in the extract including myricetin, arjunolic acid, and myricanone-exhibit strong binding affinities toward key inflammatory targets, including cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). Tumor necrosis factor-alpha (TNF-a), a central mediator in inflammation, is a common therapeutic target in the treatment of arthritis and other inflammatory conditions.[78, 79]

Antimicrobial activity: *Myrica esculenta* extracts' antibacterial activities are extensively recognized. Studies have demonstrated that these extracts can prevent the growth of a wide range of harmful microorganisms, including bacteria and fungi. This makes the plant an ideal option for producing natural antibacterial drugs. The presence of bioactive compounds including flavonoids and phenolic acids has been linked to the plant's antibacterial properties. *The* antibacterial potential of *Myrica esculenta* is primarily associated with its bioactive constituents, including flavonoids, phenolic acids and terpenes. These chemicals have been shown to exhibit antimicribial activity against different type of microorganism, including bacteria & fungi. *M. esculenta* antibacterial activity was assessed through various in vitro and in vivo approaches, including agar well diffusion and broth microdilution methods.[78, 80, 81]

Anticancer properties: Fruit extracts of *Myrica esculenta* (Bayberry) have been studied for their anticancer potential, most notably by[82] who focused on various cervical cancer cell lines such as C33A, HeLa, and SiHa. Several in vitro studies have validated these findings, showing that the extracts demonstrate considerable anticancer activity. The extracts have been proven to cause apoptosis (programmed cell death) and decrease cancer cell proliferation. These benefits are mostly due to the presence of bioactive phytochemicals, particularly phenolic & flavonoids compounds, recognize for their antioxidant and anticancer potential. Importantly, the suppression of cancer cell proliferation was found to be dose-dependent, with larger concentrations producing stronger effects. Notably, the extracts did not cause cytotoxicity in non-cancerous cells, indicating a degree of selectivity that could be useful in therapeutic applications. The outcome point of *Myrica esculenta* as a promising plant based source of anticancer therapeutics and warrant further investigation through in vivo studies and clinical evaluations.[83] One of the main phytoconstituents of M. esculent and related species is myricanol. Myricanol's antiproliferative efficacy in A549 cells was reported by Dia et al., with an IC50 of 4.89 µg/ml. The compound demonstrated anti-apoptotic activity by changing the expression of caspase 3, caspase 9, Bax, and Bcl-2, which amply supports its anticancer action Myricanol's anti-proliferative effects on lung cancer using an A549 xenograft were also demonstrated by in-vivo investigations. At a dosage of 40 mg/kg, a maximum tumour inhibition rate of 38.5% was noted. The TUNNEL assay confirmed the results, showing a notable rise in the pro-apoptotic population in addition to changed expression of apoptotic proteins and enzymes, which are indicators of apoptosis.[84]

Anti-diabetic properties: The plant has been shown to have anti-diabetic properties, including improved insulin sensitivity and lower blood glucose levels. According to certain research, chemicals from *Myrica esculenta* can imitate the action of insulin, making them potentially effective for the therapeutic management of type 2 diabetes. *M. esculenta* antidiabetic activity has also been shown to be beneficial in the treatment of numerous form of diabetes, including type 2. The methanolic leaf extract of *M. Esculenta* exhibited a significant blood glucose lowering effect in high-fat diet-fet rats and in those with type 2 diabetes induced by a single dose of streptozotocin. An extract also increased skeletal muscle glucose absorption by activating IRS-1/PI3K/Akt/GLUT4 signalling both in vitro and in vivo. The presence of bioactive constituents like flavonoids and phenolic acids has been associated with the plant's anti-diabetic properties.[85, 86]

Hepatoprotective effect: *Myrica esculenta* extracts have also been found to protect liver cells from toxins, enhancing liver health and function. Bioactive components like phenolic acids and flavonoids are believed to underlie the hepatoprotective nature of the plant. The ethanolic extract of *Myrica Esculenta* leaves has been found to provide considerable hepatoprotection against liver damage caused by plant characteristics. Carbon tetrachloride (CCl4) in rats.[87] Researchers observed that administering the extract at doses of 200 and 400 mg/kg body weight led to a significant reduction in elevated serum biochemical markers, including serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP), in CCla-treated animals. Additionally, the extract appeared to restore diminished levels of total protein and albumin in the liver.[88, 84]

Anti-asthmatic activity: Extensive study has demonstrated the strong anti-asthmatic effects of *Myrica esculenta*, notably its stem bark extract. Studies, including those published on Science Direct, have shown that the crude extract significantly reduces the proliferation of eosinophils in mice, a crucial indicator of allergic inflammation connected with asthma. Furthermore, the extract was demonstrated to inhibit plasma exudation generated by acetic acid, increasing its anti-allergic potential. Ethanol based extraction of *M. esculenta* bark has revealed its potential anti-asthmatic properties via a variety of pathways, including anti-anaphylactic, antispasmodic, and bronchodilatory activities. These combined results indicate that the plant may help treat different asthma symptoms, including airway inflammation, smooth muscle contraction, and hypersensitivity responses. Overall, *M. esculenta* shows

potential as a natural therapeutic agent for asthma control, requiring more pharmacological and clinical research.[89, 87]

Anti-psychotic activity: An ethanolic extract of Myrica esculenta was tested for its antipsychotic properties utilising established animal models, including such as apomorphine-induced stereotypy, Cook's pole climbing device, and catalepsy brought on by haloperidol in rats. These behavioural models are commonly used to evaluate dopaminergic activity and the efficacy of antipsychotic medications. In addition, levels of important neurotransmitters, notably noradrenaline and dopamine, were examined to support the pharmacological findings. Phytochemical analysis of an extract comprising various bioactive constituents, including glycosides, flavonoids, volatile oils, proteins, saponins, phenolics and tannins, which may contribute to its neuroactive effects. The extract exhibited considerable antipsychotic activity, implying possible antidopaminergic actions. While these findings are intriguing, more neurochemical research are required to completely understand the processes by which *M. esculenta* exerts its effecting the central nervous system and validate its potential as a plant-based antipsychotic drug.[90]

Hypotensive activity: *Myrica rubra*, sometimes known as Chinese bayberry, and its genus, *Myrica esculenta*, have demonstrated promise hypotensive action, particularly in preclinical trials involving spontaneous hypertensive rats (SHR). These studies show that extracts from *Myrica rubra* can effectively control high blood pressure levels. The underlying mechanisms for this action appear to include both metabolic and circulatory routes. One of the extract's primary activities is the suppression of GLUT1, a glucose transporter responsible for cellular glucose uptake, which may lead to enhanced vascular function. In addition, the extract promotes the NO/Akt/eNOS signalling pathway. This activation increases nitric oxide generation, resulting in vasodilation and increased blood flow, which helps to lower blood pressure. These data show that *Myrica rubra* has the potential as a natural medication for treating hypertension, pending further study.[91]

Antihelmentics activity: The current research was carried out to assess the anthelmintic properties of a saturated ethanolic extract from the bark of *Myrica esculenta* (commonly known as cassava) against adult Indian earthworms (Pheretima posthuma), which serve as an appropriate in vitro model due to their anatomical and physiological similarities to human intestinal parasites. The comparative standard was piperazine citrate, a well-known anthelmintic drug used in clinical practice. During the experiment, earthworms were exposed

to different quantities of the plant extract, and the metrics of interest-paralysis time (PT) and death time (DT)-were meticulously recorded. Notably, at a dosage of 12.5 mg/ml, the extract displayed a substantial anthelmintic efficacy, producing paralysis in an average of 20.11 minutes and leading in worm death in 41.25 minutes. These findings indicate that the hydroethanolic extract of *M. esculenta* bark exhibit notable anthelmintic properties, even at low concentrations. Furthermore, the reported impact at 12.5 mg/ml was found to be more strong than the conventional medication, piperazine citrate, causing both paralysis and death more quickly. This suggests that the extract could be a promising natural therapy for helminthic illnesses. subject to further pharmacological and toxicological studies.[2, 92, 93]

Nephroprotective The extract from the fruit juice of *M. esculenta* was reported to possess nephroprotective properties in gentamycin induced-nephrotoxic rat models. Extract (400 mg/kg) treated rats showed lower levels of serum creatinine (0.074±0.02 mg/dl) and urea (62.46±3.74 mg/dl) in comparison to gentamycin (toxic control) treated group. This was further validated by histopathological findings where the extract was able to restore glomerular structure whereas the gentamycin treated group showed damaged kidney tissue and deformed glomerulus[94] All these activity show in **Figure 3**.

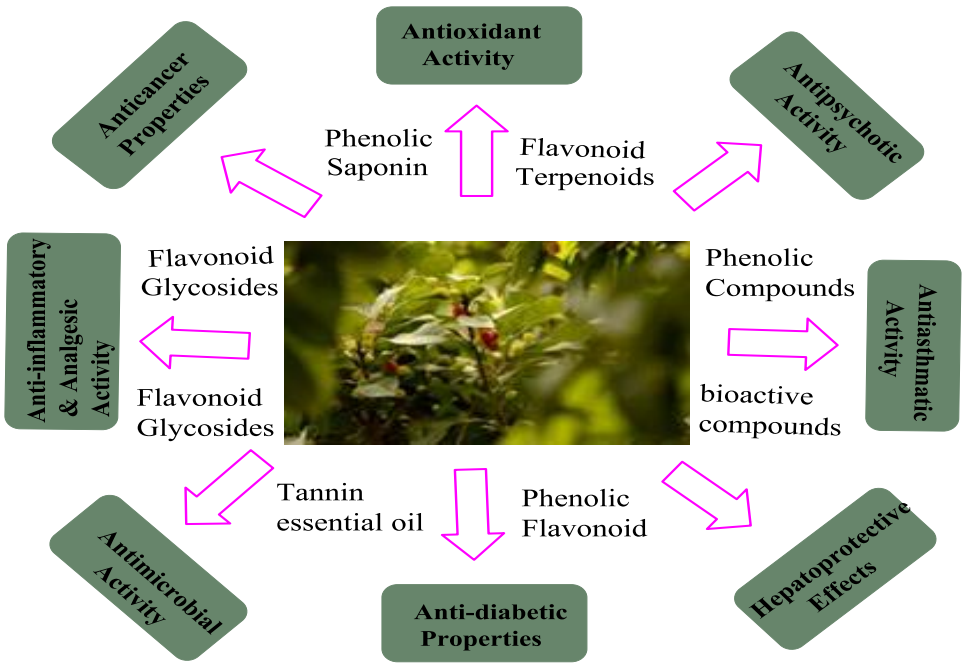


Figure 4: Various Pharmacological Activities of Myrica Esculenta Extract.

The pharmacological activities of different parts of Myrica esculenta along with their corresponding methods have been summarized in table form.

Table 10: For Presentation or Summary Chart.

Parts of plants	Activity	Methods	References
Plant Extract	antioxidant activity	Percolation technique	[95]
Methanolic extract	Robust antioxidant and Anthelmintic activities, antidiabetic	Soxhlet extraction or maceration with methanol.	[96, 97]
Ethanolic extract of bark	Anti-inflammatory antioxidant	maceration and percolation	[98]
Bark	Anticancer, antipsychotic	Soxhlet's extractor (continuous hot percolation process	[99, 100]
Stem Bark	antiallergic activity,	ultrasound-assisted extraction (UAE)	[101]
Bark Root	anti-inflammatory & antioxidant activity	continuous hot Soxhlet apparatus	[102]
Leaves	antioxidant properties	Soxhlet apparatus	[103, 28]
Ethanolic extract of bark	antihelmintic activity anxiolytic and antidepressant activity.	Soxhlet apparatus	[104, 4]
Seed	Antioxidant activity	supercritical fluid, pressurized fluid & microwave-assisted extraction	[105]
Fruits	antioxidant and antimicrobial activities	ultrasonic-assisted extraction, Cold pressing mechanical extraction method	[106]
Root	Anti-inflammatory	soxhlation	[103]

CONCLUSION

In conclusion, *Myrica esculenta* (Kaphal) extraction procedures have a significant impact on the production and efficacy of its phytochemical and pharmacological qualities. Depending on the target chemical and its bioactivities, various isolation approaches including solvent based extraction, supercritical fluid extraction & microwave-assisted extraction, have varied advantages. Solvent extraction is still the most frequent due to its simplicity and effectiveness, although modern technologies such as supercritical CO2 extraction promise greater selectivity and little degradation of bioactive chemicals. The plant's various chemical ingredients, which include flavonoids, tannins, alkaloids, and terpenoids, highlight its potential for a variety of therapeutic applications that include antioxidant, anti-inflammatory, antibacterial, neuroprotective activity and anticancer properties.

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Conflicts of Interest

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