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Specific analytical methods for the extraction of common phytochemical constituents of *Vitex negundo* Linn: A mini-review

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Abstract
Analytical chemistry is a branch of science that deals with chemical analysis responding to changing conditions. It is related to phytochemistry that deals with plant phytochemicals which are beneficial for preventing illnesses and maintaining long-term health. This review comprehensively studied the plant *Vitex negundo* Linn (Lagundi) by exploring different analytical methods utilized in extracting its common active secondary metabolites depending on its efficiency, selectivity and convenience. In this paper, most studies that had been gathered employed high yield, efficiency, and selectivity, making it difficult to determine a standardized method for *V. negundo* Linn due to the optimal environmental conditions and solvents that are being utilized. Hence, optimization remains vital for the variety of applications in extracting bioactive compounds from *V. negundo* Linn to achieve a minimum level of impurities and an acceptable degree of reproducibility and repeatability of bioactive compounds of interest.

Keywords: *Lagundi*, secondary plant metabolites, phytochemistry, analytical chemistry

Introduction
Analytical chemistry is used in a variety of applications in our society, including drug development, industrial process control, environmental monitoring, medical diagnostics, food production, and forensic surveys. It is also vital in a variety of study fields, as it is a branch of science dedicated to generating new knowledge so that chemical analysis can be enhanced to better respond to changing conditions [1]. Utilizing the principle of analytical chemistry in the stated variety of applications can be done by studying natural products or phytochemicals. This is typically a branch of science called phytochemistry that deals with plant products assessing their importance in the environment [2].

Plant phytochemicals have been found to be beneficial for illness prevention as well as for long-term health. A primary metabolite, or central metabolite, is a type of metabolite found in a wide range of organisms and cells that plays a direct role in normal development, growth, and reproduction. It usually serves a physiological purpose in the body. The following are some examples of primary metabolites: saccharides, coumarins, lignans, flavonoids, terpenoids, and steroids are commonly found in plants [3]. These kinds of pure phytochemicals must be obtained via extraction and isolation due to the complexity of plant chemical ingredients before going into structure identification, bioactivity screening, and other procedures. In recent years, a more advanced, modern technology can perform more efficient extraction and isolation that can also provide data for analysis and identification of these phytochemicals, as such conventional methods are convenient as well [3]. Extraction is the first phase in phytochemistry research. It is always a requirement for isolating effective constituents. The goal of extraction is to extract as much of the intended chemical ingredients as possible while avoiding or reducing the solution of undesirable constituents. Extraction of target constituents can be purified into monomer compounds using physical and chemical processes through solvent extraction, precipitation, crystallization, fractional distillation, salting-out, or dialysis. These methods are known for phytochemical separation [3].

In this review, *Vitex negundo* Linn (Lagundi) is the plant that will be comprehensively studied, wherein it is one of the commonly studied plants in the field of phytochemistry for its bioactive compounds.

It has been recognized by the Department of Health (DOH) as an effective clinically proven medicinal plant in the treatment of respiratory problems such as cough, asthma, sore throat, and fever [4]. Due to the prevalence of common respiratory problems, it has become a burden to millions of people worldwide. In the early years, most children could acquire multiple respiratory infections while asthma remains to be the most common respiratory condition even with adults [5]. Most of the Philippine population rely on herbal products (e.g., Lagundi) as their primary source for their healthcare needs as healthcare remains inaccessible and unaffordable in the country [6]. However, these studies also mentioned that some traditional medicines, if not ineffective, were found toxic, and may cause more serious complications. Hence, further studies that validate the efficacy of herbal medications and develop safe and low-cost drugs that are efficient in treating common respiratory infections are needed. This is where the importance of analytical techniques such as extraction to studying its bioactivity on different plants comes into place as they are vitally important in the field of studying herbal medicines, or specifically connected to medicinal and industrial fields related to phytochemistry.

Unfortunately, there is still no standardized extraction method for *V. negundo* Linn as this plant relies on different extraction techniques until the present. Yet, several studies have shown that most different extraction techniques exhibit a high yield of phytochemicals from *V. negundo* Linn. Thus, this review study will explore the different analytical methods and techniques utilized in extracting common active secondary metabolites from *V. negundo* Linn.

Vitex negundo Linn

Characteristics, Taxonomy and Locality of *Vitex negundo* Linn

V. negundo Linn is a tall, branching tree or shrub that can reach a height of 5 meters. It has a single thick, woody stem that resembles a trunk. As for its characteristics, the leaves of *V. negundo* Linn are palmately arranged, with five-pointed leaves that splay out like a hand's fingers. Furthermore, the leaves are 5-foliate in shape, with just three leaflets in rare cases, and are 4-10 cm long and hairy on the bottom. Meanwhile, its flowers are blue to lavender in color and are 6-7 mm long. The flowers produce a globose fruit that is about 4 mm in diameter and becomes black when ripe [7]. For its description and taxonomic classification, both are shown in Figure 1 and Table 1, respectively.

Fig 1: *Vitex negundo* Linn [8]

Table 1: Taxonomic Classification of *V. negundo* Linn

Kingdom:	**Plantae**
Division:	Tracheophyta
Class:	Magnoliopsida
Order:	Lamiales
Family:	*Lamiaceae*
Genus:	*Vitex*
Species:	*Vitex negundo*

For its locality, *V. negundo* Linn is found to be indigenous in tropical and subtropical areas of Eastern and Southern Africa and Asia, such as Afghanistan, Bangladesh, Bhutan, Cambodia, China, India, Indonesia, Japan, Kenya, Madagascar, Malaysia, Mozambique, Myanmar, Nepal, Pakistan, Philippines, Sri Lanka, Taiwan, Tanzania, Thailand, and Vietnam. This plant can be easily located near bodies of water, recently disturbed land, grasslands, and mixed forests [9]. As of now, it is widely cultivated in Europe, Asia, North America, and the West Indie [10]. Within these locations, they are studied for their phytochemical constituents and their pharmacological applications in the present.

Phytochemical Constituents of *Vitex negundo* Linn and its Importance

Several studies show that *V. negundo* Linn extracts in its overall structure consist of important phytochemicals such as terpenoids, lignans, polyphenols, steroids, saponins, phenolic compounds, and flavonoids [11, 12]. Shown in Table 2 are the extracted phytochemicals from the different parts of *V. negundo* Linn.

Table 2: Phytochemicals present from different parts of *V. negundo* Linn

Part of *V. negundo* Linn used	**Phytochemical Constituents Extracted**
Leaves [13, 14, 15, 16]	- 3′-Benzoyloxylhydroxy-3,6,7,4- tetramethoxyflavone - 6′-*p*-hydroxybenzoyl mussaenosidic acid; 2′-*p*-hydroxybenzoyl mussaenosidic acid - Artemetin - Carotene - Casticin - Friedelin - Hydroxy-3,6,7,3′,4′-pentamethoxyflavone - Stearic acid - Vitamin C - Agnuside - Negundoside - Nishindaside - Aucubin - Luteolin - Terpinen-4-ol - α-Terpinene - *p*-Cymol

	- γ-Terpinene - α-Terpinolene - Linalool - cis-Sabinene hydrate - Terpenyl-ester - α-Terpineol -β-Caryophyllene - Neophytadiene - Spathulenol
Seeds [15, 17, 18]	- 5-Oxyisophthalic acid, 3, 4- dihydroxybenzoic acid - 6-hydroxy-4-4-hydroxy-3-methoxyphenyl -3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaldehyde - *β*-Sitosterol - *n*-Tritriacontane - *n*-Hentriacontanol - *n*-Hentricontane - *n*-Pentatricontane - *n*-Nonacosane - *p*-Hydroxybenzoic acid
Roots [15]	-6-Hydroxy-4-(4-hydroxy-3-methoxy)-3-hydroxymethyl-7-methoxy-3,4-dihydro-2-naphthaledehyde - Agnuside - Negundin A - Negundin B - *R*-dalbergiphenol - Vitexoside - Vitrofolal E
Flower buds [19, 20]	- *β*-Caryophyllene - 2,3-dihydrobenzofuran - Flavones 4'-OH,5-OH - 7-di-O'glucoside -Kampferol-3-O-rutinoside5-hydroxy-3,6,7,3',4'-pentamethoxy flavones - Linalool - Naphthalene - Phenol - Phenol,2,3-Bis (1,1- dimethyl) - Viridiflorol - 4-Terpineol
Essential oil of fresh leaves, flowers, and dried fruits [15, 21]	- *α*-Cedrene - α-Guaiene - *α*-Selinene - *β*-Caryophyllene - *β*-Selinene - Ethyl-hexadecenoate; - (*E*)-Nerolidol - Guaia-3,7- dienecaryophyllene epoxide - Germacren-4-ol; caryophyllene epoxide - Germacrene D - Globulol - Hexadecanoic acid - Linalool - *p*-Cymene - Valencene - Sabinene - 4-terpineol

These several phytochemicals that are found in nature (specifically in *V. negundo* Linn) act as bioactive nutrients which may provide desirable health benefits in humans and reduce the risk of major chronic diseases. Therefore, most phytochemicals are applied in pharmacological, medicinal, and industrial fields, which may provide potential benefits in society [22].

Applications of *Vitex negundo* Linn - Medicinal and Industrial

Natural products are one of the sources of medicines in the pharmaceutical industry, and medicinal plants are one of the most notable sources of natural products. Medicinal plants are known to cure a variety of disorders and could be used to develop new medications. *V. negundo* Linn is one of the ten herbal medicines that the DOH has approved as an effective herbal medicine with clinical value [23]. The extracts from the leaves and roots of the plant are thought to have the greatest health benefits. Flavonoids, casticin, chryso-splenol, and vitexin, as well as chrysophanol D, nishindine, and hydrocotylene, are the main components of this plant. The monoterpenes agnuside, eurostoside, and aucubin are also present. These components have anti-inflammatory, antioxidant, antinociceptive, anti-ulcer, free radical scavenging, hepatoprotective, and other pharmacological properties [23]. A study also concluded that some of the chemicals extracted from *V. negundo* Linn have potential antibacterial, antifungal, and analgesic properties [24].

Phenolic compounds make up the largest class of phytochemicals and are found across the plant kingdom.

Phenols, which are the most abundant bioactive compounds in fruits, are important antioxidants. Phenolic substances, such as phenolic acids and flavonoids, are found in the flesh of fruits, whereas flavonoids and lignans are found in the seeds or kernels. Anti-aging, anti-inflammatory, antioxidant, and anti-proliferative effects are all dependent on phenolic compounds [24]. Flavonoids are secondary metabolites found in plants that have a number of beneficial properties. Flavonoids are beneficial to human health because of their antioxidant, antibacterial, antiviral, and anti-inflammatory properties, as well as the fact that they serve as free radical scavengers and potential reducing agents that protect against oxidative damage due to the presence of hydroxyl groups. Furthermore, flavonoids have been utilized in the food industry to preserve foods, add color and flavor, and create dietary supplements, among other major industrial applications [25].

In a chemical investigation of *V. negundo* Linn seed by Zheng *et al*. (2011) [18], they mentioned a phenylindene-type lignan, vitexdoin F that attenuates inflammation by the downregulation of iNOS protein level and NO production. This compound also modulates the bone formation and resorption process by increasing osteoblast proliferation and production of alkaline phosphatase as well as increasing OPG/RANKL protein. The investigation exhibited the potential anti-inflammatory and anti-osteoporotic activities *in vitro* of Vitexdoin F which can be considered as a lead compound for promising anti rheumatism agent.

Analytical Methods for Extraction of Common Phytochemicals present in *Vitex negundo* Linn

Phytochemicals or secondary bioactive metabolites found in nature are rich in most plants such as the *V. negundo* Linn, which proved to have the potential for preventing and tackling diseases. However, obtaining phytochemicals follows a procedure that simplifies the complexity of plants' chemical constituents. Among the important and several applications of *V. negundo* Linn in medicinal and industrial fields, accurate and precise analytical methods is important as this is a vital step for experimenting and studying different phytochemicals. This is done through extraction before isolation, structure identification, and bioactivity screening against different biological phenomena. Extraction of common phytochemicals is the first process in the field of natural product chemistry. Modern techniques of extraction have emerged from conventional methods to accelerate the extraction process of phytochemicals [27]. In this review, conventional and modern extraction techniques for *V. negundo* Linn are comprehensively studied.

Conventional Extraction Techniques

Following are the conventional extraction methods of common phytochemical constituents from different parts of *V. negundo* Linn: (1) Maceration, (2) Percolation, (3) Decoction, and (4) Soxhlet Extraction.

Maceration

Maceration is an extraction process from different dried parts of the plant which is placed inside a container. It involves soaking samples in the container with a solvent at room temperature without disturbing for about a minimum of 3 days. The days of soaking are intended to break and soften the cell wall of the sample to release the phytochemicals that are soluble to the solvent. After 3 days, the mixture is filtered, and heat is applied by transferring through convection and conduction. This will determine the type of compound extracted depending on the choice of solvent regardless of polarity [28]. For better extraction, there are considerations wherein the plant must be properly washed and separated from unnecessary materials (soil, rocks, etc.), preventing further contamination of phytochemicals. The part of the plant may be cut into small pieces to increase solvent extraction efficiency [29]. Big pieces of parts of a plant will make the solvent not be able to penetrate the cell wall. Further grinding of the sample would increase volatility, making the separation of active ingredients to be difficult. Any solvents can be used in this method depending on the chemical nature of the compounds contained in the sample. Different types of alcohol is the most chosen solvent as this can extract both hydrophilic and lipophilic compounds in nature due to its amphiphilic property [30].

Its advantages are that it is simpler than any other methods which uses non-complicated equipment, less energy utilization, low solubility to solvent only needs prolonged contact to it (ideally), suitable for less potent and cheap drugs. Unfortunately, the disadvantage of this method is that it has a long extraction process, and more solvent is required due to its low extraction efficiency [30].

In the study conducted by Abidin *et al.* (2014) entitled Comparative assessment of extraction methods and quantitative estimation of luteolin in the leaves of *Vitex negundo* Linn. by HPLC, luteolin was extracted with the use of maceration from the leaves of the sample utilizing different solvents (methanol, ethanol, chloroform, and dichloromethane [DCM]) [13]. For the study of Patil and Laddha [31], the leaves of *V. negundo* Linn were macerated using a solvent system of water and acetonitrile, extracting agnuside. Moreover, the study of Nagarsekar *et al*. (2016) [16] entitled Antioxidant and Antilipid Peroxidation Potential of Supercritical Fluid Extract and Ethanol Extract of Leaves of *Vitex negundo* Linn. extracted negundoside utilizing the solvent ethanol from the leaves of the sample. In the study "*In vitro* Antioxidant and Cytotoxic Effects of Methanol Extracts of *Vitex negundo*, *Lantana camara*, *Bauhinia variegata* and *Bauhinia racemosa* on Human Cancer Cell lines" by Badguhar *et al*. [32], *V. negundo* Linn leave extracts from cold maceration were phenols and flavonoids utilizing the solvent methanol.

Percolation

Percolation is a novel extraction technique established for extracting active ingredients in the preparation of tinctures and fluid extracts through complete removal of all soluble constituents from comminuted plant material through the extraction of crude drug with the use of a fresh solvent [36]. As for this procedure's method, glassware with a narrow, cone-shaped vessel open at both ends, that is a tapered glass or metal container with a stopcock at the base which controls solvent elution's rate, is generally used. This glassware is known to be the percolator [36]. A percolator has a top opening with a circular drilled lip which allows the liquid to pass and to subject the materials to slight pressure. Percolation can be performed as a batch process in two different ways through different types of percolators namely the large and small scales [37]. Such solid ingredients are moistened with the use of a sufficient amount of solvent, allowing it to stand for approximately 4 hours in a well-closed container, after which the mass is packed, and the top of the percolator is closed. The solvent will then be added for the formation of a shallow layer above the mass allowing the mixture to macerate in a closed percolator for 24 h. The percolator's opening will then

be opened for the liquid to drip slowly. Another additional solvent will be added as required, up until the percolate measures about half of the required volume of the finished product. The collected extract is then pressed, and the liquid is added to the percolate. Another sufficient amount of solvent is added to the process to produce the desired volume. The mixed liquid is processed through filtration or by decanting. The whole concept of adding sufficient amounts of solvent is repeated until a solvent is dropped from the percolator when evaporated and does not leave a residue [30].

Percolation is an efficient extraction method due to its special attributes which are: (1) less time consuming, (2) does not require much manipulation, (3) can use either a hot or cold solvent. [33]. In contrast to these attributes, this method also has its drawbacks including the requirement of a large amount of solvent and special attention on the particle size of material and throughout the process [29].

In the study conducted by Nagarsekar *et al*. (2011) entitled "Antioxidant and Antilipid Peroxidation Potential of Supercritical Fluid Extract and Ethanol Extract of Leaves of *Vitex negundo* Linn", it shows that through the use of conventional extraction by percolation, the presence of flavonoid was detected through TLC separation of ethanol extract of *V. negundo* Linn [16].

Decoction

Decoction is a process that utilizes boiling of plant material, dissolving chemicals present from it. It is a method that is suitable for extracting constituents soluble in water and is not vulnerable to the effects of heat. It is primarily a water-based preparation prior to extraction of bioactive compounds from plants. It is typically used only for tough and fibrous plants such as barks and roots, and plants with water-soluble chemicals [29].

In the process of decoction, the plant sample is ground into small pieces, which undergo different methods. Commonly, the Ayurvedic method is used wherein small pieces of the sample are placed in earthen pots or tinned copper vessels with clay on its exterior part. Water is then added to the pot which is heated. Afterward, the mixture is boiled on a mild flame until it is reduced from its starting volume. After boiling, extracts obtained are cooled and filtered [33]. Its advantage is that it is suitable for extracting thermolabile compounds/heat-stable compounds, cheap, and easy. Unfortunately, heat-sensitive constituents or phytochemicals are not advised in this kind of extraction method [34].

Unfortunately, there were no studies yet that conducted decoction on different parts of *V. negundo* Linn for phytochemical analysis. However, it was found that performing decoction on different parts of *V. negundo* Linn has potential pharmacological activities. Decoction of roots is known to cure respiratory disorders, wounds and ulcers, and malaria [35]. For the stem and bark, it was known to cure burns and have anti-cancer properties [35]. For the leaf, it is known to treat eye diseases, tooth disease, also wounds and ulcers, and respiratory diseases [36].

Soxhlet Extraction

A Soxhlet extractor is an apparatus designed to extract lipid from a solid material. Typically, it is only required when the desired compound has limited solubility in a solvent, and the impurity is not soluble in the solvent. This method was employed for the dried and pulverized parts using a sequential method with solvents in increasing polarity, such as hexane, dichloromethane, chloroform, ethyl acetate, and methanol. Under reduced pressure, the residue is obtained through evaporation or removal of solvent [37].

An advantage of this extraction method is that only one batch of solvent is recycled instead of many portions of warm solvent being passed through the sample. Compared to the general soaking method, Soxhlet extraction has the advantages of high efficiency, small solvent dosage, and complete extraction. However, a further disadvantage is its requirement of a concentration or evaporation step. Also, a high degree of contamination is possibly introduced into the sample with solvent impurities. This method cannot be used for thermolabile compounds since prolonged heat may result in the degradation of compounds [38].

In the study by Abidin *et al*. (2014) entitled, Comparative assessment of extraction methods and quantitative estimation of luteolin in the leaves of *Vitex negundo* Linn. by HPLC, luteolin was extracted utilizing Soxhlet extraction from the leaves of *V. negundo* Linn by using methanol, ethanol, dichloromethane, and chloroform [13]. The quantity of luteolin was determined through High-Performance Liquid Chromatography. Moreover, a drug ratio of 10:1 (mg/L) using methanol and solvent was used to extract agnuside from *V. negundo* Linn leaves [31]. The methanolic leaf extracts on the study by Khonde (2020) were also studied by taking 30 grams of air-dried powder and extracting 300 mL of methanol using the Soxhlet apparatus [39].

Modern Analytical Techniques

Knowing the conventional analytical techniques used in extracting phytochemicals from *V. negundo* Linn, modern analytical techniques have now emerged in the present. Following are the modern extraction methods of common phytochemical constituents from different parts of *V. negundo* Linn: (1) Supercritical Fluid Extraction, (2) Pressurized Liquid Extraction, (3) Microwave and Ultrasound-Assisted Extraction, (4) Ionic Liquids, (5) Enzyme-Assisted Extraction, and (6) Hydrodistillation and Steam Distillation.

Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) is a modern technique that is popular for green extraction techniques that shows various advantages compared with traditional extraction processes. It is a process of separating the extractant from the matrix of the sample of interest by utilizing supercritical fluids as the extracting solvent. Extracting solvents are utilized at varying temperatures and pressures above their critical points. Critical points are described by a critical pressure and temperature, which is a property of a substance that when reached, variations are induced which effectively modify the properties of the compounds [41].

The extracting solvents used in this method are carbon dioxide (CO_2), water (H_2O), methanol, ethylene, ethane, n-butene, and n-pentane. Commonly, supercritical fluids have mixed properties between liquids and gases, having the viscosity to be similar to gas and density is close to liquids. These liquids are appropriate in this kind of method due to their appropriate temperature and pressure conditions. Primarily, CO_2 is the most applied in this kind of method due to its accessible and safe requirements. However, its polarity is very low, so extracting polar compounds is limited. Addressing this problem is increasing its polarity by adding ethanol or methanol as modifiers [41], and optimizing the pressure and temperature conditions and solvent-to-feed ratio. Optimizing the pressure and temperature conditions will enhance the solubility of sample components, and the solvent-

to-feed ratio optimization will maximize the extraction yield from the sample.

The advantage of employing an optimization process over other methods is that it can be used for green extraction techniques, inorganic solvents can be used which makes the extract to be very pure and extracted lipids can be used immediately [42]. Moreover, it has the ability to maintain the quality of the final product as this is only used at low operating temperatures for about 40 to 80 ^{O}C, has low viscosity and high diffusivity, and is free from contamination of heavy metals and inorganic salts. Aside from its advantages, some drawbacks were observed in this method as it is very expensive and complex due to its high pressures, a little to no polar substances were extracted, and it has high electricity consumption [42].

Likewise, SFE was used as a method for *V. negundo Linn* leaves to assess its antimicrobial activity, and its antioxidant and anti-lipid peroxidation potential [16, 42]. In the study entitled Evaluation of Composition and Antimicrobial Activity of Supercritical Fluid Extract of Leaves of *Vitex negundo* by the group of Nagarsekar *et al*. (2010), leaves of *V. negundo* Linn were collected and undergone SFE. SFE was carried out in a high-pressure apparatus that has a 51 V extractor vessel, wherein samples will be put. The extractant used was CO_2 kept at an appropriate temperature and pressure. wherein the SFE extracts obtained were α-terpinene, *p*-cymol, γ-terpinene, α-terpinolene, linalool, *cis*-sabinene hydrate, terpenyl-ester, α-terpineol, β-caryophyllene, neophytadiene, and spathulenol. Primarily, the major phytochemical present was terpinen-4-ol. The same compounds obtained from the study of Nagarsekar *et al*. (2010) were same in his study in 2011 entitled Antioxidant and Antilipid Peroxidation Potential of Supercritical Fluid Extract and Ethanol Extract of Leaves of Vitex negundo Linn.

Pressurized Liquid Extraction

Pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE), is a method of isolation wherein it uses organic solvents as an extractant at elevated pressure at a conventional temperature. This method helps to increase the solubility of the analytes and improve the desorption from the matrix as it maintains the solvent in a liquid state [43]. The sample is put in an extraction cell with an organic solvent at an elevated temperature and pressure. Generally, the sample is dispersed in an inert material such as a drying agent and placed in the mixed sample present in an extraction vessel. This introduces the solvent into the vessel then followed by heating and a no-flow extraction step (static extraction step). After 0.5 to 20 minutes, sample flow starts (dynamic extraction step) and will be collected in a sample vial [44, 45].

The method's advantages over different methods are fast extraction time and lower solvent consumption, focused on varying nonpolar compounds (some are polar analytes depending on the solvent used), and it is mainly used for plants. This also has a very high yield of extraction due to modifications in temperature (200 ^{o}C) and pressure (4 - 200 atm), as it helps to enhance solubility and lower the viscosity and surface tension of the solvents. However, it has been said that most extracts are very dirty and require further purifications. This method is mostly partnered with SLE as a cleanup method [46]. Unfortunately, there are no studies that used PLE for extracting bioactive compounds from *V. negundo* Linn. Instead, PLE can be used as a reference for extracting bioactive compounds with low polarity from *V. negundo* as this method usually uses organic compounds such as hexane and toluene [46, 47]. This is because polar solvents (such as ethanol and water) are considered more viscous than nonpolar solvents, which can be difficult to permeate through the sample even at modified temperature and pressures [44, 45].

Microwave and Ultrasound-Assisted Extraction

Microwave-Assisted Extraction (MAE) is a novel automated green extraction technique established for the extraction of bioactive compounds from medicinal plants through microwave energy [48]. These are electromagnetic fields with a frequency ranging from 300 MHz to 300 GHz, consisting of two oscillating perpendicular fields namely magnetic and electric fields [48]. Fundamentally, MAE necessitates the utilization of microwave radiation to heat the extraction solvent which improves the solvent dispersion to the sample and advancing the compounds' partitioning, from solid to the solvent or liquid to the solvent [49]. The technique's mechanism revolves around these three steps: (1) temperature and pressure are increased to separate the solute from the active sites of the sample matrix; (2) solute will then be released to the solvent from the sample matrix; (3) and lastly, the solvent will then be diffused in the sample matrix [50]. As for the specific methods, an aqueous solution of the Ionic Liquids (hydrophilic) is added to the solid sample. The mixture is then placed in a microwave oven for a certain period of time procedurally shorter compared to other conventional liquid-phase extraction methods. Finally, the solid is thrown out and the IL aqueous solution with the extracted compounds is set for analysis [49].

MAE's efficiency depends on numerous factors, including the components being extracted, the properties of the solvent used, and the sample itself. As for the optimization, the parameters to alter are the solvent's polarity and volume, temperature and time, the sample size, and the microwave power [48]. Moreover, this extraction method offers several advantageous factors such as; (1) its ability to reduce extraction time and solvent consumption, (2) simultaneous extraction of multiple samples (up to 40), thus improving the sample throughput. With these factors, MAE adheres to the required criteria for modern sample preparation techniques and serves as an efficient alternative for conventional approaches for extraction matters specifically for organic and organometallic compounds. On the other hand, MAE's low selectivity, the impending reaction in high temperature, and demand for special equipment are recognized as its drawbacks [51].

Ultrasound-Assisted Extraction (UAE) is a technique with an operational procedure similar to MAE, with essential applications in different fields such as chemistry, pharmaceutical, cosmetic and alimentary fields [52]. The ultrasound itself contains a mechanical effect that utilizes shock waves and hydrodynamic force toward the solid surface, which results in an increase in infiltration of solvent towards the biological organization. It uses a frequency of 20 kHz in generating local hotspots at a macroscopic scale together with high shear stress and temperature through the production of cavitational bubbles. When the bubbles burst and are then dispersed across the surface of the sample matrix, shockwave-induced damage to the plant cell wall improves transferring of mass of phenolic compounds to the cellular membranes into solution [53]. Comparable to MAE, UAE also shortens the extraction time, reduces solvent consumption and temperature, and lowers the energy input. It is also an efficient alternative to conventional techniques for it

can easily be coupled with other extraction techniques. Due to UAE's ability to function under room temperature, prevention of oxidation and decomposition of target natural products occur [54].

As for procedures, in UAE, the sample and solvent with a ratio of 20:1 (50% ethanol in water and a solvent (mL) to biomass (g) ratio) are enclosed in a flask placed under a temperature-controlled ultrasonic bath and was sonicated for 10 to 60 minutes at 40 kHz at temperatures below 100°C. Subsequent to this, filtration and extraction are done to separate the extract from the plant residue. Optimization of UAE extraction protocol normally refers to factors including solvent to biomass ratio, the solvent itself, and temperature [53].

In a study conducted by Patil and Laddha (2018) entitled Extraction Efficiency of Agnuside from V. negundo Leaves Using Different Techniques and its Quantitative Determination by HPLC, UAE was carried out for 20 minutes using 1g of leaf powder soaked in ethanol with solvent: with a ratio of 10:1 (mL/g) under ambient temperature in a sonicator. It was done for various extraction times 10, 20, 30, 40, and 50 min. Further processing of the extracts was done in the similar manner discussed above. On the other hand, MAE was carried out at 350 W for 10 minutes with a solvent: drug ratio of 10:1. It was done for various extraction times 1, 5, 10, 20, and 30 min. As a result, MAE and UAE showed the highest extraction efficiency for the phytochemical agnuside (a type of iridoid/monoterpenoid) present in *V. negundo* Linn leaves. Other than agnuside, negundoside, nishindaside, and aucubin were extracted also [31].

Ionic Liquids

Ionic liquids (ILs) are known to be an environmentally benign reaction media for extraction and separation of bioactive compounds from diverse origins due to their nonflammability, thermal stability, and controlled miscibility properties. Due to this, ILs are now used as soluble support in immobilizing reagents and catalysts resulting in considering it as a green alternative for conventional synthetic methods [55]. ILs typically consist of unsymmetrical organic cations and organic or inorganic anions with a melting point of below 100°C which acts as diluents and/or extractants for the solvent extraction. However, the usage of ILs has a disadvantage of being toxic when released to soil or water-courses. Recently, ILs are acknowledged for its ability to dissolve biomass, extract, and purify natural-derived compounds. Moreover, it can also dissolve a wide range of biomass matrices resulting in easier access to the compounds being studied. IL's aqueous solution also improves solvation performance due to its hydrotropic nature allowing an advanced extraction [43].

The solubility data of compounds is one of the most essential factors needed to be considered to be able to perform a successful extraction and separation process. Due to natural compound's low solubility in water, solvents including alcohols, chloroalkanes, alkanes, and ethers are mostly used. However, considering the target substance to be extracted, a certain solvent should be selected if the solubility data of the target compound is known. On the other hand, when such information is not available, one should follow the principle "like dissolves like" wherein the solvent used should be with the same polarity to the compound of interest. This similarity suggests that ILs are good candidates for VOC substituents in dissolving natural products of different polarities [56].

Some unique properties of this solvent extraction are low vapor pressure, wide liquid range, high thermal stability, and low combustibility which makes it a good alternative for volatile organic compounds in solvent extraction [57]. On the other hand, densities of ILs are reported to vary between 1 and 1.6 g cm^3 and results to be the least sensitive physicochemical property to variations in temperature and impurity content. A high viscosity of solvent is undesirable for extraction methods for it inhibits mass transfer of the solute of interest. Reports show that ILs have a higher viscosity, ranging from 10 to 500 mPas at room temperature compared to other molecular solvents mentioned thus, making this factor considered as a drawback [58].

In a study conducted by Xiao *et al*. (2018), entitled *Ionic* Liquid Solutions as a Green Tool for the Extraction and Isolation of Natural Products, results show that ILs could be a better fit for the extraction of special structures. Due to its unique properties, ILs show advantages in disrupting cell walls and selecting targets through chemical selectivity in order to extract and efficiently improve products within just a short period of time hence, resulting to the isolation of 236 natural products from plants including all the typical and important bioactive types of NPs were included, such as flavonoids (compounds 1–65), alkaloids (compounds 66–129), terpenoids (compounds 130–187), phenylpropanoids (compounds 189–215), quinones (compounds 216–233), and others (compounds 234–236) [59].

Enzyme-Assisted Extraction

The high demand for novel phytochemicals has intensified the development of plant-derived compounds, or bioactive compounds. Enzymes have the ability to disrupt or degrade membranes and cell walls resulting in better release and more efficient extraction of bioactive compounds [60]. This method has been shown to achieve high yield of extraction for compounds which include oil, natural pigments, polysaccharides, and medicinal compounds. In comparison to non-enzymatic methods, enzyme-assisted extraction has also been shown to have higher recovery, reduced usage of solvent, faster extraction, and lower consumption of energy [61].

Although all parts of *Vitex* species are used as a medicament in different indigenous systems of medicine, its leaves are the most potent for medicinal use and studies. Heeman *et al*. (2019) studied the bioactive compounds from *V. negundo* Linn leaves as an alternative green technology through enzyme-assisted extraction [62]. The commercial blend of arabanase, beta-glucanase, hemicellulase, xylanase, and cellulase, corresponded to 1µmol glucose per minute at pH 5.0 and 30 °C in 30 minutes.

V. negundo Linn contains polyphenolic compounds, which possess numerous bioactive properties [63]. The efficiency of extracting polyphenols from *V. negundo* Linn leaves is influenced by pH, time, temperature, agitation, particle size, and solvents. The conventional method of extracting polyphenols uses solvents, such as methanol or derived aqueous solutions. However, the usage of enzyme-assisted extraction to obtain the polyphenols increases the nutritional value of substances [61].

Cellulases, proteases, pectinases, and xylanases are enzymes that act in degrading the plant cell walls which improve the extraction of polyphenols from *V. negundo* Linn leaves. The cellulases degrade the cellulosic compounds through catalyzing the breakdown of ß (1 → 4) glycosidic bonds that bind the necessary glucose molecules for the formation of cellulose. The aforementioned enzymes are produced by

various microorganisms, which include bacteria, actinomycetes, and fungi [64].

Enzyme-assisted extraction is a potential alternative to the conventional solvent-based methods of extraction, as the enzymes are ideal catalysts for assisting in the extraction or synthesis of complex bioactive compounds. Moreover, this method is based on the inherent capability of enzymes to catalyze reactions with exquisite regioselectivity, specificity, and ability to function under mild processing conditions in aqueous solutions [65]. It also offers the possibility of greener chemistry or sustainable chemistry. Green chemistry involves the reduction or elimination of the use of hazardous substances in generating intermediates of products [66]. Although enzyme-assisted extraction has the advantage of being a green alternative to conventional methods [67], it is not always feasible on an industrial scale as enzymes are relatively expensive for large industrial production, and their behavior is rigidly limited by environmental conditions [61].

Hydrodistillation and Steam Distillation

Essential oils are plant-based volatile oils that have strong aromatic components obtained through mechanical pressing or distillation [68]. These could be biosynthesized in different plant organs as secondary metabolites, and be used in perfumery, aromatherapy, medicine, cosmetics, and incense. Essential oils are complex mixtures of volatile compounds, such as terpenes, alcohols, and phenolics. Some of the terpenes are potent drugs against diseases and cancers [69].

V. negundo Linn is used in traditional medicine as a cure for vermifuge and arthritis. Khokra *et al*. (2008) reported pesticidal, antibacterial, and antifungal properties on the extracted essential oils from the leaves of *V. negundo* Linn via hydrodistillation [70]. Hydrodistillation is the traditional method of extracting essential oils, making it one of the oldest and easiest techniques. This type of extraction is based on isotropic distillation. The setup consists of a condenser and a decanter for the collection of condensate and the separation of essential oils from water. The process is multilateral that can be utilized by small and large industries. However, only a small amount of essential oil is produced when distillation is prolonged [69]. Khokra *et al*. (2008) isolated the essential oils from *V. negundo* Linn leaves by subjecting the leaves to hydrodistillation separately at 8 hours using a closed-type of Clevenger apparatus. In cases of leaves oil extraction, the leaves were treated with 10% KOH for 3 hours then washed twice with fresh water prior to hydrodistillation [70].

A study by Kaul *et al*. (2011) was also able to identify the major compounds from the hydrodistilled 0.23% essential oil of *V. negundo* Linn flowers in a Clevenger-type apparatus for five hours. Analysis of gas chromatography-mass spectrometry (GC/MS) was carried out, and the main constituents of the oil were sabinene, linalool, 4-terpineol, *β*-caryophyllene, α-guaiene, and globulol [21].

Steam distillation is another type of distillation for plants that are sensitive to temperature, such as natural aromatic compounds. It is the most widely used method for extracting essential oils near 100 °C. It is subsequently followed by the condensation to form an immiscible liquid from which the essential oil can be separated in a clarifier [69]. The principle of this technique is that the combined vapor pressure equates to the ambient temperature near the normal boiling point so that the volatile components with higher boiling points from 150 to 300 °C can be evaporated. This method allows the control of temperature and the amount of steam to be applied to the plant material. However, appropriate selection of distillation technique plays a vital role in determining the yield and quality of end products [71].

A study by Marathe *et al*. (2010) showed that the essential oils present in the leaves of *V. negundo* Linn have antibacterial activities responsible for bactericidal effects [72]. The chemical composition of present essential oils and antimicrobial properties of the essential oils from *V. negundo* Linn leaves were analyzed by GC/MS. The essential oils were extracted from air-dried leaves through steam distillation to yield 1.6% v/w essential oils using Clevenger apparatus. Then, it was dried over anhydrous sodium sulfate, and analyzed via GC/MS. The analysis of Marathe *et al*. (2019) identified 10 compounds of which 5 were monoterpenes, 3 were sesquiterpenes, and 2 were fatty acids. Volatile oil from the flowering twigs of *V. negundo* Linn from India was also determined by GC/MS, where it indicated the presence of 94 compounds. The main compounds identified were viridiflorol, *β*-caryophyllene, 4-terpineol, and linalool. The oil from the flowering twigs was shown to have a higher amount of viridiflorol than from the leaves [71]. Thus, it can be a better source for natural viridiflorol [72].

In utilizing this type of distillation, major conditions are observed. These conditions include (1) extraction of temperature-sensitive compounds, (2) chemically non-reactive substances with water, and (3) immiscible material to be extracted [69]. Since the steam can remain at the boiling point of water, it requires less fuel for the steam boiler. In addition to that, steam distillation works well for immiscible substances. Thus, it works as a cost-effective method to invest in extracting a diverse array of immiscible substances. However, it has the disadvantage of having a higher initial cost for investing in equipment and necessary extra care to work with heat sources, pressurized containers, high temperatures, and volatile oils [73].

Summary of Methods for Extraction of *Vitex negundo* Linn and Phytochemicals Extracted

Table 3: The Comparison of Each Method (Conventional and Modern) used for Extraction

Method	Advantage	Disadvantage	Environmental Conditions
Maceration [28, 29]	- Simple - Less energy utilization - Cheap - Polar and non-polar solvents	- Long term extraction - Low extraction efficiency (needs more solvent)	- Room temperature - Subjects to a warm bath (37 ^{o}C)
Percolation [29]	- Short time and more complete extraction - Requires less time than maceration. - Suitable method for potent and costly drugs	- More solvent is required. - Special attention should be paid on particle size of material and throughout the process.	- Room temperature, occasionally under heat
Decoction [29]	- Suitable for extracting thermolabile compounds - Cheap - Effortless extraction - mostly polar due to the usage of water as a solvent	- Heat-sensitive phytochemicals are not advised in this kind of extraction method (volatile)	- Room temperature - Subjects to boiling (depends on the solvent used)

Soxhlet Extraction [38]	- Only one batch of solvent is recycled - High efficiency - Small solvent usage - Complete extraction	- Requires concentration or evaporation step - High degree of contamination is possibly introduced into the sample with solvent impurities - Cannot be used for thermolabile compounds	- Room temperature - Under heat, depending on solvent used
SFE [41]	- Used for green extraction techniques - Utilization of inorganic solvents - Maintains the quality of the final product - Low viscosity - High diffusivity - Prevents contamination from heavy metals and inorganic salts	- Expensive - Complicated process due to optimization of environmental conditions - Little to no polar substances can be extracted - High electricity consumption	- Optimized temperature (40 ºC to 80 ºC) and pressure (approximately 73 atm; extraction requirement is approximately 349 atm)
PLE [46]	- used for green extraction techniques - Fast extraction - Lower solvent consumption - Extracts mostly nonpolar compounds - High yield of extraction - Enhances solubility - Lowers viscosity and surface tension of solvents	- Contaminated extracts - Requires further clean-up method or purifications	- Optimized temperature (200 ºC) and pressure (approximately 40 to 200 atm)
MAE/UAE [51]	- Ability to reduce extraction time and solvent consumption - Simultaneous extraction of multiple samples (up to 40). - Lowers the energy input	- Low selectivity - Impending reaction in high temperature - Demand for special equipment	- Room temperature to less than 100ºC
ILs [53,57]	- Ability to dissolve biomass - Improves solvation performance - Low vapor pressure - Wide liquid range - High thermal stability - Low combustibility	- High viscosity - Toxicity, could become pollutants and pose environmental risks	- Room temperature
Enzyme-Assisted Extraction [72, 69, 61]	- Better release and more efficient extraction of bioactive compounds -Higher recovery -Reduced usage of solvent -Faster extraction -Lower consumption of energy -Green alternative to conventional methods	- Not always feasible - Expensive for large industrial productions - Behavior is limited by environmental conditions	- Room temperature, or heated after enzyme treatment
Hydrodistillation/ Steam Distillation [71, 74, 69]	- One of the easiest techniques - Allows the control of temperature and the amount of steam to be applied to the plant material - Multilateral, can be utilized by small and large industries - Cost-effective	- Only a small amount of essential oil is produced when distillation is prolonged - Higher initial cost - Necessary extra care	- Room temperature

Table 4: Phytochemicals Extracted from *V. negundo* Linn using Different Extraction Methods

Method Used	Source (Part of *V. negundo*)	Solvent/s	Phytochemical Constituent/s Extracted
Maceration [13]	Leaves	- Methanol - Ethanol - Chloroform - DCM	- Luteolin
Maceration [31]	Leaves	- Water and acetonitrile (solvent system)	- Agnuside
Maceration [42]	Leaves	- Ethanol	- Negundoside
Maceration [32]	Leaves	- Methanol	- Phenols (constituents not stated) - Flavonoids (constituents not stated)
Percolation [42]	Leaves	- Ethanol	- Flavonoids (constituents not stated)
Soxhlet Extraction [13]	Leaves	- Methanol - Ethanol - Chloroform - DCM	- Luteolin
Soxhlet Extraction [31]	Leaves	- Methanol	- Agnuside
Soxhlet Extraction [39]	Leaves	- Methanol	- Methanolic leaf extract (constituents not stated)
SFE [42]	Leaves	- Carbon dioxide	- Terpinen-4-ol - α-Terpinene - *p*-Cymol - γ-Terpinene - α-Terpinolene - Linalool - cis-Sabinene hydrate

			- Terpenyl-ester - α-Terpineol -β-Caryophyllene - Neophytadiene - Spathulenol
MAE/UAE [31]	Leaves	- Ethanol	- Aucubin - Agnuside - Negundoside - Nishindaside
ILs [59]	Leaves	- Water	- Alkaloids - Flavonoids -Phenylpropanoids - Quinones - Terpenoids
Enzyme-Assisted Extraction [63]	Leaves	- Water	- Polyphenols (constituents not stated)
Hydrodistillation [70]	Leaves	- Potassium hydroxide	- Essential oils
Hydrodistillation [21]	Flowers	- Water	- α-Guaiene - *β*-caryophyllene - Sabinene - Globulol - Linalool - 4-Terpineol
Steam Distillation [72]	Leaves	- Water	- Monoterpenes - Sesquiterpenes - Fatty acids
Steam Distillation [73]	Flowering twigs	- Water	- *β*-caryophyllene - Linalool - Viridiflorol - 4-Terpineol

Conclusion and Recommendation

It can be said that the most important step in experimenting with natural products is the extraction of target compounds, as failure in extraction causes lower yields of bioactive compounds or contamination. This review focused on different classical and modern methods used for the extraction of bioactive compounds from *V. negundo* Linn and discussed its convenience focusing on the said plant species. It can prove that plants like *V. negundo* Linn are rich in phytochemicals regardless of what extraction method is used. Moreover, the focus of the study is the selectivity of methods used for *V. negundo* Linn, which regards efficiency and convenience explained by its advantages and disadvantages, and environmental conditions.

For the common methods used for extraction of phytochemicals from *V. negundo* Linn, only decoction (conventional method) and PLE (modern method) were still not used for the phytochemical analysis of compounds. The conventional methods used were maceration, percolation, and Soxhlet extraction, while the modern methods used were SFE, UAE/MAE, ILs, enzyme-assisted extraction, and hydro distillation and steam distillation. Commonly, the leaves, flower, and flowering twigs of *V. negundo* are primarily used for different kinds of extraction. Leaves are the most common among the three stated parts of the plant which proves to have the greatest amount of phytochemicals extracted, regardless of extraction method used. Moreover, different extraction techniques employed a high yield and selectivity of what they wanted to study on.

Conventional methods, such as maceration, are proved to have low extraction efficiency rather than percolation and Soxhlet extraction, wherein Soxhlet extraction exhibits high efficiency of extraction. Both maceration and percolation are more convenient as both methods use less complex procedures than Soxhlet extraction. Furthermore, for environmental conditions, all methods are conducted under room temperature yet need further application of heat for a better extraction yield efficiency. However, it can be said that all procedures can be convenient which can depend on the solvent used, fewer errors, and optimization of equipment for better extraction results.

For the modern methods, all exhibit various advantages and disadvantages with one another. For SFE, it exhibits a high efficiency extraction yield for nonpolar substances as it utilizes primarily the inorganic solvents. Yet, it is not that convenient as it is expensive, uses high energy consumption, and optimizes temperatures and pressures above the critical points of solvents and compounds of interest. For MAE/UAE, it is used for fast and simultaneous extraction using low solvent consumption and lower energy, making it time-efficient for the results, but it is only selective for certain compounds. It can be also done at room temperature but demands for a special equipment which makes it somehow, not convenient for the researchers to easily extract the compound of interest. Next are the use of ILs, wherein it makes it convenient as it is done in room temperature and has a high efficiency due to improving of solvation performance, but yielded extracts were expected to be contaminated. For the enzyme-assisted extraction method, it also utilizes room temperature, or heated after enzyme treatment for faster extraction which makes the result to have a high recovery of bioactive compounds. It is yet expensive and the behavior of the sample must match with the environmental conditions of the enzyme, which makes it less convenient. Lastly, the hydrodistillation or steam distillation method is one of the easiest techniques and cost-effective which make it convenient for research study purposes. It just requires attention because yield of extracts is expected to be low when distilling is prolonged.

In conclusion, it is difficult to determine a standardized method for *V. negundo* Linn, as most studies that the researchers gathered employed a high yield, efficiency, and selectivity of what they wanted to focus on. This can be proved that each extraction procedure is unique depending on the sample of interest that a researcher wants to study. It can be suggested that extraction methods must be consistent with

the objectives, samples, and target compounds. As for the extraction technique to be efficient, different methods must incorporate optimal conditions such as the environment they work on, and the solvents that they use. Optimization is very important for the variety of applications in extracting bioactive compounds from *V. negundo* Linn, in such that to achieve a minimum level of impurities and an acceptable degree of reproducibility and repeatability of bioactive compounds of interest.

References

1. Bergquist J, Turner C. Analytical chemistry for a sustainable society – trends and implications. Analytical and Bioanalytical Chemistry 2018;410(14):3235-3237.
2. Egbuna C, Ifemeje JC, Kryeziu TL, Mukherjee M, Shah H, Rao GMN, *et al*. Phytochemistry. Edn 1, Apple Academic Press/CRC Press, Taylor & Francis, Anambra State 2018;I:1-29.
3. Feng W, Li M, Hao Z, Zhang J. Analytical Methods of Isolation and Identification. Phytochemicals in Human Health 2019, 1-27.
4. Alegado-Bagaoisan DM, Castro MC, Purificacion J. A Systematic Review on Vitex negundo (NIRPROMP formulations) for the Treatment of Acute Cough of Mild to Moderate Severity in Pediatric Patients. Acta Medica Philippina 2020;54(1):36-43.
5. Asthma. https://www.who.int/news-room/fact-sheets/detail/asthma. 3 May 2021.
6. Olivar JE, Alaba JPE, Atienza JF, Tan JJ, Umali M, Alejandro GJ. Establishment of a standard reference material (SRM) herbal DNA barcode library of *Vitex negundo L.* (lagundi) for quality control measures. Food additives & contaminants: Part A 2016;33(5):741-748.
7. Gill BS, Mehra R, Navgeet, and Kumar S. *Vitex negundo* and its medicinal value. Molecular Biology Reports 2018;45(6):2925–2934.
8. Uses of *Vitex negundo* L. https://nif.org.in/VITEX-NEGUNDO-L 2020.
9. Bano U, Jabeen A, Ahmed A, Siddiqui M. Therapeutic Uses of *Vitex negundo.* World Journal of Pharmaceutical Research 2015;4(12):589-606.
10. Zheng C, Qin L. Nuts and Seeds in Health and Disease Prevention. Academic Press, Shanghai 2011, 479–486.
11. Koirala N, Dhakal C, Munankarmi NN, Ali SW, Hameed A, Martins N, *et al. Vitex negundo* Linn.: phytochemical composition, nutritional analysis, and antioxidant and antimicrobial activity. Cellular and Molecular Biology 2020;66(4):1-7.
12. Zheng C, Li H, Ren S, Xu C, Rahman K, Qin L, *et al*. Phytochemical and Pharmacological Profile of *Vitex negundo.* Phytotherapy Research 2015;29(5):633-647.
13. Abidin L, Mujeeb M, Mir SR, Khan SA, Ahmad A. Comparative assessment of extraction methods and quantitative estimation of luteolin in the leaves of *Vitex negundo* Linn. by HPLC. Asian Pacific Journal of Tropical Medicine 2014;7(1):S289-S293.
14. Roy S, Bairwa K, Grover J, Srivastava A, Jachak S. Analysis of Flavonoids and Iridoids in *Vitex negundo* by HPLC-PDA and Method Validation. Natural Product Communications 2013;8(9):1241-1244.
15. Suganti N, Dubey S. Phytochemical constituents and pharmacological activities of *Vitex negundo* Linn. Journal of Chemical and Pharmaceutical Research 2016;8(2):800-807.
16. Nagarsekar KS, Nagarsenker MS, Kulkarni SR. Antioxidant and Antilipid Peroxidation Potential of Supercritical Fluid Extract and Ethanol Extract of Leaves of *Vitex negundo* Linn. Indian Journal of Pharmaceutical Sciences 2011;73(4):422-9.
17. Bansod M, Harle U. *Vitex negundo* L: Phytochemical Constituents, Traditional Uses and Pharmacological Properties. Pharmacologyonline 2009;1:286-302.
18. Zheng C, Zhang X, Han T, Jiang Y, Tang J, Brömme D, *et al*. Anti-inflammatory and anti-osteoporotic lignans from *Vitex negundo* seeds. Fitoterapia 2014;93:31-38.
19. Singh V, Dayal R, Bartley J. Chemical Constituents of the Volatile Oil from *Vitex negundo* L. flowering twigs. Indian Perfumer 2000;44(22):41-47.
20. Keerti G, Padma K. Evaluation of Phytochemical and Antimicrobial study of Extracts of *Vitex negundo* Linn. International Journal of Drug Development and Research 2012;4(4):192-199.
21. Kaul P, Rao B, Bhattacharya A, Singh K, Syamasundar K, Ramesh S. Essential Oil Composition of *Vitex negundo* L. Flowers. Journal of Essential Oil Research 2011;17(5):483-484.
22. Jimenez-Garcia S, Vazquez-Cruz M, Garcia-Mier L, Contreras-Medina L, Guevara-González R, Garcia-Trejo J. *et al*. Therapeutic Foods. Academic Press, Guanajuato 2018, 397-427.
23. Rana S, Rana K. Review on Medicinal Usefulness of *Vitex negundo* Linn. Open Access Library Journal 2014;1(3):1-13.
24. Pawar S, Kamble V. Phytochemical Screening, Elemental and Function Group Analysis of *Vitex negundo* L. leaves. International Journal of Pharmacy and Pharmaceutical Sciences 2017;9(6):226-230.
25. [Rasul MG. Conventional Extraction Methods Use in Medicinal Plants, their Advantages and Disadvantages. International Journal of Basic Sciences and Applied Computing 2018;2(6):10-14.
26. Ruiz-Cruz S, Chaparro-Hernández S, Hernández-Ruiz K, Cira-Chávez L, Estrada-Alvarado M, Ortega L, *et al*. Flavonoids – From Biosynthesis to Human Health. Intech Open, Sonora 2017, 354-369.
27. Feng W, Li M, Hao Z, Zhang J. Analytical Methods of Isolation and Identification. Phytochemicals in Human Health 2019, 1-27.
28. Abubakar A, Haque M. Preparation of Medicinal Plants: Basic Extraction and Fractionation Procedures for Experimental Purposes. Journal of Pharmacy & Bioallied Sciences 2020;12(1):1-10.
29. Pawar S, Kamble V. Phytochemical Screening, Elemental and Function Group Analysis of *Vitex negundo* L. leaves. International Journal of Pharmacy and Pharmaceutical Sciences 2017;9(6):226-230.
30. Zhang Q, Lin L, Ye W. Techniques for extraction and isolation of natural products: a comprehensive review. Chinese Medicine 2018;13(1):20.
31. Patil SP, Laddha KS. Extraction Efficiency of Agnuside from *Vitex negundo* Leaves Using Different Techniques and its Quantitative Determination by HPLC. International Journal of Health Sciences and Research 2018;8(8):129-135.
32. Badgujar NV, Mistry KN, Chudasama PN, Patel JS. *In vitro* Antioxidant and Cytotoxic Effects of Methanol Extracts of *Vitex negundo, Lantana camara, Bauhinia variegata and Barringtonia racemosa* on Human Cancer

Cell lines. Indian Journal of Pharmaceutical Sciences 2017;79(3):431-437.
33. Mukherjee P. Quality Control and Evaluation of Herbal Drugs. Edn 1, Elsevier, Kolkata 2019, 195-236.
34. Percolation. https://www.medicinalplants-pharmacognosy.com/pharmacognosy-s-topics/extraction-methods/percolation/. 2014.
35. Neha B, Jannavi R, Sukumaran P. Phyto-pharmacological and Biological Aspects of *Vitex negundo* Medicinal Plant - A Review. Journal of Pharmaceutical Research International 2021;33(29A):17-32.
36. Basri F, Sharma HP, Firdaus S, Jain P, Ranjan A. A Review of Ethnomedicinal Plant-*Vitex negundo* Linn. International Journal of Advanced Research 2014;2(3):882–895.
37. Bai SM, Prasad P. Extraction of Phytochemicals from the Stem and Leaves of the Ayurvedic Medicinal Plant *Vitex negundo* as Possible Therapeutic against *Vibrio cholera*. International Journal of Latest Trends in Engineering and Technology 2015;5(1):96-100
38. Lao RC, Shu YY, Holmes J, Chiu C. Environmental Sample Cleaning and Extraction Procedures by Microwave-Assisted Process (MAP) Technology. Microchemical Journal 1996;53(1):99-108.
39. Khonde V. The studies on the effect of plant extract of *Vitex negundo* Linn against different bacterial species. Journal of Pharmacognosy and Phytochemistry 2020;9(4):537-539.
40. Mendiola J, Herrero M, Castro-Puyana, M, Ibáñez E. Natural Product Extraction: Principles and Applications. Edn 1, RSC Green Chemistry, RSC Green Chemistry, Campinas 2013;XXI:196-230.
41. Khawli FA, Pateiro M, Domínguez R, Lorenzo J, Gullón P, Kousoulaki K, *et al*. Innovative Green Technologies of Intensification for Valorization of Seafood and Their By-products. Marine Drugs 2019;17(12):1-20
42. Nagarsekar KS, Nagarsenker MS, Kulkarni SR. Evaluation of Composition and Antimicrobial Activity of Supercritical Fluid Extract of Leaves of *Vitex negundo*. Indian Journal of Pharmaceutical Sciences 2010;72(5):641-3.
43. Soria AC, Brokl M, Sanz ML, Martínez-Castro I. Sample Preparation for the Determination of Carbohydrates in Food and Beverages. Chemistry, Molecular Sciences and Chemical Engineering 2012;4:213-243.
44. Lehotay SJ & Schenck FJ. Encyclopedia of Separation Science, Edn 1, Vol. I, Academic Press, San Diego 2000, 3409–3415.
45. Otero P, Quintana S, Reglero G, Fornari T, García-Risco M. Pressurized Liquid Extraction (PLE) as an Innovative Green Technology for the Effective Enrichment of Galician Algae Extracts with High Quality Fatty Acids and Antimicrobial and Antioxidant Properties. Marine Drugs 2018;16(5):1-14
46. Basheer C, Alhooshani K, Nuhu AA, Kanimozhi S, Lee HK. Sample Preparation of Complex Biological Samples in the Analysis of Trace-Level Contaminants. Chemistry, Molecular Sciences and Chemical Engineering 2012;3:681-700.
47. Picó Y. Recent Advances in Sample Preparation for Pesticide Analysis. Chemistry, Molecular Sciences and Chemical Engineering 2012;3:569-590.
48. Kataoka, H. Encyclopedia of Analytical Science, Edn 3, Vol I, Elsevier, Amsterdam 2019, 231-255.
49. Pacheco-Fernández I and Pino V. Liquid-Phase Extraction, Edn 1, Elsevier, Amsterdam 2019;V:499-537.
50. Ani TA, Calinescu I, and Lavric V. Microwave Extraction of active principles from medicinal plants. UPB Scientific Bulletin, Series B: Chemistry and Materials Science 2012;74(2):129-142
51. Llompartn M, Garcia-Jares C, Celeiro M, Dagnac T. Encyclopedia of Analytical Science, Edn 3, Elsevier, Amsterdam 2019;I:67-77.
52. Rostagno M, Prado J, Mudhoo A, Santos D, Forster-Carneiro T, Meireles MA. Subcritical and supercritical technology for the production of second generation bioethanol. Critical Reviews in Biotechnology 2015;35(3):302-312.
53. Al Jitan S, Alkhoori S, Yousef L. Studies in Natural Products Chemistry, Edn 1, Elsevier, Amsterdam 2018;LVIII:389-417.
54. Yolmeh M, Habibi Najafi M, Farhoosh R. Optimisation of ultrasound-assisted extraction of natural pigment from annatto seeds by response surface methodology (RSM). Food Chemistry 2014;155:319-324.
55. Paike V, Sun C. Green Synthetic Approaches for Biologically Relevant Heterocycles, Edn 2, Elsevier, Amsterdam 2015;II:495-515.
56. Bogdanov M. Alternative Solvents for Natural Products Extraction, Green Chemistry and Sustainable Technology, Edn 1, Springer-Verlag Berlin Heidelberg, Berlin 2014;XII:127-166.
57. Qi D. Hydrometallurgy of Rare Earths. Edn 1, Elsevier, Amsterdam 2018;I:187-389.
58. Ola P, Matsumoto M. Metal Extraction with Ionic Liquids-Based Aqueous Two-Phase System. Recent Advances in Ionic Liquids 2018, 146-158.
59. Xiao J, Chen G, Li N. Ionic Liquid Solutions as a Green Tool for the Extraction and Isolation of Natural Products. Molecules 2018;23(7):1-23.
60. Marathe S, Jadhav S, Bankar S, Singhal R. Enzyme-Assisted Extraction of Bioactives. In: Puri M. (eds) Food Bioactives 2017, 171-201
61. Nadar S, Rao P, Rathod V. Enzyme assisted extraction of biomolecules as an approach to novel extraction technology: A review. Food Research International 2018;108:309-330.
62. Heemann ACW, Heemann R, Kalegari P, Spier MR, Santin E. Enzyme-assisted extraction of polyphenols from green yerba mate. Brazilian Journal of Food Technology 2019;22:1-10.
63. Saklani S, Mishra AP, Chandra H, Atanassova MS, Stankovic M, Sati B, *et al.* Comparative Evaluation of Polyphenol Contents and Antioxidant Activities between Ethanol Extracts of *Vitex negundo* and *Vitex trifolia* L. Leaves by Different Methods. Plants 2017;*6*(4):1-11.
64. Behera BC, Sethi BK, Mishra RR, Dutta SK, Thatoi HN. Microbial cellulases - Diversity & biotechnology with reference to mangrove environment: A review. Journal Genetic Engineering and Biotechnology 2017;15(1):197–210.
65. Puri M, Sharma D, Barrow C. Enzyme-assisted extraction of bioactives from plants. Trends in Biotechnology 2012;30(1):37-44.
66. Altemimi A, Lakhssassi N, Baharlouei A, Watson D, Lightfoot D. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants 2017;6(4):1-23.

67. Mushtaq M, Sultana B, Akram S, Anwar F, Adnan A, Rizvi S. Enzyme-assisted supercritical fluid extraction: an alternative and green technology for non-extractable polyphenols. Analytical and Bioanalytical Chemistry 2017;409(14):3645-3655.
68. Essential Oils 2021. https://www.niehs.nih.gov/health/topics/agents/essential-oils/index.cfm. 9 September.
69. Rassem H, Nour A, Yunus R. Techniques for Extraction of Essential Oils from Plants: A Review. Australian Journal of Basic and Applied Sciences 2016;10(16):117-127
70. Khokra SL, Prakash O, Jain S, Aneja KR, Dhingra Y. Essential Oil Composition and Antibacterial Studies of *Vitex negundo* Linn. Extracts. Indian Journal of Pharmaceutical Sciences 2008;70(4):522-526.
71. Dilworth L, Riley C, Stennett D. Plant Constituents Carbohydrates, Oils, Resins, Balsams, and Plant Hormones. Pharmacognosy 2017, 61-80.
72. Marathe S, Jadhav S, Bankar S, Singhal R. Enzyme-Assisted Extraction of Bioactives. Food Bioactives 2017, 171-201
73. How to Make an Essential Oil Distiller from Kitchen Equipment 2021. https://www.leaf.tv/articles/how-to-make-an-essential-oil-distiller-from-kitchen-equipment/.