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MECHANOCHEMICAL ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS

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ABSTRACT

The green method known as mechanochemical aided extraction (MCAE) provides a definite benefit in terms of extracting the required elements from natural goods in aqueous medium at room temperature and without the use of any hazardous, poisonous organic solvents. Additionally, the polysaccharides recovered using this approach have a different composition than those obtained using more traditional techniques. The following processing settings were optimized: Liquid-solid ratio of 2:1, extraction time of 48 minutes, and milling time of 30 minutes. Response surface approach design experiments were used to investigate the MCAE parameters. The experimental valueof (11.40 0.12)% matched the model's prediction under these circumstances. Common names for the evergreen Mediterranean plant Laurus nobilis L. include bay, sweet bay, bay laurel, Roman laurel, and daphne. L. nobilis leaves include lignans, proanthocyanidins, phenolic acids, and flavonoids. The successful industrial use of these important chemicals requires the development of appropriate extraction techniques that give the highest yields and extract quality. exploiting mechanochemical aid, By removing the phenolic component from L. nobilis leaf extracts, future research planning may be done more effectively, and the process of using this plant for industrial purposes can be streamlined.

KEYWORDS: MCAE, Laurus Nobilis, Bamboo Leaves, polysaccharides, proteins, phenolic compounds.

INTRODUCTION

The MCAE technique is used to extract the natural components found in plants, such as flavonoids, phenols, alkaloids, polysaccharides, proteins, and essential oils.[1] Alkaloids from dendronium werealso isolated using this technique.[2] It has been shown that MCAE is a successful technique for obtaining triterpene acids from the needles of Siberian fir.[4] Volatile organic solvents such as petroleum ether, ethanol, and ethyl acetate make up the majority of conventional extraction solvents. Still, utilizing these organic solvents has a number of drawbacks, including high solvent consumption, extended extraction times, poor extraction efficiency, environmental contamination, aswell as carcinogenic and neurotoxic effects. There are several opportunities to address the need for more plant product extraction by looking for non-toxic, ecologically friendly solvents that can take the place of traditional ones.[6,7] DESs are used in nanotechnological processes as well as the separation of phytoconstituents in plants to obtain natural products.

Therefore, a decrease in the number of solution components and a simplification of the processes for recovering the extracted chemical are predicted to increase extraction efficiency.[8] The use of a novel extraction method called mechanochemical-assisted extraction (MCAE) has been growing in popularity. MCAE produces mechanochemical composites by performing mechanochemical processing on the material using solid reagent, followed by extraction in solvent.[9] Polymerized monosaccharides are joined to form polysaccharides via glycoside bonds. These natural molecules are abundant and vital, serving a multitude of biological purposes such as constructing cell membranes. They are also stored as nutrients.[8] due to their profound biological effects, which include immunomodulatory, radiation-protective, antiviral, hypolipidemic, anti-tumor, and anti-oxidant properties. Polysaccharides obtained from many Chinese plant species have garner significant attention in recent times. It is an interesting phenomena to produce distinct polysaccharide fractions from various batches of the same plant with only minor variations in monosaccharide contents, glycosidic linkages, molecular weight, and biological activity. Even with these differences, a number of purified, documented, and published bioactive polysaccharides have been obtained from several types of traditional Chinese herbs.[3] Reserve chemicals known as polysaccharides can be

found in the cytoplasm or as parts of the membrane and cell wall of an organism.[43,44] They may be found in a variety of natural resources, including bacteria, fungus, algae, plants, mammals, arthropods, and more.[19] They do not harm living things and are biocompatible and biodegradable. Because of their features, they have the potential to be used in a variety of ways, such as medicinal and food packaging. Polysaccharides may be utilized to create films for food packaging, coating formulas for paper, and edible and bioactive materials for active and intelligent packaging.[45,46]

Polysaccharides, which are an essential component of diets, have been shown to display a wide rangeof biological activities that have drawn a lot of interest from researchers in the domains of biochemistry and medicine and provide a wealth of possibilities for more study. More importantly, several studies have demonstrated the minimal toxicity and great efficacy of polysaccharides in the treatment of metabolic diseases.[47]

Exopolysaccharides (EPS) are important for bacterial protection from environmental stressors andtheir biofilm matrix, cell identification, and cell adhesion to inorganic surfaces and tissues.

Microorganisms produce extracellular polymeric substances (EPS) as a capsule or loosely adhered slime layer.[48,49] Over the last two decades, exopolysaccharides derived from milk fermentation by lactic acid bacteria (LAB) have garnered significant attention owing to their numerous health benefits, exceptional biocompatibility, high viscosity, ability to stabilize emulsions, safety, and potential for use in medicine.[50,51,52] Polysaccharides come in several forms.[53] Heteroglycansare polysaccharides made up of two or more unique monosaccharide units. A kind of carbohydrate known as a diheteroglycan consists of two distinct monosaccharide units; a triheteroglycan has three distinct monosaccharide units, and so on. It is possible to distinguish between two types of polysaccharides: A homo- polysaccharide is a single kind of monosaccharide molecule. Examples include glycogen, starch, and cellulose. Polymers made up of two or more different monosaccharide units are called hetero- polysaccharides. One substance that provides extracellular support to organisms is hyaluronic acid. Most commonly, polysaccharides made by three main processes: 1, 2, and 3 ring-opening polymerization, sequential glycosylation, and condensation polymerization.

Three different processes are generally used to create polysaccharides: 1] sequential glycosylation, 2] polymerization via condensation, and 3] polymerization with ring opening.[54]

Fruits, vegetables, legumes, tea, wine, and coffee are the main sources of phenols, which are responsible for the organoleptic properties of plant-based foods. Similar to this, phenolic compounds contribute to fruit bitterness through their interaction with salivary glycoprotein. Phenolics can also be used to improve the color of many fruits and vegetables. It is acknowledged that the differences incolor and flavor across different wine brands are caused by phenolics.[55,56] Plants generate phenolic chemicals in response to several physiological and environmental stresses, such as illness, insect assault, UV radiation, and damage.[57,58] An aromatic ring with one or more hydroxyl groups is the fundamental structural component of phenolic substances.[59] Based on the quantity ofphenol units in the molecule, two classes of plant phenolic chemicals exist: simple phenols and polyphenols. Plant phenolics are therefore made up of lignins, lignans, coumarins, condensed and hydrolyzable tannins, phenolic acids, and flavonoids.[60]

In the kingdom of plants, phenolic chemicals make up a significant class of secondary metabolites. The shikimate route produces several compound families of plant phenolics, which are then biosynthesized.[61] Phenolic compounds are common dietary phytochemicals that may be found infruits, vegetables, and grains. Food phenolics may act as a preventing measure against degenerative illnesses, according to epidemiological studies.[62] Since antioxidant activity is a necessary for life,the majority of the positive effects of phenolic compounds have been linked to it.[63] Eating more whole grain meals can reduce your chance of developing ischemic heart disease.[64] Furthermore, studies have demonstrated that eating the required three servings of whole grains per day significantly reduces the risk of ischemic heart disease in adults.[65] Therefore, whole grains are among the healthiest foods one can eat and provide a wide range of health advantages.[66] Whole grains are also rich in fiber and phytonutrients.

Numerous plants that are edible include phenolic chemicals. They are essential for sustaining themeals' oxidative stability as well as their organoleptic qualities.[67]

MCAE OF POLYSACCHARIDES

1) From Bamboo Leaves

Polysaccharides were extracted from the tissue of bamboo leaves using the mechanochemical -assisted extraction (MCAE) technique.[5] Numerous tropical and subtropical places around the world are home to bamboo, a perennial woody grass. It belongs to the subfamily Bambuseae and thefamily Gramineae. China's Zhejiang Province's Suichang County is where the moso bamboo leaves were collected.[10,11,12,13,14] Before being utilized, they were oven-dried and kept in a dry, dark spot. They brought glucose, sodium bicarbonate, and acetic acid.

Fig No. 1- Bamboo Leaves.

2) MATERIAL AND METHOD

A) Material and Reagent

The Suichang region of Zhejiang province, China, is where the moso bamboo leaves were collected. They were baked to dry them, then until they were needed, they were kept in a dry, dark spot. The Chinese Medical and Biological Products Institute in Beijing, China provided standard glucose for the experiment. The Tianjin Yongda Chemical Reagent Development Center, situated in Tianjin, China, supplied sodium bicarbonate, acetic acid, and other analytically grade chemicals.

B) MCAE METHODS

Sodium bicarbonate was mixed with dried bamboo leaves that had been crushed into a coarse powder (20.0 g). The Russian AGO-2 high-intensity planetary activator was then equipped with the combination. Grinding for many minutes resulted in the production of a powder with a particle size of around 200m. After being extracted for a while at a certain temperature using a sufficient volume of water, the powder was separated by filtering at a low pressure. To concentrate the filtrate, a rotary evaporator was employed. After adding a four-fold amount of anhydrous ethanol and precipitating the concentrated solution with acetic acid, it was incubated at 4ºC for 24 hours. Centrifugation was used to separate the crude polysaccharides, namely the anhydrous ethanol precipitate, which was then lyophilized and subjected to a UV-Vis spectrophotometer test for analysis. The yield is stated in the following way: Yield (%)=m/M*100% M is the weight of bamboo leaves (g), and m is the weight of polysaccharides (g) that were examined using UV analysis (14). Along with these structural and property changes, the authors also discuss how mechanochemical processing affects the polysaccharides themselves. According to gel permeation chromatography evidenc, the polysaccharide macromolecules in the rotary (roller) mill are not destroyed by mild mechanical treatment.[18]

4. Seperation and Purification

Many contaminants, including protein, lignin, and inorganic ions, can be found in the polysaccharides that are isolated from natural sources. Certain procedures must be followed in order to separate the crude polysaccharides since Evaluating the structure-activity connection of these compounds is challenging. To create a single polymer with the same level of spatial conformation and polymerization, several polysaccharides must be combined.

4.1 Eliminating Pollutants From Polysaccharides

4.1.1. Elimination of Proteins

Polysaccharides and proteins are examples of complex hydrophilic biopolymers with a wide range of structural changes. In order to purify and separate polysaccharides, it is essential to remove proteins from crude polysaccharides. The idea behind both the trichloroacetic acid approach and the Sevag technique is that the reagent precipitates proteins rather than polysaccharides by denatureing them. However, the Sevag method requires a lot of time and work.[20] Protease-secreting microbes like Saccharomyces cerevisiae can be used in protein removal processes in addition to the direct injectionof a common protease.[21] The combination of Sevag and enzymes may effectively compensate for the inadequacies of a single approach by limiting the degradation of polysaccharides.

4.1.2. Removal Of Pigments

Accurate polysaccharide identification is hampered by the colors produced by the phenolic chemicals extracted from natural polysaccharides, which are known to impact chromatographic analysis. Particularly, animal crude polysaccharides are darker than plant crude polysaccharides. These three methods—activated carbon, hydrogen peroxide oxidation, and resin process—are often used for decolorization. Since ion exchange resin (also known as adsorption resin) has a high decolorization rate and stable characteristic group structures after decolorization, it has been increasingly popular, especially in recent years.[22,23]

4.2. Polysaccharide Purification

After being extracted from the cell, a polysaccharide is a combination of molecules with different levels of polymerization rather than a single molecule. Studying the connection between the structure and the biological function therefore starts with thorough purification. Three kinds of purification procedures may be distinguished based on the process of separation: chemical precipitation, chromatographic purification, and physical purification. A single purification technique has not been utilized much in recent years; instead, a variety of devices and separation techniques have been combined to boost the purification outcomes.[24,25,26]

4.2.1 Chromatographic Seperation

A) Anion-Exchange Column Chromatography

Crude polysaccharides are usually purified using Anion-exchange column chromatography as the initial step[27], and it is predicated on the partition and adsorption chromatographic concepts. Ion exchange resin chromatography is accomplished via adsorption, electron-dipole interaction, or reversible exchange between the ions of the mobile phase, the sample, and

the stationary phase's surface charged groups. It is standard procedure to use exchange medium containing DEAE- cellulose, such asDEAE-dextran gel and DEAE-sepharose, to separate various acidic, neutral, and mucopolysaccharides.[28,29] Neutral polysaccharides cannot be adsorbed on the exchanger at pH 6, in contrast to acidic polysaccharides. Alternatively, the various acidic polysaccharides can be eluted using a buffer with the same pH but a varied ionic composition. How much neutral polysaccharides can be adsorbed depends on the alkalinity of the column being utilized and the amount of acidic groups in the molecule.

B) Gel Column Chromatography

Based on the molecular sieving activity of the gel, gel column chromatography is a technique used to separate a wide variety of polysaccharides with varying molecular sizes and shapes from one another. Small molecules and inorganic salts can be eliminated prior to purification using a gel with tiny holes. Commonly employed as the stationary phase are gels like agarose, polyacrylamide, and dextran gel, and the eluent is usually deionized water or diluted salt solutions. Ionic strength of the eluent needs to be higher than 20 M in order to prevent tailing. For polysaccharides with varying molecular weights, various gels are suitable. As a result, the particular gel column should be chosen based on the target polysaccharide's relative molecular mass.[30]

As seen in figure 2, Gel column chromatography is often used as the first step after anion-exchange chromatography.[31,32,33] When it comes to the separation of sticky, viscous polysaccharides, the softness and simplicity of use of this combination technique could be helpful.

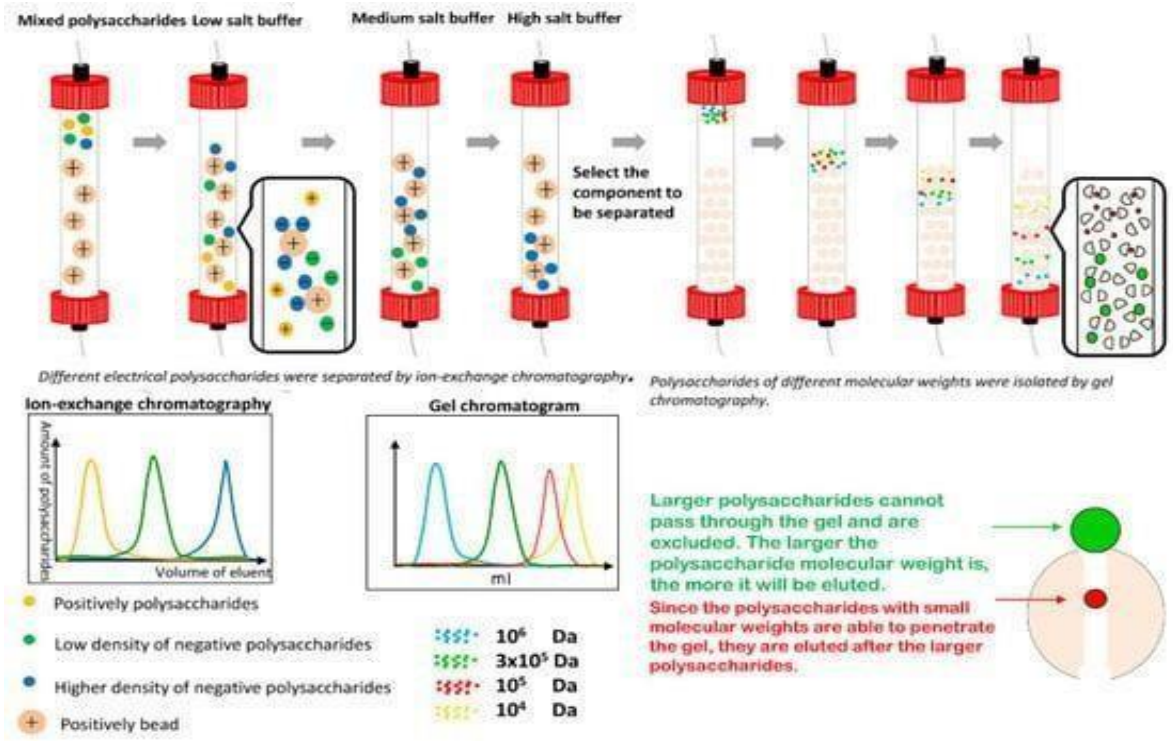

Fig. No. 2: Diagrammatic representation of gel column chromatography and ion exchange.

5. Polysaccharide Analysis

The composition of polysaccharides plays a major role in their biological activity because it is well known that the molecular structure and a number of physicochemical characteristics, such as water solubility, molecular weight, composition of monosaccharides, glycosidic bonds in the main chain, etc., frequently affect polysaccharide biological activity.[34] The study of TCM materials is highly challenging since they frequently contain a variety of Biochemical bases, lipids, proteins, and more components in addition to polysaccharides. As a result, the analysis might be significantly impacted by the extraction and purification. There are several ways for analysis available right now. The total quantity of polysaccharide is commonly measured using colorimeters equipped with chromogenic systems, such as the sulfuric acid pairs phenol.[35,36], anthracenone[37], and carbazole.[38] The most efficient separation techniques, chromatography, have been extensively employed in conjunction with other structural analytical techniques such as infrared, mass spectrometry, and others to examine the structure and composition of polysaccharides.[39] Because TCM polysaccharides are intricate, pretreatments must be carried out before analysis. There are several methods for pretreating polysaccharides, includingperiodate oxidation, Smith degradation, and methylation analysis and then the different analytical procedures indicated above can be used to determine their content and structural makeup. Additionally, For polysaccharide analysis, the

electromigration method is widely used because to its excellent separation efficiency. A growing number of approaches will be developed and used to the analysis of polysaccharides as a result of the advent and development of current analytic techniques.[40,39,41,42]

MCAE Of The Phenolic Chemicals

1) From Laurus Nobilis Leaf Polyphenols

The evergreen shrub Laurus nobilis L., sometimes referred to as bay, sweet bay, bay laurel, Roman laurel, or daphne, is a member of the Lauraceae family, which encompasses between 2500 and 3500 plant species that are native to East Asia's subtropics and tropics as well as South and North America.[68] The Mediterranean region, which has a high yearly precipitation rate, is where this plant's native habitats are found.[69] As a result, For a very long time, The fruits and leaves of L. nobilis have been used in traditional medicine to treat a variety of conditions, including rheumatism, coughs, diarrhea, and viral infections.[70,71] Laurel leaves include a wide variety of polyphenols, such as organic acids, sugars, polysaccharides, alkaloids, norisoprenoids, and essential oils. Differentsubstances, such lignans and phenolic acids, have different levels of structural complexity than flavonoids. It has been demonstrated that these substances possess antibacterial[74],anti-inflammatory, and antioxidant properties.[72,73] Since polyphenols have redoxcharacteristics that enable them to function as antioxidant agents.[77]

It is an evergreen tree that is considered to be fragrant. The perfumery and cosmetic industries utilize bay essential oils primarily as ingredients in fragrances and soaps. Bay is used as a food preservativein the food industry. because of its antibacterial and insecticidal properties.[76] In the cosmetics and culinary industries, Laurus nobilis is also utilized as a scent ingredient.[77]

Fig. No. 3:- Laurus nobilis leaves.

1) MATERIAL AND METHOD

A) Material and Reagent

Plant sample preparation and target chemical preservation from degradation are the initial steps in every plant extraction process. Plant material that has been fresh, dried, or frozen can be used to extract phenolic chemicals. When the fresh, undried plant material is used, intact enzymes are able to breakdown flavonoids, in particular glycosides, which are present in large quantities in L. nobilis leaves.[78] According to a report, in order to keep samples fresh, the interval between harvest and experimental use should be no more than 3 hours.[79] For the extraction of bioactive chemicals, dried and frozen plant material is often recommended. Plant material can be dried in a variety of methods, such as The methods of drying that include air, oven, microwave, and freeze (lyophilization). Depending on the kind of plant material, air drying at ambient temperature for 36 hours[80] to several months or even a year[81] is the most often used approach that doesn't require any specific equipment. Despite its complexity, lyophilization is the second most preferred procedure since it often yields final extracts with greater TPC.[82,83] However, compared to freeze-dried extracts, air-dried L. nobilis leaf extracts contained higher total flavonoid content (TFC) and TPC.

B) METHOD

Recently, a novel method known as MCAE has surfaced to address the issues with purification resulting from poor selectivity and solvent residues from earlier intricate extraction processes. This technique is based on studies of how chemicals change physically and chemically when subjected to mechanical force, such as while being ground in a ball mill.[85,86] Plant material must be mechanochemically treated in a ball mill with a solid reagent—typically carbonated salts—under extremely insensitive mechanical pressure prior to solvent extraction.[87] Cell walls rupture as a result of this process, making it possible to extract target compounds with improved water solubility.[88] The purifying process can be expedited and extraction costs decreased by using water instead of other common solvents. Depending on their alkaline strength and the chemical characteristics of the target molecules, solid alkali reagents such as NaCO, NaHCO, and NaOH are frequently utilized.[89] According to certain studies.[90,91] MCAE produces more flavonoids while taking less time, avoiding organic solvents and utilizing lower extraction temperatures. Rincon et al. (1992) used solid reagents such as Na2CO3, BaCO3, Li2CO3, CoCO3, K2CO3, and CaCO3 in excess of 25 or 50% before extracting L. nobilis leaves with ethanol. It has been demonstrated that a 25% surplus produces a greater TPC than a 50% excess. The greatest TPC was obtained by adding 25% Li2CO3. The entire extraction process in MCAE took 40 minutes.

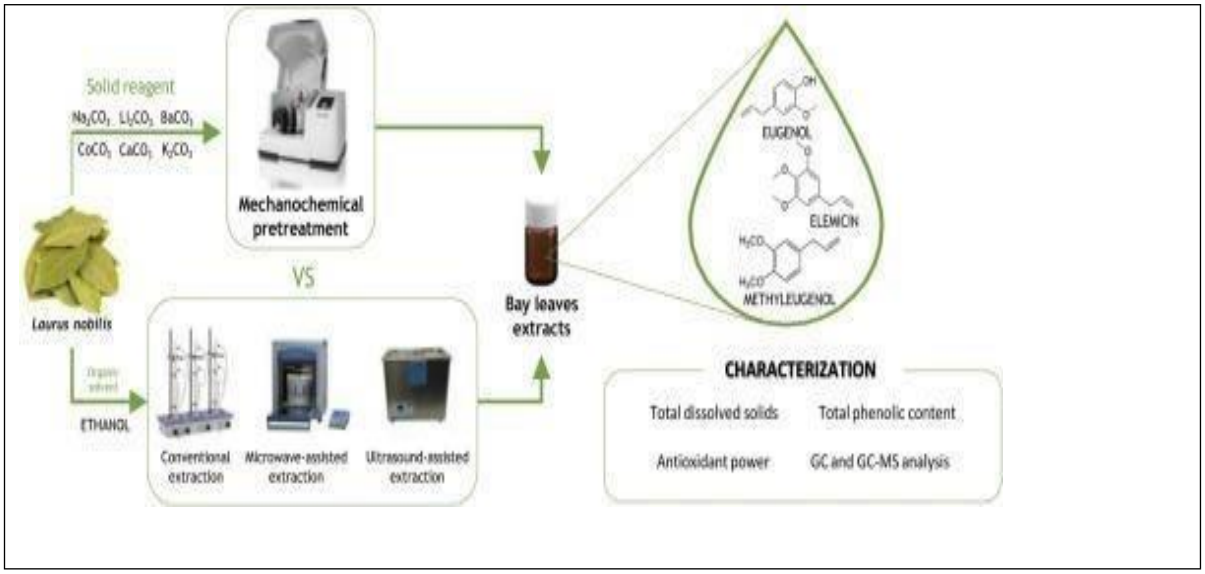

Fig. No. 4: - Diagramatic Presentation Of MCAE Of Phenolic Compound.

2) Phenolic Compound Content Determination

Total Phenolic Content

Using the Chandler and Dodds method, the total phenolic content of the methanolic extract of Laurus nobilis leaves was ascertained. In 1983, the Folin-Ciocalteu reagent was utilized.[93] This substanceis a blend of sodium phosphotungstate (Na3PW12O40) and sodium phosphomolybdate (Na3PMo12O40). The process relies on a redox reaction that creates a blue chromophore whose maximum absorbance is influenced by the quantity of phenolic chemicals. A wavelength of 710 nm was used in this investigation. It may be detected using a spectrophotometer between the wavelengths of 690 and 710 nm.[94] Furthermore, Sigma-Aldrich supplied gallic acid. In short, 46 mL of distilled water, 1 mL of Folin-Ciocalteu reagent, and 1 mg of the extract in an aliquot of 0.1 mL of extract solution were added to a volumetric flask. The flask was then shaken vigorously. After adding three milliliters of the 2% Na2CO3 solution, the mixture was stirred sporadically for two hours. The absorbance was measured at 760 nm. By repeating the same procedures for all standard gallic acid solutions (0-1000g in 0.1mL), a standard curve that satisfied the equation [0.0012* gallic acid (g)] + 0.0033 equals the amount of absorption was produced.

Flavonoid Quantification

Dewanto et al. (2002)[95] state that a colorimetric approach was used to determine the total flavonoids. At 510 nm, an absorbance measurement was taken. The standard range is produced using a variety of concentrations, from 50 to 500 mg/L, using catechin. The catechin equivalent in milligrams (mg/g DW) per gram of dry weight flavonoids are measured.

Tannin Quantification

Via a reaction with vanillin, strong sulfuric acid depolymerizes condensed tannins to produce red anthocyanidols, which may be quantified using spectrophotometry.[96] The absorbance was measured at 500 nm. Catechin was used to generate a calibration curve at concentrations ranging from 50 to 600 mg/l. The amount of condensed tannin contained in grams of dry weight (mg/g DW)was represented as mg catechin equivalent.

Analysis And Seperation Of Phenolic Compound

A) Gas Chromatography

GC is a useful method for the identification, quantification, and separation of several phenolic compounds present in plants, such as tannins, flavonoids, and anthocyanins. It employs the evaporation temperature particular to each compound to separate it from the solution by passing the sample through a heated column that is divided between an inert gas under pressure and a thin layer of nonvolatile liquid covered with an inert substrate inside the column.[97] The primary elements identified by GC are phenolic compounds' derivatization and volatility. In order to identify carvacrol derived from Thymus pulegioides L., Vaiciulyte et al.[98] used GC-FID (flame ionization detector). Recently, GC has been employed extensively to analyze complex compounds in combination with MS detectors due to its excellent quantification sensitivity and selectivity. For instance, GC-MS has been used to characterize the low-molar-mass fraction of lignans, which makes up the majority of the hydrophilic extracts in Norway spruce knotwood.[99] The most used capillary columns for the GC method of phenolic compound analysis are 30 m in length, with an outer diameter of 0.25 to 0.30 mm and an inner diameter of 0.25 m. Helium is usually used as the carrier gas.[100]

RESULT

For Polysaccharides

METHOD	MCAE	UAE	SFE
Solvent	water	water	Ethanol
Time of extraction(min)	48	48	120
Temperature of extraction.(°c)	60	90	50
ratio of liquid to solid(mL/mg)	21	15	-
Yield(%) (g/g)	11.40	10.2	2.47
Polysaccharides content(%)	31.12	29.5	-
Pressure(Mpa)	-	-	40

For Phenolic Compounds

METHOD	MCAE	UAE	SFE
Solvent	ethanol	water	ethanol
Time of extraction(min)	40	30	60
Temperature of extraction(°c)	55	60	60
ratio of liquid to solid(mL/mg)	-	15	-
Yield(%)	6.60±0.20	-	-
Phenolic content(%)	7.5	4.14	5.16
Pressure (Mpa)	-	-	25

CONCLUSION

This work enhanced the MCAE settings for the raw polysaccharides from bamboo leaves. Comparing the optimal circumstances to the UAE and SFE, we obtain the maximum yield and content of polysaccharides. In a nutshell, the MCAE is an excellent option for effectively extracting polysaccharides from bamboo leaves. In the current work, we've also covered how MCAE of bay leaf extracts was carried out employing mechanochemistry and several solid reagents. Comparative extraction methods like UAE and SFE were used to evaluate this unique extraction process. Total phenolic content, solvent type, extraction duration, temperature, yield, and other factors were all taken into account while characterizing the extracts. The key benefits of MCAE, according to the data, are an improv edextraction rate, less solvent use, a shorter extraction duration, a reduced extraction temperature, and high efficiency.

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