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Medicinal Properties of Herbal Combination and *M. officinalis* Phytochemistry, Anti -Allergic Effect, Extraction Techniques, Remedial Applications

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Abstract

An everlasting herb of the Lamiaceae family, lemon balm (*Melissa officinalis*) is well known for its soothing and medical qualities. The botanical features, phytochemical nature and pharmacological benefits of *M. officinalis* are carefully examined in this review, that focuses on significant bioactive ingredients such as flavonoids, phenolic acids (including rosmarinic and caffeic acids), essential oils (like the citral and citronellal) and triterpenoids. The efficiency of extraction, the preservation of bioactivity and the sustainability of acquiring these bioactive compounds have all been substantially improved due to advanced extraction techniques including ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), pressurized liquid extraction (PLE), supercritical fluid extraction (SFE) and matrix solid-phase dispersion (MSPD). The anti-allergic abilities of a combined herbal preparation comprised of the bur-marigold (*Bidens tripartita*), calendula (*Calendula officinalis*) and hawthorn (*Crataegus monogyna*) are presented in this review. Traditionally, these plants were historically used to support the immune balance, sooth tissues that are inflammatory and lessen inflammation. All three herbs have large amounts of flavonoids, polyphenols, triterpenoids and antioxidants that assist in controlling allergic responses, based on phytochemical analysis. The combined use of the extract reduces inflammatory symptoms, and protect tissues from damage due to oxidation, according to the experimental study. These herbs can serve as an effective natural treatment to treat allergies because of their cooperative activity, so enhances their overall medicinal effect.

Keywords: Anti-allergy or antioxidant activity combined herbal extract, extraction technique, bioactive compound.

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1. Introduction

A period of hundreds of years, people have been learning about the health benefits and therapeutic applications of lemon balm (*Melissa officinalis*), the everlasting plant in the Lamiaceae family. It appears in the Mediterranean and a few countries of Asia. For hundreds of years, people of these regions have farmed it and employed it in traditional formulations [1]. This plant is a natural treatment for digestive issues, anxiety and depression among other conditions. As therefore, its soothing and mood-enhancing benefits have been thoroughly explored. A valuable medicinal herb, *M. officinalis* is usually utilized as a powerful sedative. Allergopathologies are currently on increase worldwide every single year and are often brought on by adverse environmental factors, changes in the body's immune system anxiolytic, and anticonvulsant [2]. Aromatic plants and their physiologically active substances are among several natural substances that can enhance medications or medicinal properties based on recent scientific research [3-5]. Many bioactive molecules include the phenolic acids, flavonoids , rosmarinic acid (RA) and essential oils (EO) can be observed in *M. officinalis* [6-7]. which have antiviral, neuroprotective, anti-inflammatory, antioxidant and antibacterial benefits [8].

The yield and bioactivity of plant extracts are greatly affected by the means of extraction applied as determined by a number of studies. For example, when compared to traditional technique microwave-assisted extraction (MAE) has been providing to improve the processing of phenolic compounds from *M. officinalis* [9]. Similarly by more effectively dissolving cell walls and improved solvent absorption ultrasound-assisted extraction (UAE) boosted the antioxidant properties of *M. officinalis* extracts [10]. It has also been confirmed that the use of CO_2 for supercritical fluid extraction (SFE) produce extracts with better antibacterial activity and more volatile compounds [11]. Analysis had revealed that *M. officinalis* ability to regulate various neurotransmitters, especially gamma-aminobutyric acid, accounts for its anxiolytic and antidepressant effect [12] and malnutrition. Statistics show that 20% of people today have some form of seasonal health issue. Studies on the epidemiology of allergies issues show that they are increasing continuously and globally [13]. Herbal natural products contain a range of physiologically active chemicals, so these products can have a mild impact on the body, reduce several aspects of the complex causes of allergies and repair irregular immune response capabilities. Plants in the genus *Grataegus*, such as *Calendula officinalis*, show promise as herbal medication compositions. The primary herb from these plants that has anti-allergic properties is *B. tripartita*, sometimes known as bur-marigold. The following classes of biologically active substances (BAS) comprise the chemical composition of bur-marigold: flavonoid compounds (luteolin, luteolin-7-glucoside) (up to 1.3%), flavanones (isocoreopsin, flavanomarein), associated chalcones (including butein), aurones (sulfuretin, sulfurein, etc.), polysaccharides carbohydrates (4.5–4.7%), and hydroxycinnamic acids [14]. The bur-marigold herb contains antioxidant, anti-allergic, antifungal, antibacterial, hepatoprotective, immune stimulating and hypotensive qualities in herbal solutions [15]. Flavonoids (isorhamnetin, quercetin, isoquercetin, narcissin, and rutin) make up 0.3 to 0.8% of the flowers of *Calendula officinalis* [16]. In addition, marigold flowers have terpenoids (triterpene alcohols 1.2%, triterpene saponins 2–10%), particularly oleonolic acid glycosides-calendulosides A–H. (about 5%), precursors of faradiol which contain anti-inflammatory and anti-edematous qualities (Figure 1) [17].

2. Extraction method for compound from *M. officinalis*

- ***Ultrasound assisted extraction***

UAE is a highly efficient and sustainable technique for extracting bioactive substances from *M. officinalis*. This technique uses ultrasonic vibrations to interfere with plant cell obstacles, enhancing the release of beneficial phytochemicals. With an extraction that takes 33 minutes, an ultrasonic power of 371.7W and a ratio of solvent 40% ethanol-water, optimized UAE conditions provide remarkably high yields of rosmarinic acid, up to 86.3 mg/g of dry weight, surpassing traditional heat-based and microwave-assisted methods [18]. In addition, UAE extracts have stronger antioxidant activity, which related to improved preservation of heat-sensitive phenolic elements like flavonoids and rosmarinic acid, required for their powerful ability to neutralize free radicals [19]. Comparative studies highlight UAE's superiority over conventional methods of extraction with a considerable reduction in extraction times and compound thermal degradation [20]. Important substances like flavonoids might break down because high ultrasonic intensity or extended exposure might cause localized heating or the production of radicals [21]. In addition, UAE could lead to emulsification and make process of purification harder. Optimizing ultrasonic parameters working at lower temperatures, choosing suitable solvent solutions and employing pulsed rather than continuous waves. It has also been revealed that combining UAE with methods like the use of MAE or enzymatic pretreatment increases compound stability and efficiency (Figure 2) [22].

- ***Solvent Extraction***

Bioactive chemicals, including phenolic acids such rosmarinic acid (RA), can be taken out of *M. officinalis* using ethanol and hydroalcoholic extractions. The most effective polarity for extracting bioactive substances with powerful antioxidant properties, such as RA, ursolic acid and oleanolic acid, is provided by hydro-alcoholic solutions, especially those containing 70% ethanol. Typically, the extraction process comprises drying, grinding and use of certain solvents, such as ethanol-water mixtures. Elevated temperatures (50°C–65°C), low liquid-to-solid ratios, and moderate extraction times (30 min–2 h) all lead to increased extraction efficiency and make it easier to release bioactive chemicals from plant matrices using techniques like cold maceration, UAE, or shaking incubation (Figure 3) [23]. Efficiency and industrial-scale production can be improved by further improving ethanol concentration and extracting parameters. Water is thought to be most secure and most sustainable extraction solvent, even if it might extract less non-polar molecules. Ethanol is preferred due to its less harmful properties and food-grade status, but both methanol and ethanol are quite good at extracting phenolic chemicals. Because of its intermediate polarity and higher capacity to break down plant cell walls, acetone-water solutions generally improve polyphenol extraction. Methanol's toxicity restricts its use in consumables, despite its potent extraction capability, particularly for low molecular weight polyphenols. Ethanol is perfect for use in food and medicine because it strikes balance b/w security and effectiveness [24].

- ***Pressurized Liquid Extraction***

Accelerated solvent extraction (ASE), a substitute for pressurized liquid extraction (PLE), is now a popular method of removing bioactive substances from *M. officinalis* including flavonoids and phenolic acids. By extracting under high temperatures and pressures, the above method greatly improves solubility, diffusion rates, and extraction efficiency while keeping solvents like ethanol and water in their liquid condition above their typical boiling points. When compared with traditional method of extraction as Soxhlet or enzyme-assisted extraction, optimized PLE conditions specifically, temperatures around 150°C paired with 40%–70% ethanol concentrations have been demonstrated to increase yields of RA and other phenolics. For instance, phenolic levels as high as 193.18 mg GAE (Gallic Acid Equivalents)/g of extract can be achieved with water-based PLE, and TEAC and DPPH tests verify the intact antioxidant activity [25]. As an example, substantial yields of RA and total phenolics were obtained effectively from *M. officinalis* by ASE, indicating improved antioxidant activity preservation and extraction efficiency compared to conventional techniques. In addition, flavonoid recovery from *M. officinalis* considerably improved by PLE use of ethanol–water combinations at moderate temperatures without damaging sensitive chemicals. Results here reveal that PLE is more consistent and trustworthy for industrial applications requiring high-quality botanical extracts, UAE is efficient for small-scale fast extractions (Figure 4) [26].

- ***Super Critical Fluid Extraction***

SFE is a unique, effective, and environmentally friendly method of extracting bioactive ingredients from *M. officinalis* such as phenolic acids (RA, caffeic acid, and chlorogenic acid) and volatile oils (citral, citronellal, and geraniol). SFE use supercritical carbon dioxide (CO_2) as the solvent and works at moderate temperatures (around 35–40 °C and controlled pressures (about 10–18 MPa). These circumstances greatly improve the diffusion, extraction selectivity, and solubility of compounds without creating heat degradation. Based on earlier research, SFE successfully separates useful EOs involving citral and citronellal as well as polyphenols with strong antioxidant abilities that are confirmed by spectrophotometric analyzes and assays like Rancimat. For example extraordinarily high levels of phenolic compounds are produced under ideal extraction conditions at 10 MPa at 50°C for a period of thirty minutes [27-28]. In terms of its phenolic content and antioxidant power, SFE proved to be less efficient and more susceptible to temperature and pressure than ethanol-water extraction [29]. Such drawbacks can be resolved by pairing SFE with different methods such as UAE, or by using co-solvents [30].

- ***Microwave Assisted Extraction***

MAE is a new and highly efficient method for isolating bioactive compounds from *M. officinalis*, including flavonoids (luteolin, apigenin) and phenolic acids (RA, caffeic acid). By using microwave radiation for quickly heating solvent and plant material, MAE method significantly improves solvent penetration and speed up the extraction of essential chemicals. It has been shown that choosing hydro-alcoholic solvents (50%–70% ethanol), applying microwave power of about 371.7 W, and short extraction durations of three to five minutes are ideal conditions for maximizing RA and total phenolic yields while maintaining their antioxidant qualities [10-31]. Additionally, MAE provides sustainability advantages by minimizing energy and solvent consumption, which is in line with green chemistry ideas. MAE is becoming steadily more well-liked for producing high-purity bioactive extracts for use in pharmaceutical, therapeutic, and cosmetic applications due to its efficiency, shorter extraction periods, and environmental friendliness [32].

- ***Matrix Solid Phase Dispersion***

Matrix solid-phase dispersion (MSPD), a highly efficient and selective extraction technique for extracting bioactive substances such phenolic acids (such as rosmarinic and caffeic acids), made up of *M. officinalis*. In order to enable simultaneous extraction and preliminary purification in a single process, MSPD involves homogenizing plant material with a solid-phase dispersant, typically C18 silica or Florisil. Most effective MSPD modes include using ethanol-water mixtures as eluents, delicately combining plant powder and dispersant, and carefully controlled vacuum-assisted elution, that yields high recovery rates that frequently surpass 90%. MSPD is more economical as well as environmentally beneficial than conventional extraction methods since it greatly lowers need for solvent and improves extraction process [33].

- ***Obtaining Herbal Extract (*****Bur-marigold*****, Calendula)***

Samples of the bur-marigold plant, *Calendula* flowers, hawthorn leaves, and flowers gathered in the Ukrainian districts of Kharkiv and Zhytomyr and analyzed for compliance with national monographs of the State Pharmacopoeia of Ukraine (which completely complies with the European Pharmacopoeia) "Bur-marigold herb" [14]. The mixed extracts were filtered to remove any potential contaminants after being preserved or conserved for two days at 8 °C. The requirements for the content of flavonoids, polysaccharides, and polyphenols were used to assess the degree of BAS extraction in the extracted materials. The resultant aqueous-alcoholic extracts with DSR 1:20 were put in a reactor, where extractants were eliminated by evaporation at 60–80 °C under low pressure to a final moisture content of no more than 5% (dry extracts as components of combined herbal extract) [34].

3. Phytochemical Analysis

- ***Total Flavonoid Amount***

The quantitative determination of the flavonoid content was carried out by absorption spectrometry using HP-8453 UV VIS Spectrophotometer. The total flavonoid content was estimated as luteolin-7-glucoside equivalents some gram of dry weight. Extracts were placed in a volumetric flask and adjusted to the mark with ethanol [35].

- ***Analysis of Polysaccharides***

Ethanol was added to extracts. The resulting mixture was heated on a water bath and then it was allowed to stand for some minutes. Then, using previously adjusted to a constant weight and weighed glass filters, the precipitated polysaccharides were filtered under vacuum, washing the precipitate on the filter with a small amount of ethanol. Next, the filters were placed in an oven and dried at a temperature to constant weight[36].

- ***Analysis of Polyphenol***

The total amount of phenolic compounds was evaluated using the modified Folin-Ciocalteu reagent spectrophotometry method. Pyrogallol was used as a standard, for which the polyphenol content was calculated.

4. Uses and benefits of *M. officinalis*

- ***Medicinal Application of M. officinalis for childbirth and gynecological health***

M. officinalis has become greater attention recently due to its potential as a remedy for gynecological and birth conditions. This everlasting plant has been used historically as a traditional treatment. It shows a number of pharmacological activities and may be an effective option for managing problems that may affect women. Numerous research looked at its application to control symptoms that include postpartum depression (PPD), premenstrual syndrome (PMS), relief the pain and the distress of menopause. The successful application of *M. officinalis* in curing primary menstrual cramps is supported by several randomized controlled studies and systematic reviews.

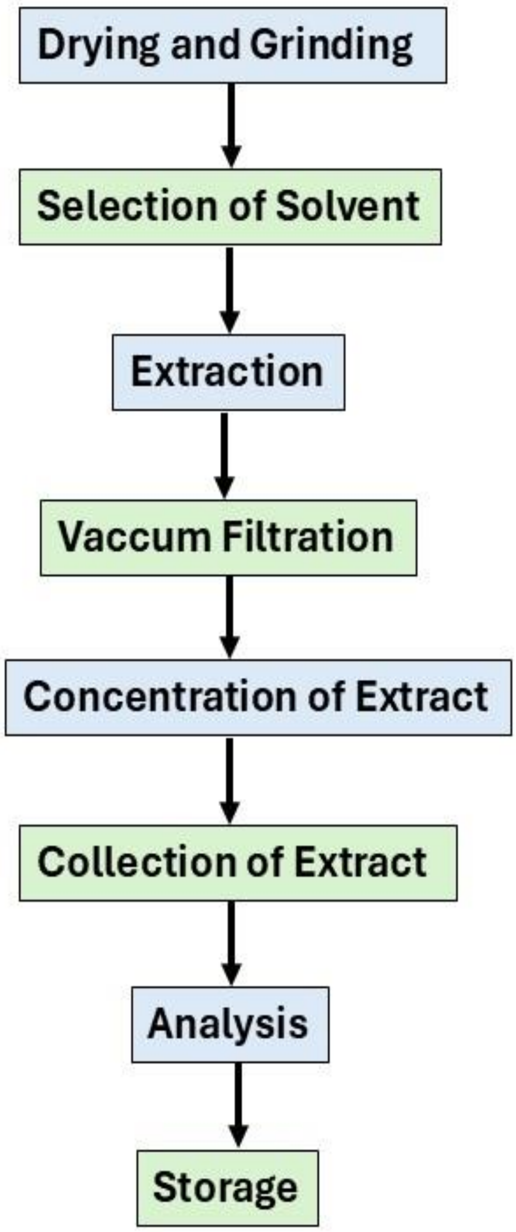

Figure 1: Steps of extracting balm from natural sources

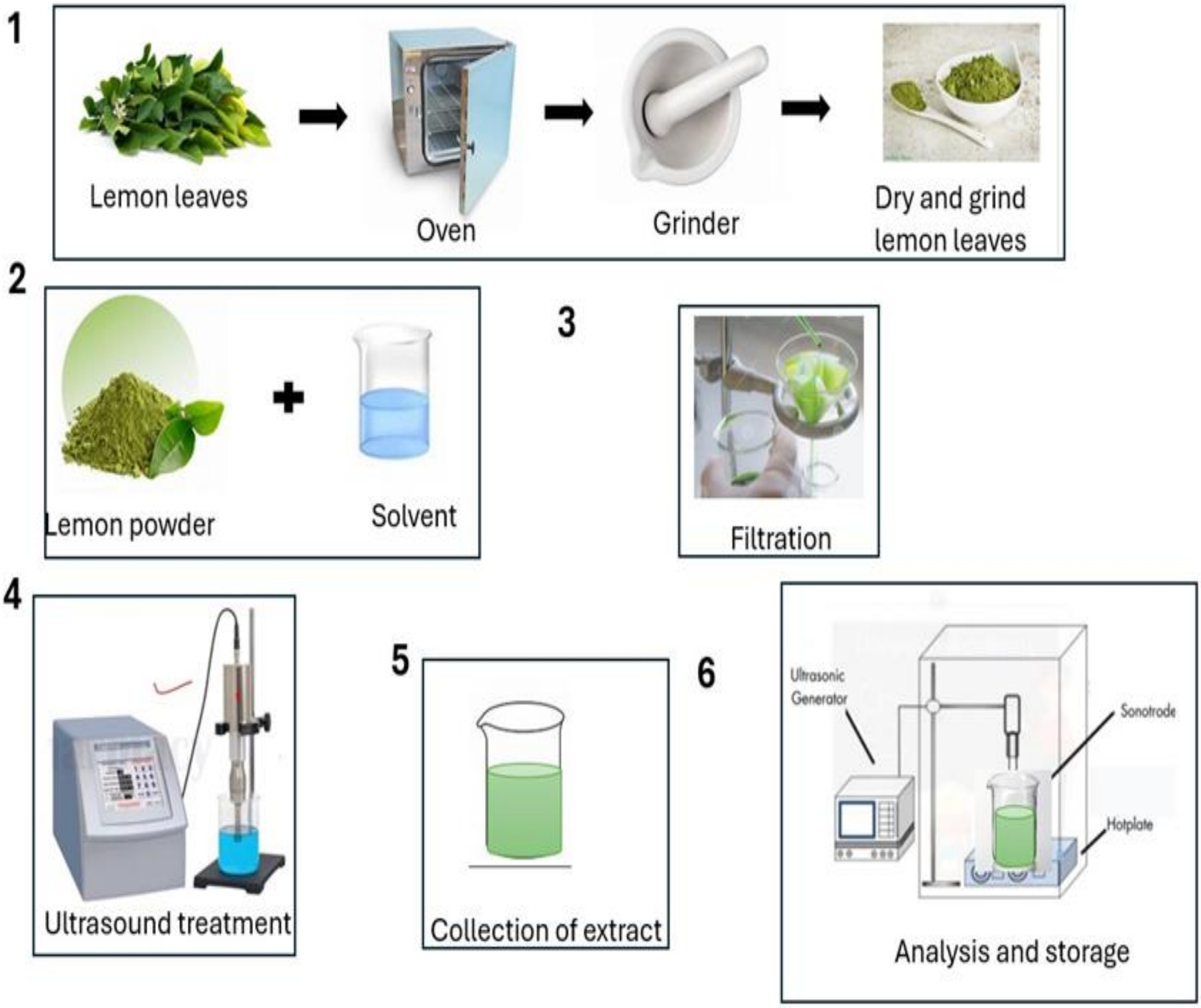

Figure 2: Schematic diagram of ultrasound assisted extraction procedure

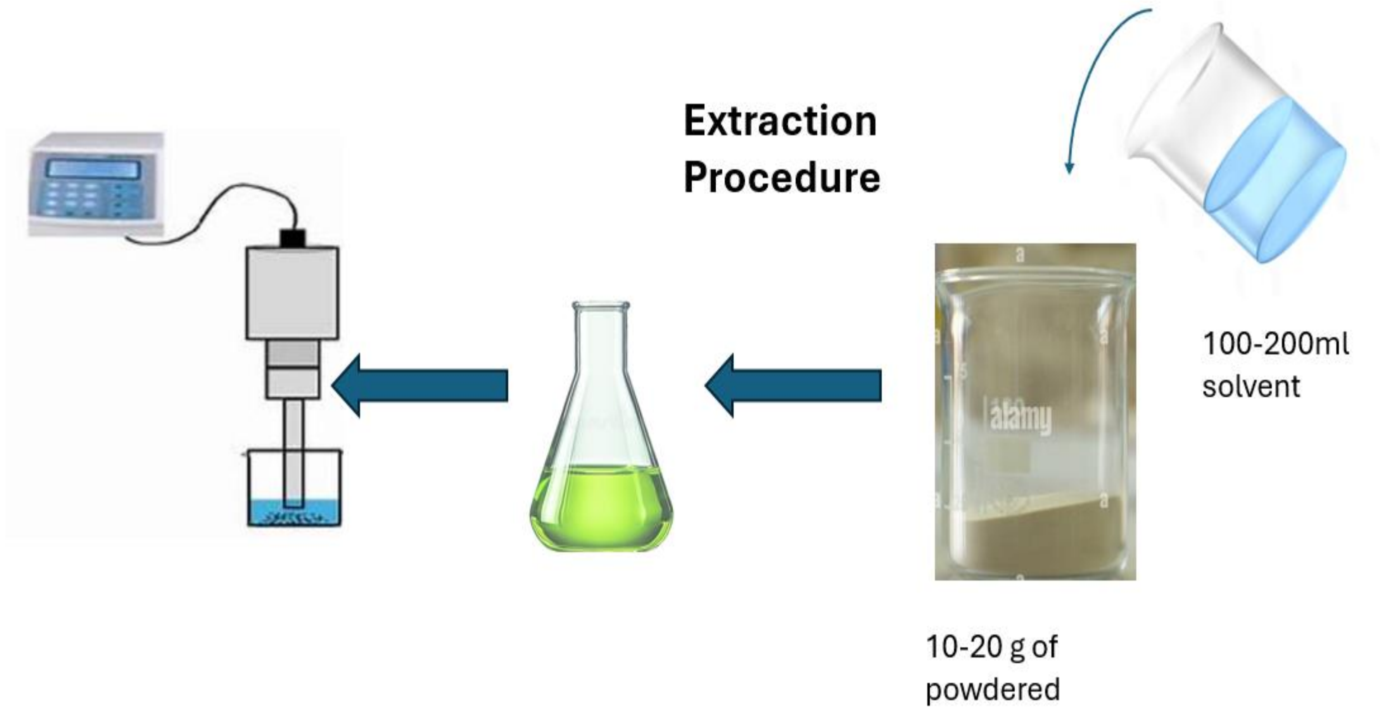

Figure 3: Extraction Method for plant materials

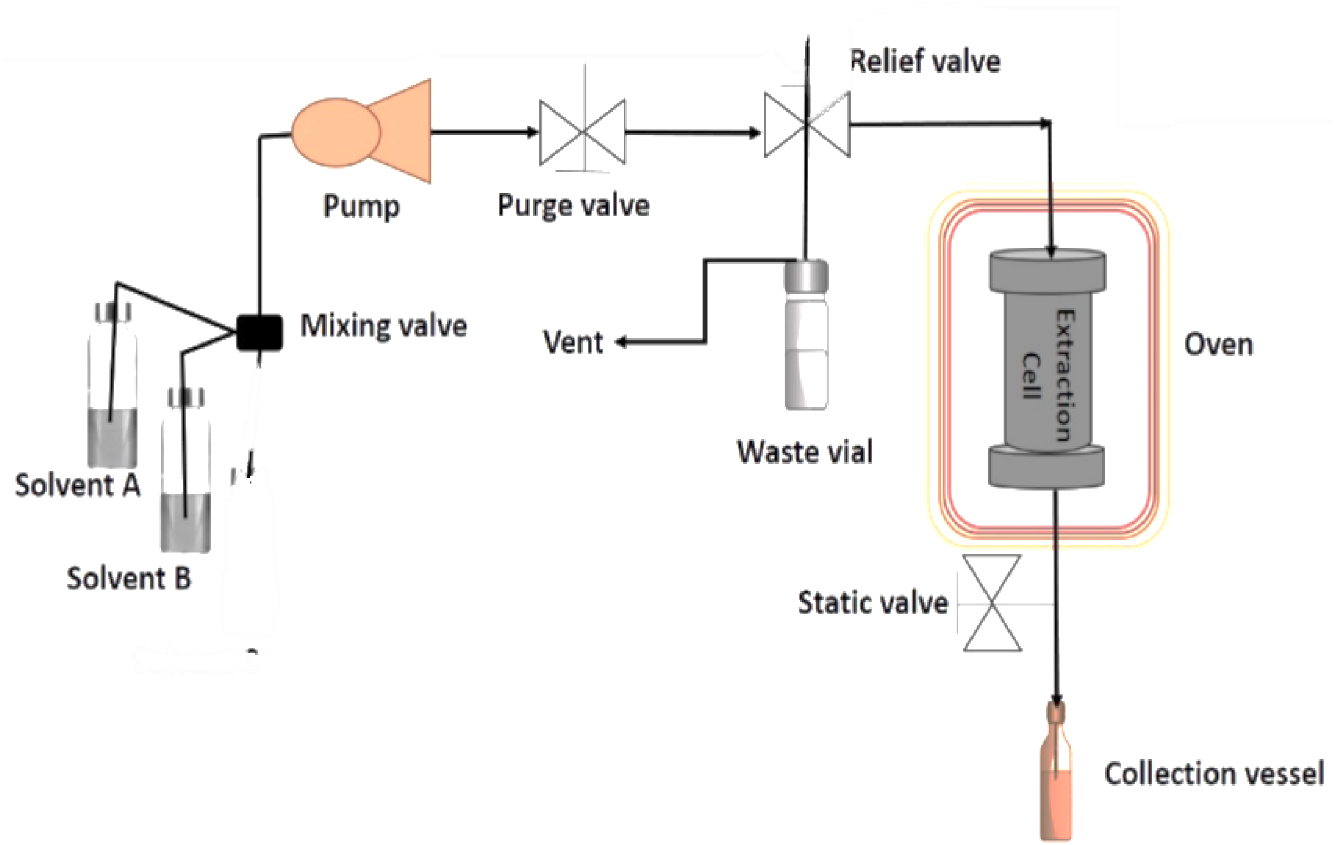

Figure 4: Diagram of pressurized liquid extraction method [37]

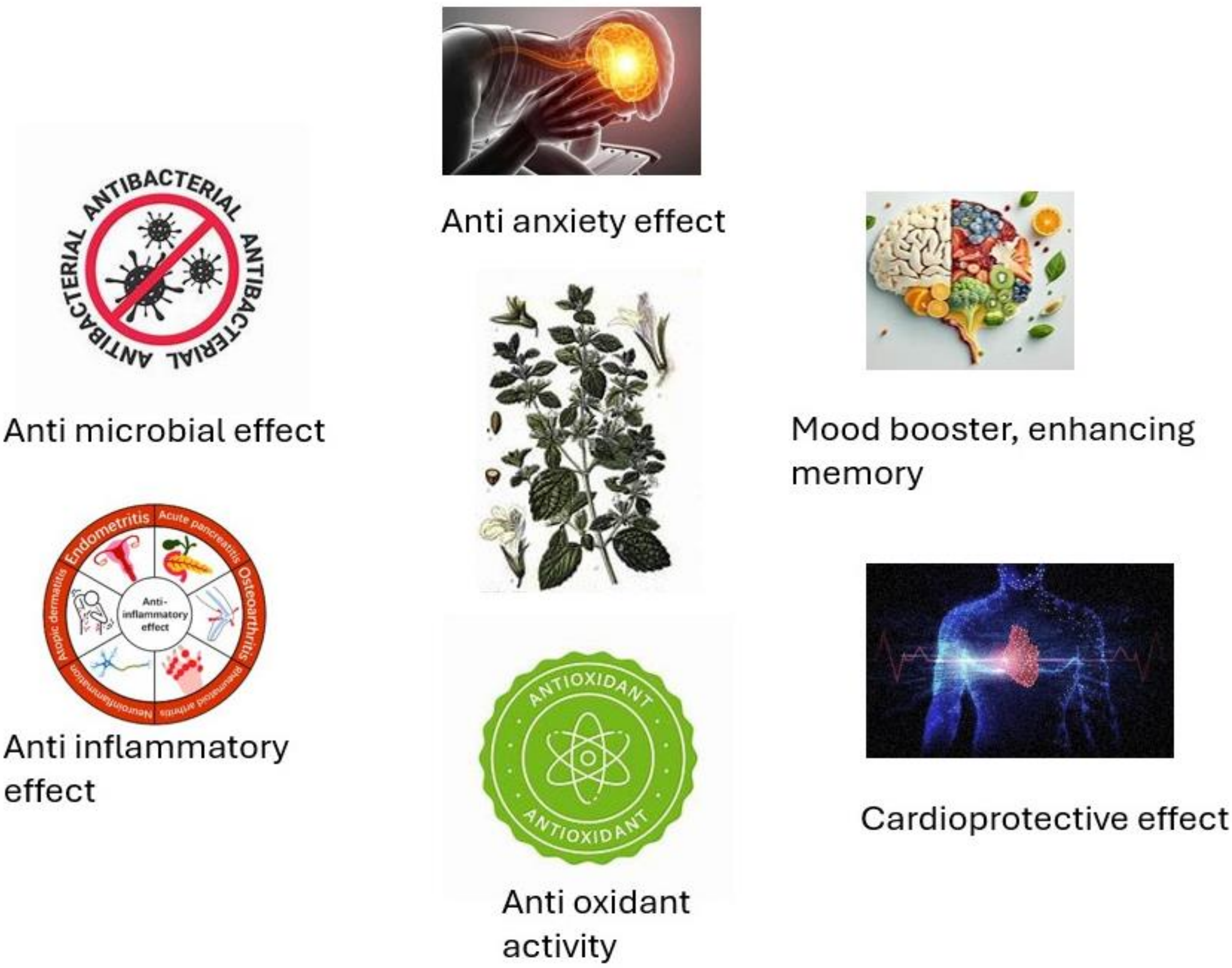

Figure 5: Applications of *M. officinalis*

Women with mild to severe pain reported that taking *M. officinalis* capsules benefited their quality of life and lessened their suffering pain during menstruation. It possesses analgesic properties, which are considered to be caused by its prostaglandin-inhibiting properties (prostaglandins are mediators of inflammation and pain related to menstruation). *M. officinalis* has also been shown to lessen the intensity of menstrual cramps. *M. officinalis* is being studied as a treatment for psychological symptoms related to several gynecological health conditions, particularly depression after delivery and PMS, in addition to period problems.

- ***Mood Elevator Effect of M. officinalis***

Ancient medical books [38]record the traditional use of *M. officinalis* as an activating and satisfying agent, which reflects its long-standing reputation as a mood booster. These traditional claims are confirmed by evidence from current study. Aqueous and methanol extracts of *M. officinalis* showed a slight reduction of monoamine oxidase (MAO)-A, an enzyme that breaking down neurotransmitters like norepinephrine, serotonin, and in an *in vitro* analysis.

- ***Neurosupportive Effect***

The medicinal potential of *M. officinalis* in treating various central nervous system (CNS) medical conditions is well- supported by *in vitro* and *in vivo* study confirming its neuroprotective characteristics. The MTT and LDH metabolic assays, that are normally used for viability and toxicity evaluations in cultured cells under conditions of oxidative stress, proved that use of *M. officinalis* methanol extracts in treating PC12 cells protects against H_2O_2-induced cytotoxicity. It has been observed that before treatment PC12 cells with the acidic parts of *M. officinalis* ethanol extracts, which are rich in polyphenols, flavonoids, and terpenoids, lowers Aβ-induced cell death and oxidative damage. Plant's strong antioxidant activity is main cause of these effects [39].

- ***Anxiolytic effect***

M. officinalis also called lemon balm has wide range of medicinal properties which have soothing, anti-inflammatory effects. Current studies have proved that their relaxing and soothing effect of lemon balm makes it a treatment of anti-anxiety issue. GABA transaminase (GABA-T) inhibitory activity has been proven by the use of *M. officinalis* and main substance, RA, in the methanol extracts, indicating that they are helpful in raising brain's concentration of GABA, a neurotransmitter that lessens anxiety {Taiwo, 2012 #6.

- ***Antioxidant property for disease prevention***

Reactive oxygen species (ROS) production exceeding the body's antioxidant defense system, causing oxidative stress, which is primary cause for the appearance of many diseases including cardiovascular disease, diabetes, cancer, and neurological diseases. Many different *in vitro* or *in vivo* research proved that the antioxidant properties of essential oil and the extract of *M. officinalis,* showing the probability of its use for treatment (Figure 5) {Miraj, 2017 #7}.

4. Future Perspective

Future research on *Melissa officinalis* should focus on improving the extraction and utilization of its most effective bioactive compounds. Refining techniques for extraction that especially improve essential elements such as rosmarinic acid for neurologic and anti-inflammatory actions, citral-rich essential oils for antibacterial effects, and ursolic acid for anticancer potential should be given top priority. A promising method to increase yields while shielding heat-sensitive chemicals or reduce solvent and energy usage is taking advantage of eco-friendly methods, specifically the combination of ultrasonic and microwave-assisted extraction. The standardization of *Melissa officinalis* extracts using proven techniques for accurately identifying essential bioactive ingredients should be the top priority for upcoming research. This will increase study repeatability and offer constant efficiency for pharmaceutical and nutraceutical applications. Further well-designed animal research and clinical studies with humans are needed to confirm efficacy, provide safe dosage ranges, and prove medicinal relevance, despite preclinical evidence suggesting benefit in neurodegenerative illnesses, anxiety, and mood control. To maximize therapeutic efficacy, inventive formulation techniques such liposomal utilization and nanoparticle-based systems should be examined to improve bioavailability and targeted delivery.

5. Conclusions

Melissa officinalis is a valuable medicinal plant due to its diverse bioactive constituents, including phenolic acids, flavonoids, terpenoids, volatile oils, and polysaccharides, which contribute to a wide range of therapeutic effects. The application of advanced extraction techniques such as UAE, MAE, PLE, SFE, and MSPD has significantly enhanced extraction efficiency, compound purity, biological activities, and environmental sustainability compared to conventional methods. Experimental studies further demonstrate that standardized herbal extracts, optimized for flavonoids, polyphenols, and terpenoid compounds, exhibit strong pharmacological activities, particularly antioxidant, neuroprotective, and anti-allergic effects. Notably, combined herbal extracts showed superior efficacy in reducing allergic responses and stabilizing mast cell membranes when compared with standard reference drugs. Despite these promising findings, further interdisciplinary research, including clinical trials, mechanistic studies, and advanced drug delivery development, is essential to fully validate efficacy and support the incorporation of *M. officinalis* based formulations into modern medicinal, pharmaceutical, and cosmetic applications.

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