Comprehensive Overview on Extraction Isolation and Evaluation Techniques for leaves of Moringa Olifera Lam.

[1]Satpute Rajshree, [2]Sachin Bhalekar, [3]Shinde Nutan, [4]Tamboli Meher, [5]Pingat Hemlata

[1]Author, [2]Co- Author, [3]Student, [4]Student, [5]Student

[1]Department of Pharmacy,

[1]Samarth Institute of Pharmacy, Belhe, Maharashtra ,India.

[1]rjshrsatpute@gmail.com, [2]siop13392@gmail.com, [3]shindenutan898@gmail.com, [4]tambolimeher10@gmail.com, [5]hemlatapingat255@gmail.com.

Abstract: The "miracle tree" or "tree of life" is the common name for the Moringa Oleifera tree, which is a member of the Moringaceae family. In English, it's also called the Drumstick tree. It has historically been used to treat wounds, heart problems, liver problems, ulcers, discomfort, and inflammation. Moringa oleifera Lam. It is becoming more and more well-known throughout the world, especially because of its high nutritional content and ability to prevent malnutrition. Along with fiber, protein, minerals, and a well-balanced mix of amino acids, the plant also contains flavonoids, isothiocyanates, phenolic acids, and tannins, among other healthy substances. It includes 13 different tree species and is found in various additional tropical countries. According to studies, the active constituents of the Moringa Oleifera plant are being employed in novel functional foods and have uses in cosmetics and pharmaceuticals. Additionally, its leaves may be ground into a powder that can be used as a nutritional supplement. Moringa's traditional and contemporary applications, as well as its therapeutic benefits and usage in herbal remedies, are becoming more and more well-known. Particularly the choice of the right extraction method needs to be carefully evaluated. There are several methods for extracting the leaves, including the dipping (maceration) process, microwave-assisted extraction Soxhlet extraction, and ultrasound-assisted extraction. Different solvents are used in these extraction procedures, which might improve the extraction efficiency and the quality of the extracted substance. The isolation and extraction methods discussed in this review will help you choose the right technique for extracting bioactive chemicals from Moringa leaves in a way that is simple, practical, and quick.

Keywords: Drumstick, extraction, bioactive chemicals, Moringa Olifera.

I. **Introduction**: Phytochemicals, which occur naturally in various components of medicinal plants, may either kill or halt the static growth of infectious pathogens. Secondary metabolites are by products of primary metabolism and are produced in a wide range of forms, including alkaloids, steroids, flavonoids, terpenoids, glycosides, saponia, tannins, phenolic compounds, and others. As a result, the fundamental phytochemical analysis of active chemicals yields evidence for the traditional usage of medicinal plants in science. Typically, Moringa Olifera is a little, fast-growing evergreen or deciduous tree that reaches a height of 10 to 12 meters and has open crowns. Its thin branches, trip inner leaves with winged leaves, and thick, corki, white bark are characteristic of its humid habitat. Moringa Olifera Lam is the name. in several nations, it is utilized as a very healthful vegetable. Its young leaves, flowers, seeds, and soft pods are frequently consumed and have certain therapeutic benefits. The growing of rice, which originated in South Asia, has Middle East, Africa, Asia, and other areas have previously embraced M.Olifera which has historically been used for food, skincare, medicine, and breast milk production. Nearly every element of M. Oleifera has potential benefit. Furthermore, M. Oleifera is employed in biomass as biodiesel, bio-pesticides, bio-stimulants, animal feed, and water purification. production in industrial and agricultural processes. Indigenous medicine utilizes different components of this plant to treat a variety of diseases. It has diuretic, antihypertensive, cholesterol-lowering, antioxidant, antidiabetic, renal, and hepatoprotective properties, as well as antitumor, antipyretic, analgesic, antiepileptic, anti-inflammatory, antiulcer, and antispasmodic effects. Its leaves have a low calorie content. and included in the diets of the obese. The Moringa plant is a unique source of zeatin, quercetin, kaempferol, and a variety of other phytochemicals.

Fig.1 Moringa Leaves And Powder

II. **Isolation:** Gathering leaves from arbitrary gardens from From Pune, Belhe, as well as from Khatgaon takli At Nagar. kept at a room temperature of 26°C.

Leaf extract preparation:

The leaves were gathered, carefully rinsed in distilled water, and air-dried for a few minutes. The leaves were then dried for two hours at in the hot air oven. 60°C. The leaves are taken out of the hot air oven after two hours and gently crushed by hand to reduce their size. The grinder is also used for this. The dried leaves were made into a powder, and the powder's weight was precisely determined.

1.The Soxhlet Extraction technique: It is widely used for the extraction of bioactive substances, such lipids, sterols, and fatty acids. a form of atmospheric liquid extraction that uses solvents at boiling temperature and low pressures (ambient pressure) to selectively extract the target chemicals. The traditional procedure of Soxhlet extraction yields the maximum amount of carotenoids.

Procedure:

1.Wash the round-bottom flask with distilled water.

2.The solution in the round-bottom flask is heated and evaporates.

3.The vapor reaches the condenser, where it cools and condenses before dripping into the extractor chamber, which is where the plant matter is housed.

4.The chamber is slowly filled with the solvent, which causes the target molecules to dissolve into it.

5. The chamber drains back down to the flask, bringing the dissolved substances with it, after it reaches a certain point.

6.The cycle repeats continuously, with new solvent coming into contact with the plant material until the extraction process is finished.

2.Maceration: It is one of the earliest and most basic extraction techniques, in which plant materials are soaked in solvents at room temperature. Because the technique is simple and only needs a few tools, it is easy to use. However, maceration has disadvantages, including the possibility of reduced output and extended extraction times. In order to get a sufficient yield from Moringa oleifera, maceration is an efficient method for extracting phenolic chemicals, but it may need a lot of solvent and a long processing time.

Process:

1.The plant material is finely ground or crushed to increase the surface area and facilitate better solvent penetration.

2. After that, the substance is immersed in a solvent such as water, ethanol, methanol, or a combination of solvents.

3. Depending on the material, the solvent is maintained in contact with it for a long period, ranging from a few hours to several days. the kind of substance, the solvent, and the target molecules.

4.The extraction process may be made more robust by sometimes shaking or stirring it.

5.Following maceration, the solvent that contains the extracted chemicals (the filtrate) is separated from the solid plant matter via decanting or filtering.

6.To get a concentrated extract, the solvent is frequently evaporated; this extract can then be used for additional research or uses.

3.Microwave assisted extraction: Microwave-Assisted Extraction, or MAE, is a quick, effective, and eco-friendly method used to extract biomolecules from Moringa oleifera leaves. It uses microwave energy to heat both the solvent and the plant material, which helps speed up the process and gives better results than traditional techniques. The process usually starts by drying and grinding the leaves, then mixing them with a suitable solvent such as ethanol. After that, the mixture is placed in a sealed container and heated with specific levels of microwave power, temperature, and time to break down the plant cells and release the useful compounds.

Process: 1.Dry and grind the Moringa oleifera leaves into a powder to increase surface area and efficiency.

2.Add the prepared leaf powder and an appropriate solvent (e.g., ethanol, methanol) to high-pressure microwave vessels.

3.Place the sealed vessels into a microwave-assisted extraction system and irradiate the mixture for a specified time and power.

4.After irradiation, allow the mixture to cool before opening the vessels. The extract can then be filtered or centrifuged to remove solid plant material.

5.For some applications, the solvent may be evaporated using a rotary vacuum evaporator to concentrate the extracted compounds.

Determination of phytochemical components: Both quantitative and qualitative chemical tests were performed on different extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water) of plants leaves extract with standard methods described for various secondary metabolites.

Qualitative Analysis:

Test for alkaloids:

1.Mayer's test:

1.Mayer's reagent (2-3 drops) was mixed with 2 mL of each plant leaves extract (methanol, ethanol, acetone, petroleum ether, chloroform, and water).

2.The appearance of a green precipitate in the solution indicated the presence of alkaloids.

2.Wagner's test:

1.Wagner's reagent (2-3 drops) was mixed with 2 mL of each plant leaves extract (methanol, ethanol, acetone, petroleum ether, chloroform, and water).

2.The appearance of brick-colored precipitate in the solution indicated the presence of alkaloids.

3.Dragandroff's test:

1.Dargendroff's reagent (2-3 drops) was mixed with 2 mL of each plant leaves extracts (methanol, ethanol, acetone, petroleum ether, chloroform, and water).

2.The appearance of a reddish-brown precipitate in the solution indicated the presence of alkaloids.

Test for flavonoids:

1.Alkaline test: 1. An equal amount of 2.0% NaOH solution was mixed with plant leaf extracts using methanol, ethanol, acetone, petroleum ether, chloroform, and water, creating a yellow mixture.

2.When 2-3 drops of diluted acid were added, the color changed to colorless, showing that flavonoids were present. A pink-to-crimson color appeared in the solution, which means flavonal glycosides were also present.

2.Shinoda test:

1.Magnesium ribbons cut into small pieces were dipped in HCl solution (2-3 drops), then mixed with 5 mL of plant leaves extract dissolved in alcohol.

2.A pinkish-red mixture showed that there was a flavone present.

Test for saponin:

1.Lead acetate test:1An equal amount of 1% lead acetate solution and plant leaves which were extracted using methanol, ethanol, acetone, petroleum ether, chloroform, and water, were combined.

2.When a white precipitate formed, it showed that saponins were present.

2.Foam formation test: 1Take a few ml plant leaves extract and the dissolved in the 20 mL distilled water.

2.Agitation for the 15 minutes and then keep it stable for 5 minutes formation of stable foam is 1 to 2 cm. It indicate the presence of saponin.

Test for Protein:

1.Biuret test: 1. The plant leaves extraction were dissolved in 2 mL of water and then filtered. After that, the filtered liquid was combined with one drop of 2% copper sulfate solution, 1 mL of 95% ethanol, and some potassium hydroxide pellets. 2.When this mixture was made, it turned pink, which showed that there were proteins present.

2.Millon's test:1.The plant leaves extract, dissolved in 2 mL of water using solvents like methanol, ethanol, acetone, petroleum ether, chloroform, and water. After dissolving, the mixture was filtered.

2.Then, 2 mL of the filtered liquid was combined with Millon's reagent, which is a few drops. If a white substance formed in the solution, it showed that proteins were present.

Test for Carbohydrates:

1.Molish's Test: 1. The same amount of alcoholic alpha-naphthol was used to test the plant leaves extracts, which included methanol, ethanol, acetone, petroleum ether, chloroform, and water.

2.Then, 1 mL of concentrated sulfuric acid (H_2SO_4) was slowly added around the sides of the test tube. When a violet ring formed at the top part of the test tube, it showed that carbohydrates were present.

Test for Tannins:

1.Feric chloride test :1.Plant leaves were soaked in different liquids like methanol, ethanol, acetone, petroleum ether, chloroform, and water.

2.These extracts were then mixed with a solution called Ferric Chloride. When the mixture turned blue, green, or blue-black, it showed that tannins were present.

Test for Terpenoids :

Libermann Burchart test :

1.The plant leaves were soaked in different solvents like methanol, ethanol, acetone, petroleum ether, chloroform, and water.

2.Then, a few drops of acetic anhydride and concentrated sulfuric acid (H_2SO_4) were added to the mixture along the side of the tube. The solution changed color from blue to a blood red, which showed that terpenoids were present.

Test for phenol :

1.Feric chloride Test :1.A few drops of Ferric Chloride (FeCl3) solution at 5% concentration were combined with 2 mL of plant leaves extract prepared using methanol, ethanol, acetone, petroleum ether, chloroform, and water.

2.The solution turned dark green or bluish black, which showed that phenol was present.

2.Glycoside's Test :1.The same amount of chloroform and acetic acid was mixed with the plant leaf extract, which was prepared using methanol, ethanol, acetone, petroleum ether, chloroform, and water.

2.The mixture was left at 4 degrees Celsius for one hour. Then, concentrated sulfuric acid was added. When the color changed from blue to green, it showed that glycosides were present.

1.Phytosteroid Test : 1.The same amount of chloroform and acetic acid was mixed with plant leaf extracts, which included methanol, ethanol, acetone, petroleum ether, chloroform, and water.

2.A few drops of strong sulfuric acid were also added. When this happened, a bluish-brown ring formed, showing that phytosteroids were present.

Phytochemical screening : Experiments were conducted for the screening and identification of eight phytoconstituient present in the Moringa oliefera and study were carried out in Methanol, Ethanol, Petroleum ether extracts by using the standard procedure described by Indain pharmacopoeia.

Thin layer chromatography studies : Solvent extract was placed to Silica gel plates for the Thin layer chromatography (TLC) analysis. The plates were labeled, and glass capillaries were used to add the sample. The TLC plates were put into a chamber with the solvent mix of Chloroform, Methanol, and water in the ratio 7:3:1, which is the mobile phase. They were left for 20 minutes to let the bands develop, and then the results were seen under UV light.After the solvent runs are done, the TLC plates are dried and then sprayed with iodine reagent to show the bands. The movement of each separated compound is shown by its retention factor, called Rf. The Rf value is calculated using a formula. The formula is Rf equals the distance the solute traveled divided by the distance the solvent front traveled on the TLC plate.

III. Discussion:

Primary and secondary metabolites are two types of organic compounds made by plants.Primary metabolites, like proteins, carbohydrates,amino acids, and lipids, help plants grow and develop. Secondary metabolites are not necessary for survival but help plants interact with their surroundings, which helps them stay alive in their environment.Secondary metabolites are grouped according to how they are made in the body because they are small molecules that come from metabolic processes. These metabolites fall into four big categories: flavonoids, steroids, and alkaloids. Alkaloids are made from amino acids, which are the basic building blocks of life, and they contain nitrogen in ring-shaped structures. In plants, alkaloids help protect against animals that eat them and diseases. Because they have strong effects on the body, about 12,000 different alkaloids are used as drugs, medicines, stimulants, and even poisons. Flavonoids are a type of polyphenolic compound.Plant chemistry is the foundation for many medicines used in the pharmaceutical industry. The different chemicals found in plants have biological effects that can help treat diseases when taken as food or medicine. These chemicals are also used in the cosmetic and agriculture industries. Some plant chemicals, like alkaloids, terpenoids, and phenylpropanoids, are being studied for making new drugs. But because we don't know much about the chemicals in plants, we can't fully understand how useful they might be for medicine.

IV. Conclusion:

In the end, this study was selected to examine the different phytochemical compounds found in Moringa oleifera. The results showed that using solvents with different levels of polarity during the extraction process greatly affected the amount of secondary metabolites in M. Oleifera. These findings provide solid scientific support for the use of Moringa oleifera in the pharmaceutical and nutritional industries. However, more biological tests are needed to understand its possible benefits and check for any harmful effects.

V. References :

1. Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. Molecule. 2016;21(5).

2.Pandey A, Tripathi S. Concept of standardization, extraction and pre phytochemical screening strategies for herbal drug. J Pharm Phytochem. 2014;2(5):115-9.

3.Senthilkumar A, Karuvantevida N, Rastrelli L, Kurup SS, Cheruth AJ. Traditional Uses, Pharmacological Efficacy, and Phytochemistry of Moringa peregrina (Forssk.) Fiori. -A Review. Front Pharm. 2018;9:465.

4. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Yoga Latha L. Extraction, isolation and characterization of bioactive compounds from plants' extracts. African J Tradition Comp Alt Med. 2011;8(1):1-10.

5.Nanna RS, Banala M, Pamulaparthi A, Kurra A, Kagithoju S. Int J Pharm Sci Rev Res. 2013;22(1):11-8.

6.Singh V, Kumar R. Study of Phytochemical Analysis and Antioxidant Activity of Allium sativum of Bundelkhand Region. Int J Life Sci Res. 2017;3(6):1451-8.

7.Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. Int Pharm Sci. 2011;1(1):98-106.

8.Ezeonu CS, Ejikeme CM. Qualitative and Quantitative Determination of Phytochemical Contents of Indigenous Nigerian Softwoods. New J Sci. 2016;1-9.

9.Yang L, Wen KS, Ruan X, Zhao YX, Wei F, Wang Q. Response of plant secondary metabolites to environmental factors. Molecule. 2018;23:762

10.Sanchita A, Sharma A. Gene Expression Analysis in Medicinal Plants under Abiotic Stress Conditions. Plant Metabolite Reg Environ Stress. 2018;2:404-14.

11.Pradeepa M, Kalidas V, Geetha N. Qualitative and quantitative phytochemical analysis and bactericidal activity of Pelargonium graveolens .Int J App Pharm. 2016;8(3):7-11.

12.Paliwal R Sharma, V Pracheta. A review on horse radish tree (Moringa oleifera): A multipurpose tree with high economic and commercial importance. Asian J Biotechno. 2011; 3:317-28.

13.Vongsak B, Sithisarn P, Mangmool S, Thongpraditchote S, Wongkrajang Y, Gritsanapan W. Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method. Industrial crops and products. 2013 Jan 1; 44:566-71.

14.Bako SS, Okere JU, Etonihu AC, Mohammed Y, Olanisakin OA, Atolaiye BO, Mau PC. Medicinal values of Moringa oleifera leave extracts. Raw materials research and development council: Moringa national crop for economic growth and development. 2010; 107:114.

15. Brockman HG, Brennan RF. The effect of foliar application of Moringa leaf extract on biomass, grain yield of wheat and applied nutrient efficiency. Journal of Plant Nutrition. 2017 Nov 26;40(19):2728-36.