



Recent Advance Study Of Isolation And Extraction Of Moringa Oleifera And Pongamia Pinnate

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Abstract :

Aim of this paper was to perform a phytochemical analysis on Moringa oleifera's aqueous and ethanolic extract in order to determine the plant's antifungal properties. A remarkable plant with numerous therapeutic benefits, Moringa oleifera Lam. has been utilized historically as drug that is both herbal and nutraceutical. Data on the phytochemical and antifungal properties of leaf extracts based on different solvents are patchy. The goal of the research was to assess the potential medicinal benefits of Moringa oleifera Lam's seeds and leaves as herbal remedies. The antifungal qualities of Pongamia pinnata were studied using a variety of plant parts, including leaves, bark, roots, and seeds. Ethyl alcohol, ethyl acetate, and distilled water served as the solvents for extracting powdered plant parts. The study highlights the potential antifungal properties of Pongamia Pinnata, emphasizing the need for further research to identify and characterize the active (antifungal) substances. These substances hold promise for eco-friendly control of fungal pathogens.

Keywords:Moringa oleifera,Millettia Pinnata biological analysis,phytochemical analysis,analytical study

.Introduction :

For countless years, fungi have shared the Earth with humans, evolving in tandem over an extensive period. Approximately two million diverse fungal species thrive in outdoor environments soil, plants, trees, animals, and mammals. They also inhabit human skin and various indoor surfaces [1,2].

Fungal infections present an on going and significant risk to human health and well-being [3]. These infections can be categorized into three groups: (a) allergic reactions to fungal proteins; (b) toxic responses to specific fungi's toxins; and (c) infections (mycoses). Individuals in good health may experience various superficial, cutaneous, subcutaneous, and occasionally systemic infections leading to a spectrum of conditions from severe, life-threatening disseminated diseases like histoplasmosis to athletes developing nail and foot infections[4]. Proactive pathogens, either endogenous (Candida infections) or acquired from the environment (such as Cryptococcus and Aspergillus infections), contribute significantly to the occurrence of fungal infections[3].

Moreover, fungi can induce superficial infections, impacting both the skin and mucosal surfaces. Although these infections are not life-threatening, they occur more frequently than invasive fungal infections (IFI) and significantly diminish the affected person's quality of life [5, 6]. Antifungal medications, also known as antifungals or antifungal agents, either eliminate or impede the growth of fungi, including molds and yeasts.

They fall into two primary categories: targeted site-specific antifungals (ii) and nonspecific antifungals. Nonspecific antifungals, acting as disinfectants and antiseptics, are particularly useful for localized or superficial treatments of the skin or mucosa [7].

Phytochemical isolation is the procedure of physically and chemically separating each of the components of plant materials or therapeutic components individually and filtering them to produce monomer complex.

The initial step in isolating desired natural compounds from raw components is extraction. Following the extraction principle, techniques encompass solvent extraction, distillation, pressing, and sublimation. Solvent extraction is a particularly popular method. The extraction of natural products involves four stages: (1) diffusion of the solute out of the solid matrix; (2) dissolution of the solute in the solvent; (3) absorption of the solvent into the solid matrix; and (4) retrieval of the extracted solutes. Enhancements facilitating spread and dissolution in the preceding steps streamline the extraction process. The effectiveness of extraction depends on the solvent's characteristics, particle size of raw materials, solvent-to-solid ratio, extraction temperature, and extraction duration [8,9,10,11].

Fig . :1 [12]

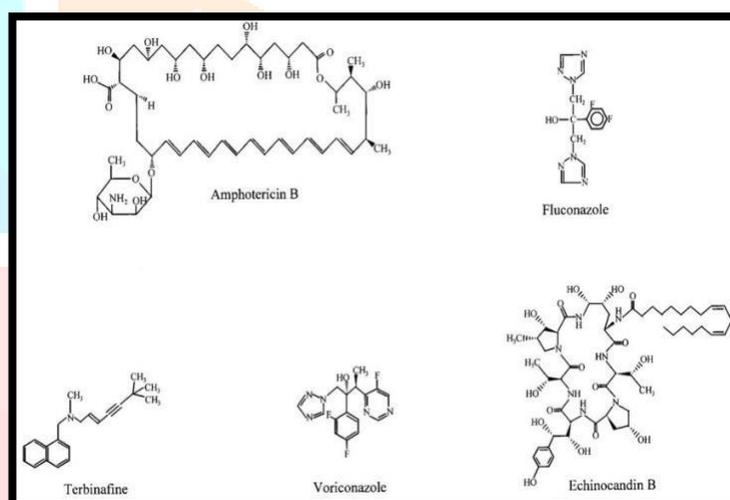


Fig: Structures of representative antifungal agents

Mechanism of Action:

Griseofulvin:

It acts in a fungistatic manner against the majority of dermatophytes and is ineffective against bacteria or yeast. Its efficacy against deep mycoses is reduced [13]. Griseofulvin was the first inhibitory agent that was unique to fungi (Fig. 2). It is currently unknown how exactly this compound works [14]. For dermatophyte infections, griseofulvin is useful when the serum concentration is less than 1 µg/ml. Certain dermatophyte strains can be grown with escalating concentrations of the medication to cause in vitro resistance to the medication [13]. An inhibitor of microtubule assembly is griseofulvin. It affects how the mitotic spindle forms by interacting with microtubules. In dermatophytes mitosis is eventually inhibited by this interference. Using this method, griseofulvin acts as a fungistatic substance against species of *Microsporum*, *Trichophyton*, and *Epidermophyton* [15]. Griseofulvin exhibits only moderate selective toxicity for fungi, with rare acknowledged risks of liver toxicity. Its primary activity is confined to dermatophyte fungi. The reasons behind athlete's foot and ringworm. However, it is known that certain other kinds of compounds, like those found in *C. neoformans*, can disrupt the assembly and operation of microtubules in pathogenic fungi [16]. Griseofulvin needs to be taken for a longer amount of time to be effective because the body eliminates it out quickly [15].

Fig:2 [17]

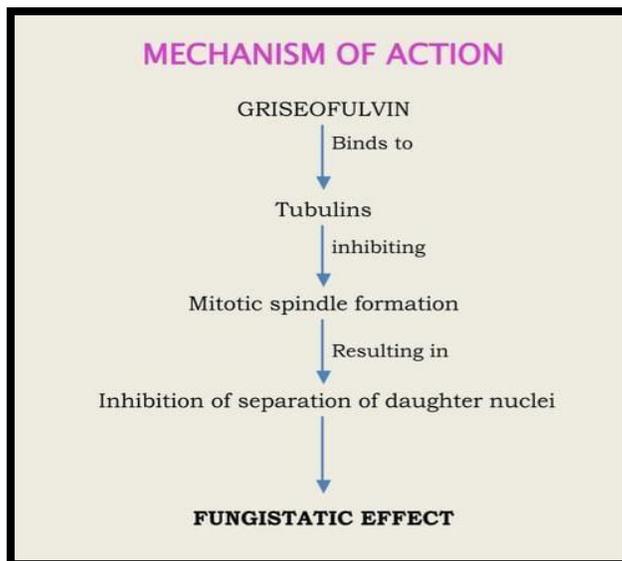


Fig: 3 [18]

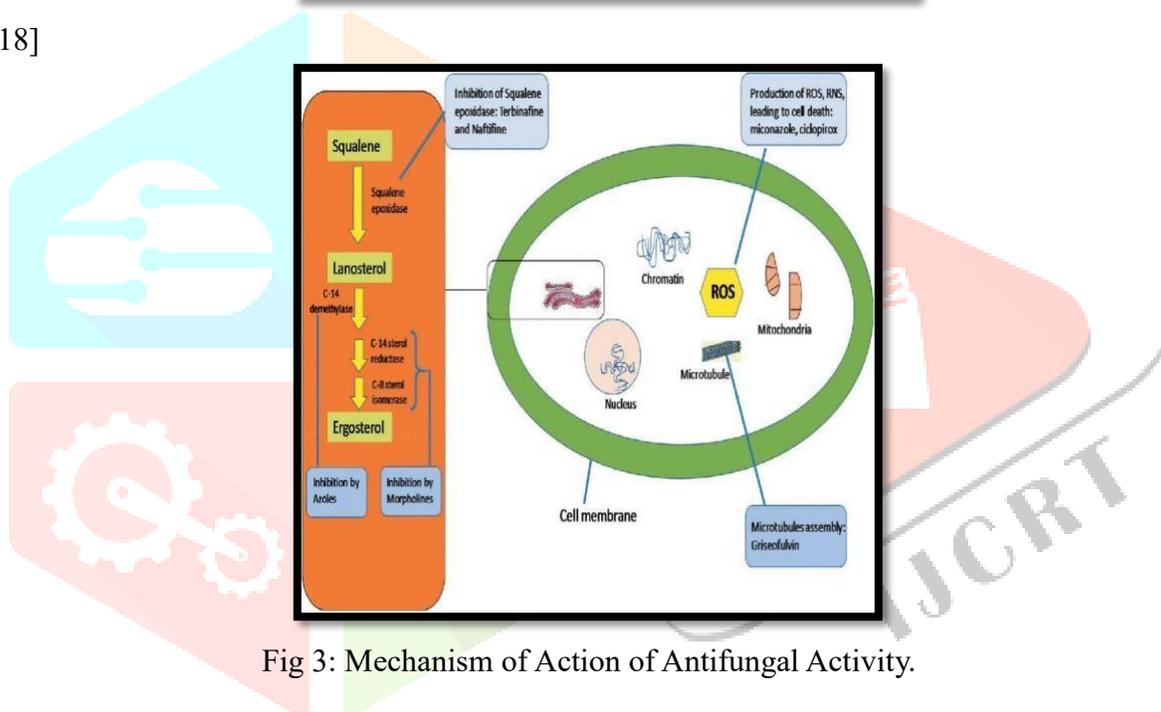


Fig 3: Mechanism of Action of Antifungal Activity.

Moringa oleifera:

"Drumstick" is a common name for *Moringa oleifera* [19]. It is referred to as a "Miracle tree" since practically every section of it contains goods that are beneficial to people. Eaten are the pods and leaves. The plant has traditional use in the treatment of and is significant in medicine for poisonous bites, rheumatism, ascites, and as a stimulant for the heart and circulation [20]. Moreover, leaves are recognized for their antioxidant properties and efficacy in addressing conditions such as asthma, cramps, dry tumors, and illusions [21].

Fig: 4 [22,23]

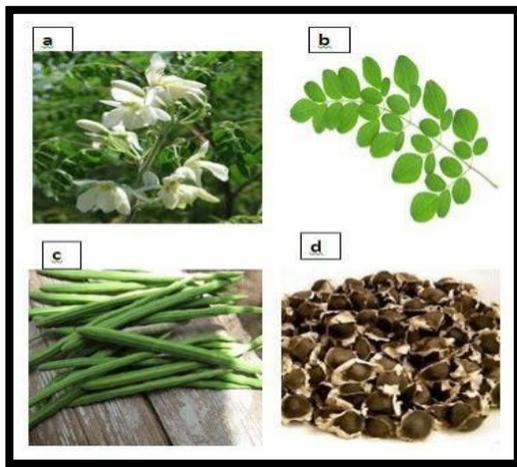


Fig 4: Moringa Oleifera

Biological Analysis :

Toxonomical classification : [24]

- Kingdom - Plantae.
- Sub kingdom - Tracheobionta.
- Super Division - Spermatophyta.
- Division - Magnoliophyta.
- Class - Magnoliopsida.
- Sub class - Dilleniidae.
- Order - Capparales.
- Family - Moringaceae.
- Genus - Moringa.
- Species - Oleifera.

Botanical Description :

Synonyms :

There are numerous names for the plant *Moringa oleifera* in the world. Here are some synonyms for the terms [25].

- Latin - *Moringa oleifera*
- Sanskrit - Subhanjana,

- Hindi - Saguna, Sainjna
- Unani - Sahajan
- Ayurvedic - Haritashaaka, Raktaka, Akshiva
- English - Drumstick tree, Horseradish tree

Chemical Constituents:

A unique blend of zeatin, quercetin, kaempferol, and various other phytochemicals is offered by the moringa plant [26]. When consumed in their raw form, the leaves serve as an excellent source of vitamin C and vitamin A, ranking among the top plant sources for minerals and vitamin B [27]. The ethanolic extract of *Moringa oleifera* leaves contains Niazininins A and B, niazirin, and niazirin [28]. Benzoic acid, gallic acid, and beta benzaldehyde can be isolated from the methanolic extract of *Moringa oleifera* leaves [29].

Fig: 5 [30]

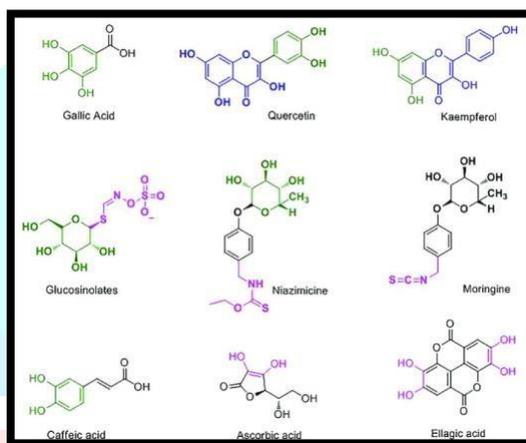


Fig : Structure of Chemical Constituents of Moringa Oleifera.

Moringa oleifera is distributed in the Sub-Himalayan Tracts, Assam, Bengal, and Peninsular India [31]. Additionally, it thrives in tropical climates. Utilizing the leaves, barks, flowers, fruit, seeds, and roots, various medicinal products can be derived.

Phytochemical analysis :

The extracts underwent phytochemical analysis to qualitatively identify various compounds, including alkaloids, flavonoids, steroids, volatile oil, glycoside, reducing sugar, tannins, and saponins.

• Alkaloids :

Wagner's test : By adding diluted iodine solution (Wagner's reagent) to the drug solution. Perform Dragendroff's test by combining the drug solution with potassium bismuth iodide reagent.

Hager test : Mix the drug solution with a small quantity of saturated aqueous Hager's reagent, leading to the resolution of picric acid.

Mayer's Test: By adding a small amount of K_2HgI_4 Mayer's reagent to the drug solution [1].

• Saponins :

Frothing test: In a test tube, agitation of 0.1 g of extract and 5 ml of distilled water resulted in the formation of persistent honeycomb foam for five minutes, serving as initial indication of the presence of saponins [33].

• Flavonoids :

Lead sub-acetate test: Dissolving 0.5 g of extract in 5 ml of distilled water, the mixture was heated for five minutes and subsequently filtered. Upon cooling the filtrate for five minutes, the addition of two to three drops of lead sub-acetate solution resulted in the formation of a yellow precipitate, indicating the presence of flavonoids [34].

Ferric chloride test: Combining 1 ml of ethanol with around 0.1 g of extract, followed by the addition of 1 ml of 10% ferric chloride, revealed the presence of flavonoids through the formation of a brown solution accompanied by a murky green precipitate [33].

• Tannins :

Mixing 1 milliliter of distilled water with 0.5 g of plant extract, the resulting mixture was filtered, and the filtrate was then combined with a few drops of ferric chloride. The observation of a green, blue-black, or blue green precipitate served as evidence for the presence of tannins [35].

• Steroids :

In an alternate test tube, the combination of 1 ml of each extract and 5 drops of concentrated H₂SO₄ occurred [36].

• Glycosides:

To a test tube, 5 ml of extract, 25 ml of diluted sulfuric acid, and a boiling period of 15 minutes were introduced. Following cooling, neutralization with 10% NaOH took place before the addition of 5 ml of Fehling solution [32].

• Reducing Sugars :

The application of a water bath facilitated the heating of 0.5 ml of plant extracts, along with 1 ml of water and 5-8 drops of Fehling's solution [32].

• Volatile oil :

Shaking 2 milliliters of the extract with 0.1 milliliters of diluted NaOH and a small quantity of diluted HCl was performed [32].

Analytical Study :**• Collection of Plant Material:**

Collecting the fresh stem bark of *Moringa oleifera* involved cutting the cleaned samples into small (1-2 cm) pieces and air-drying them before grinding. The mechanically ground fine powder, totaling 20g, was steeped in carbon tetrachloride, petroleum ether, ethyl acetate, and dried powder for 72 hours. The resulting separate extracts were then filtered, centrifuged for 20 minutes at 5000 rpm, and concentrated to a gummy substance under decreased pressure using a rotating vacuum evaporator at 50°C. After that, the gelatinous materials were gathered and dried as normal in a small container. As a result, crude extracts were produced [36].

• Preparation of plant sample :

Following the collection of leaves, a meticulous cleaning process with water ensued, followed by chopping, air drying in the shade at room temperature, oven drying, and subsequent grinding into a powder using an electric grinder. The resulting powder was preserved in air-tight containers for future use [37].

- **Preparation of leaf extract:**

- i. **Maceration Extraction Method:**

Originally employed in wine-making processes, maceration is now widely used in research on plant extraction. The coarse or powdered plant materials were immersed in solvents, such as ethanol, methanol, or acetone, for a minimum of three days at room temperature, with regular stirring [38].

The principles of osmosis and diffusion serve as the foundation for the maceration process. The plant's softened cell wall facilitates the release of phytochemicals through this process. Three days later, the mixture was filtered using filtration. The maceration technique was employed on Moringa leaves, involving the maceration of powdered, dried leaves for 72 hours at room temperature with intermittent shaking in 70% ethanol (1:40, w/v) [39].

Following the filtration of the extract, the marc or extraction residue underwent additional extraction using the same solvent and technique until the process was finished. This method resulted in the highest extract yield (40.50%, w/w) with maximum total phenolic contents of 13.23 g CAE/100 g extract and maximum total flavonoid contents of 6.20 g IQE/100 g extract [39]. Maceration is a traditional technique, but at effective concentrations, this method appears easily understood and manageable [40].

A high yield of crude extract can be produced by improving the extraction efficiency with the right type and strength of solvent. Effective waste management is necessary due to the substantial volume of solvent employed in the extraction procedure.

- ii. **Soxhlet Extraction Method :**

The Soxhlet extraction method was used to extract plant material in methanol and ethyl acetate[41]. 30g of gathered, powdered leaf and seed extracts that were weighed, extracted using 70% ethanol, 80% petroleum ether, and 90% methanol aqueous (distilled water), prepared to a capacity of 250 ml, and refined using a Soxhlet device [42]. Using a rotary evaporator, the leftover solvent from the extraction process was evaporated to yield the crude extract. For antifungal activity, the crude extract was kept in a refrigerator at 40 degrees Celsius [41]. fig: 6 [43]

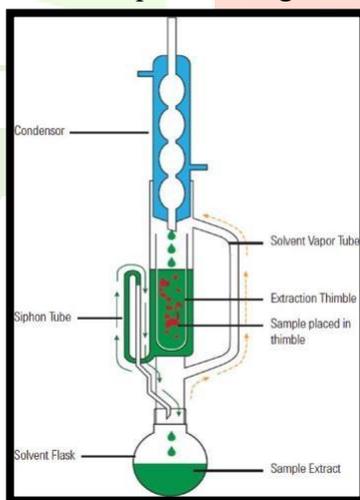


Fig 6 : Soxhlet Extraction Apparatus

- **Determination of Antifungal Activity :**

The in vitro antifungal activity of the plant's crude extracts was evaluated using the toxic food method.

To create the sample, a 10% ethanolic solution (w/v) of the extract was mixed with melted, sterilized Sabraud agar medium, aiming for a concentration of 100 g/ml. This mixture was added to previously sterilized Petri dishes. A 5-day-old fungal mycelial block (4 mm in diameter) was inoculated and placed in the center of each plate, followed by incubation at 27°C. Every experiment included a control set. The assessment of the fungus's linear mycelial growth occurred after three to five days of incubation, and the percentage inhibition of radial mycelial development for the test fungus was calculated accordingly.

$$I = (C - T) / C \times 100$$

Where,

I = Percentage of inhibition;

C = Diameter of the fungal colony in the control.

T = Diameter of the fungal colony in the treatment.

Every outcome was contrasted with the common antifungal medication nystatin [44].

Fig:7 [45]



Fig: Determination of Antifungal Activity

Side effects :

It appears that moringa has few or no adverse effects. However, since Moringa may interact with other medications, anyone thinking about using it should speak with a doctor.

Marketed Preparation :

1. .Enstylz Hand and foot cream.
2. Green Era Moringa oleifera Leaves powder capsule

Fig 8: [46,47]

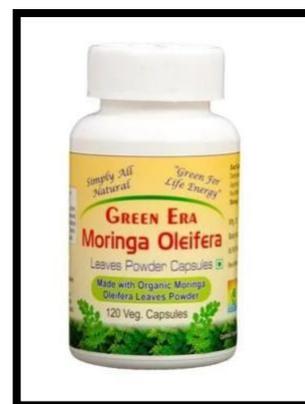


Fig : marketed Preparation of Moringa oleifera

Millettia pinnata /Pongamia Pinnata :

Millettia pinnata known by the common name Karanji, Millettia pinnata is a common medicinal plant. The product is easily accessible throughout India and has been utilized since ancient times for its beneficial properties. Its general effectiveness extends to a variety of health conditions. However, its primary use in Aaurveda is for treating worm infestations. In homeopathy, it is also utilized as medication [48].

Fig:8[49]



Fig 8 : Millettia Pinnata.

Biological Analysis :**Taxonomical classification**

- Kingdom - Plantae
- Order - Fabales
- Family - Fabaceae
- Genus - Pongamia (Millettia)
- Species - Pinnata
- Biological Name - Pongamia pinnata Linn Pierre [50].

Botanical Description:**Synonyms:**

Derris indica (Lam.) Bennett

Millettia novo-guineensis Kane and Hat

Pongamia glabra Vent - Pongamia pinnata Merr [51]

Common Name:

Hindi, Marathi and Gujarat. : Karanj, Karanja

Sanskrit. : Naktamala

English. : Indian beech [51].

Chemical Constituents:

From *Pongamia pinnata* seeds, eight fatty acids (three saturated and five unsaturated) and six compounds (two sterols, three sterol derivatives, and one disaccharide) have been isolated. Physico-chemical and spectroscopic methods were employed to elucidate their structures. Unique metabolites, including beta-sitosteryl acetate and galactoside, stigma sterol and its galactoside, as well as sucrose, were identified for the first time. The fatty acid composition comprised equal amounts of saturated and unsaturated fatty acids, with oleic acid (44.24%), stearic acid (29.64%), and palmitic acid (18.58%) being the most abundant. Additionally, trace amounts of octadecatrienoic and hiragonic acids (0.88%) were detected. Flavonoid derivatives (flavones, flavans, and chalcones) were also isolated, with the majority being flavonoid derivatives such as Karanjin, Pongapin, Pongamone C, Pongamol, along with miscellaneous compounds like triterpenes (Lupeol) and carboxylic acid (Pyperonylic acid) [54].

Fig :8 [55]

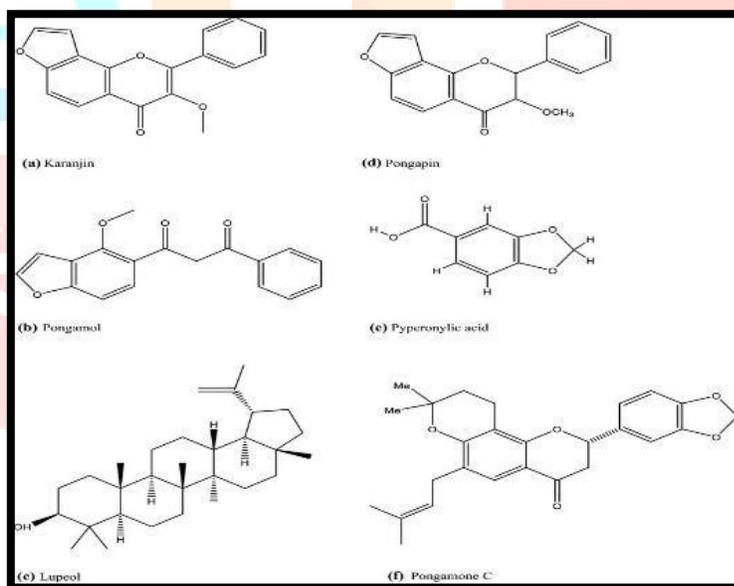


Fig : Chemical Constituents of Millettia Pinnata

Geographical Source:

There are millettia in another part of India. The majority of the species are found in the Himalayan foothills, the Western Ghats, and eastern India. This plant genus is distributed across tropical South-East Asia, spanning Bhutan, China, India, Pakistan, Nepal, Taiwan, Thailand, Bangladesh, and Myanmar to Malaysia [56].

Physicochemical Analysis :

• Test for alkaloids :

Each solvent's crude semisolid or solid extract was evaporated to produce desiccation in the water. Hydrochloric acids were used to dissolve the residues. Following filtration of the mixture, the filtrate was divided into three equal portions. A small quantity of Mayer's reagent was administered to one section, an equivalent amount of Dragendorff's reagent was administered to another, and an equal amount of Wagner's reagent was administered to a third segment. The presence of the corresponding alkaloids was indicated by the appearance of cream-colored, orange, and brown precipitates [57].

• Test for flavonoids:

Lead acetate test: Several drops of lead acetate were introduced into the unrefined extract within a test tube. One indicator that flavonoids were present was the formation of a yellow-colored precipitate [58].

• Test for tannins:

Braymer's test: In a test tube, 2 milliliters of the unrefined extract, 2 milliliters of distilled water, and a small amount of 10% FeCl₃ were combined. The formation of a green-black precipitate was considered an indication of the presence of tannins [58].

• Test for saponins:

Add 2 milliliters of the unrefined extract to a test tube, supplement it with 4 milliliters of distilled water, ensure thorough mixing, and vigorously shake. The formation of a persistent foam was considered indicative of the presence of saponins [58].

• Test for terpenoids:

Salkowski test: Within a test tube, combine 2 ml of the unrefined extract, a small quantity of chloroform, and 1 ml of concentrated sulfuric acid. The appearance of a reddish-brown complex was construed as an indication of the presence of terpenoids [59].

• Test for steroids:

Mix 0.5 ml of the unrefined extract with 2 ml of acetic anhydride and 2 ml of concentrated sulfuric acid in a test tube. A color change from violet to green or blue was considered indicative of the presence of steroids [58].

• Test for anthraquinones :

Take a dry test tube with approximately 1 milliliter of extract, fill it with chloroform, and shake the tube. Following the filtration of the extract, shake the filtrate with an equal volume of 10% ammonia solution. The presence of a pink, violet, or red shade in the ammonical layer indicates the presence of anthraquinones [57].

• Test for cardiac glycosides :

Dissolve 1 milliliter of the extract in a solution of ferric chloride and glacial acetic acid. Subsequently, add concentrated sulfuric acid. The presence of cardiooids is signified by the formation of a brown ring [57].

• Test for Proteins :

Add two to three drops of 1% CuSO₄ solution to one milliliter of the extract in a 70% NaOH solution. The presence of a purple hue indicates the presence of proteins. For an Amino Acid Test, introduce Ninhydrin reagent to 1 milliliter of the sample, and after a brief period in a water bath, violet coloration can reveal the presence of amino acids [57].

• Test for Tri-Terpenoids :

Mix one milliliter of each extract with a small quantity of sulfuric acid in chloroform to produce a reddishbrown color, signaling the presence of triterpenoids [57].

• Test for Reducing Sugar :

Introduce a few drops of Molisch's reagent to one milliliter of the extract and shake thoroughly. Add one milliliter of concentrated sulfuric acid to the sides of the test tube, resulting in a reddish-violet ring. The presence of carbohydrates is indicated at the intersection of the two layers [57].

Analytical study :**Collection of Plant Material:**

We collected *P.pinnata* bark, leaf, seed, and pod, plants material was meticulously cleaned. Cleaned with tap water and allowed to in the sun and shade for a duration of 20 to 30 days. Based on the distinct plant components, the herb material underwent meticulous drying, fine chopping, pulverizing, and subsequent storage in airtight containers [59].

Preparation of Powder :

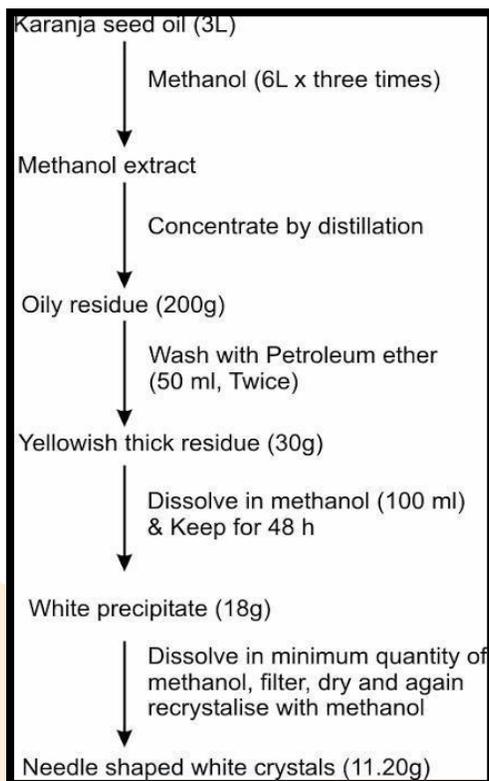
Immediately following collecting, the plant materials were prepared and used in this investigation. The gathered plant parts were shred and allowed to air dry for 72 hours at 50°C. After being dried, the materials were finely powdered and kept in airtight containers. Containers until extraction at room temperature. The same plants were used to create crude extracts, which were made by extracting 2g of dried material for 30 minutes at a time using 20ml of distilled water, ethanol, and ethyl acetate. Following filtration using Whatman No. 1 filter, the extracts underwent vacuum drying. For antifungal assessments, the samples were air-dried and subsequently redissolved in 10 milliliters of water [60].

Preparation of Extract :**i. Macerration Extraction :**

Approximately fifteen days were utilized drying 500 grams of *Pongamia pinnata* plant seeds in the shade. It is ground into a coarse powder and kept for later use in an airtight glass jar. The roughly 100 grams of seed powder soaked in aqueous Stored for approximately twenty-four hours in a conical flask. It passes through Whatmens Filter Paper No. 1 for filtering. The remaining filtrate is heated in a rotary evaporator to semi-solid state [57].

ii. **Simple Distillation Extraction :**

Fig:9 [61]

Fig : flow chart of simple Distillation extraction method for *Millettia Pinnata*

A 1:2(v/v) methanol to liquid–liquid extraction process was applied to *P. pinnata* seed oil. Three extractions were conducted. Every methanol fraction was combined as well as Focused. Upon allowing the concentrated methanol extract to stand for 48 hours, distinct layers emerged, with a dark brown oil separating at the bottom. 200 g of a yellowish-oily precipitate was extracted from the oil layer. To eliminate any remaining oil, the precipitate was twice washed with 50 milliliters of petroleum ether. The precipitate (30 g) was dried. And dissolved in an adequate amount of methanol before being set aside for a while. After some time, the product was settled (18 g). The product has needle-shaped and amorphous crystals in it. Methanol was added to the precipitate in tiny amounts until the entire needle-shaped crystals dissolved. The amorphous powder fraction exhibited lower solubility in methanol compared to needle-shaped crystals. Filtered solution was stored for an entire night. which causes needle-shaped crystals to precipitate. Repeated recrystallization with methanol was performed until 11.20 g of white crystals emerged [62].

iii. **Soxhlet Extraction :**

In each batch, 1.5 kg of material underwent comprehensive extraction using the Soxhlet extraction method with petroleum ether, chloroform, ethyl acetate, and methanol. The solvents were evaporated under pressure until all extractable solvents were removed. Subsequently, the extracted substances were labeled, individually stored at 4°C, and preserved in sealed, ambercolored containers [63].

Determination of Antifungal Activity:

The antifungal properties of various plant components, such as leaves, bark, roots, and seeds, were assessed. Powdered plant parts were subjected to extraction using three distinct solvents: distilled water, ethyl alcohol, and ethyl acetate. The antifungal efficacy against two plant pathogens (*Alternaria solani* and *Helminthosporium turcicum*) and two human pathogens (*Epidermophyton floccosum* and *Candida albicans*) was determined. Among the plant parts, seeds exhibited the most potent antifungal activity, followed by roots and bark, while leaves displayed the least activity. The most sensitive to all of the extracts was *Epidermophyton floccosum*, which was closely followed by *Candida albicans*, demonstrating the extracts' component susceptibilities. Turkey's

Helminthosporium was the most resilient to all of the extracts, This species moved carefully behind, demonstrating their familiarity with and resistance to chemicals originating from plants [64]. The fungal strains were cultured in a broth medium comprising potato dextrose for a duration of 72 hours, utilized for verifying the antifungal activity of Karanjin-mediated biosynthesized AgNPs against both saprophytic and fungi research [65].

Side effect :

It appears that Millettia Pinnata has few, or no adverse effects. However, since millettia may interact with other medications, anyone thinking about using it must consult with a doctor first.

Marketed Preparation :

- a. Pongamia 4 Cream [66]
- b. Pure karanji seed oil [67]
- c. Jiva skin fit table [68]
- d. Fungase herbal antifungal cream [69]

Fig:

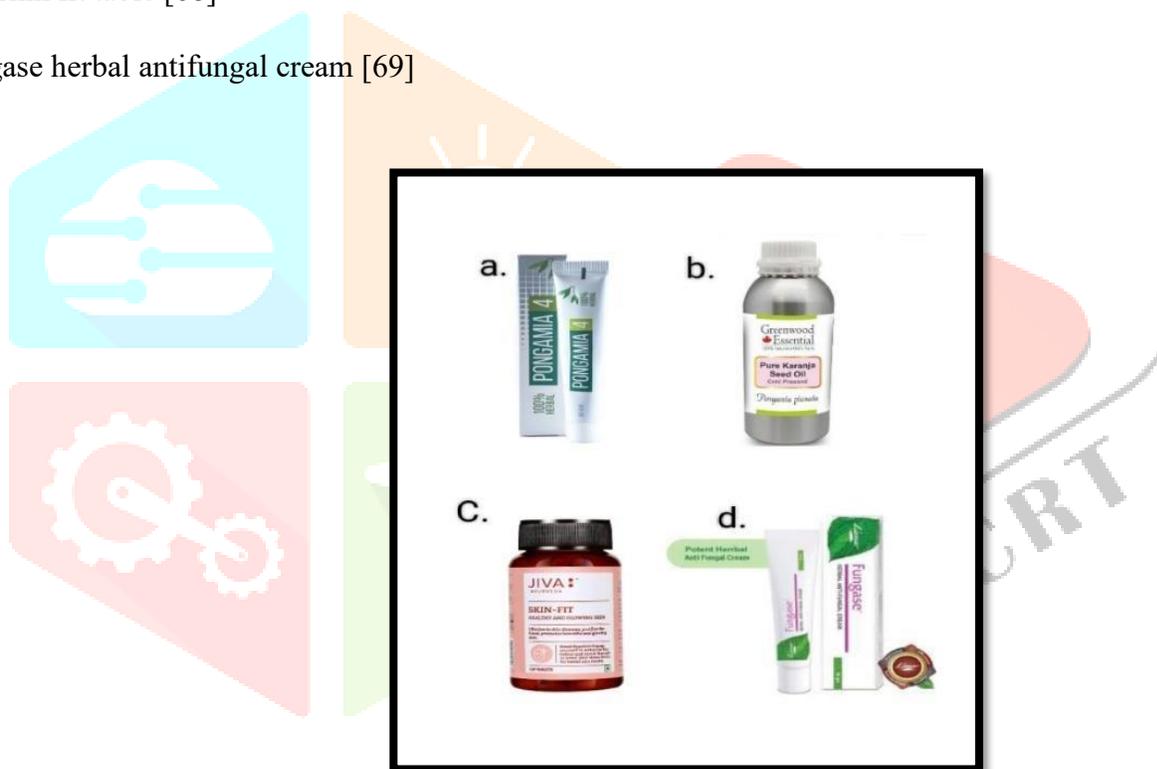


Fig: Marketed Preparation of Millettia Pinnata

Conclusion :

Investigating plant extracts' antifungal effect towards plant diseases became the primary goal of using them. In this work, we employ extracts from Moringa oliefera as herbal fungicides and environmentally friendly methods to manage fugal plant diseases. since the majority of plant extracts are easily Easily accessible, safe for the environment, less likely to cause pest resistance and the revival, and less damaging to plant growth functioning, quality, and most importantly, affordability of seeds. Each of those evaluations and discoveries provide more proof that the herbal extracts from the Moringa oliefera plants, including oil, seeds, roots, leaves, and pod coats, have the potential to develop into effective and secure substitutes for the hazardous fungicide. Hence, it can be employed as an alternative resource for the medical treatment of skin disorders induced by dermatophytes.

In traditional the medicine of Ayurveda, *Pongamia pinnata* was commonly employed as a medicinal ingredient to treat a variety of illnesses. Herbal remedies often contain focuses fruit and seed extracts. *Pongamia pinnata* seeds have been shown to include recognized flavonols with antifungal properties. actions. It have been reported that the extract has antifungal properties. These biological properties can be utilized to create new drugs which can take the place of ones that are no longer working. Still, more investigation is required for evaluating phytochemicals found in various plant parts in order to correctly connect them.

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