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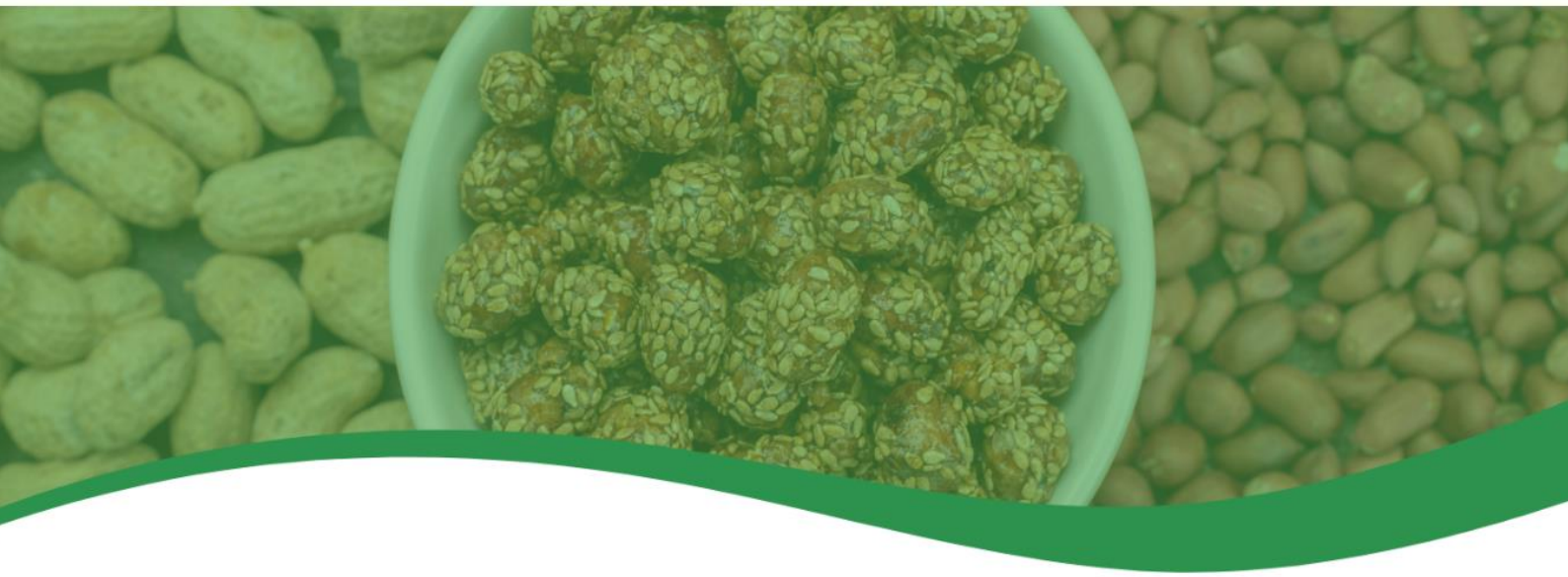

Concept of Malabar nut (Justicia adhatod) and antibacterial activity against staphylococcus

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Concept of Malabar nut (Justicia *adhatod)* and antibacterial activity against staphylococcus

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Abstract

Justica adhatoda L., commomly known as Malabar nut, is a perennial evergreen shrub belonging to the Acanthaceae family. Native to tropical regions including India, Sri Lanka, Myanmar, Malaysia, and Southeast Asia, it thrives in arid, rocky soils with minimal water availability. For over two millennia, this plant has served as a quinazoline alkaloids, such as quinazoline alkaloids (notably vasicine and vasicinone), polyphenols, flavonoids, glycosides, phytosterols, saponins, triterpenoids, and volatile essential oils—J. adhatoda exhibits a broad spectrum of pharmacological effects. These include potent antibacterial, anti-inflammatory, antidiabetic, antioxidant, antimalarial, bronchodilatory, expectorant, antitussive, hepatoprotective, cardioprotective, anti-ulcer, and insecticidal properties, largely attributed to vasicine's bronchodilatory action via β2-adrenergic stimulation and polyphenolic antioxidant scavenging. Traditionally, all plant parts, particularly the leaves, have treated respiratory ailments (e.g., asthma, chronic bronchitis, tuberculosis, and colds), inflammatory pain, and dermal wounds. Recent studies underscore its relevance in contemporary research, particularly for developing natural bronchodilators and anti-inflammatory agents amid rising antimicrobial resistance and respiratory disorders. The current study examines its phytochemical content and estimate its antimicrobial activity by the minimum inhibitory concentration assay.

Keyword: Phytochemical. Anti-inflammatory, antioxidant, quinazoline, natural bronchodilators.

1. Introduction

Justicia adhatoda L., commonly referred to as Malabar Nut and adhatodavasica, is a well-known medicinal herb. It is of the family Acanthaceae. The plant has several names in India, including; Vasaka, Adulsa and Arusa, in addition to Malabar Nut. Malabar Nuts are a shrub and provide year-long leaf cover, as well as have a long history of use in both Unani, Siddha and Ayurveda medicine systems. Plants have been used since ancient times to provide various forms of treatment using traditional medicine. Many researchers have tried to learn more about Indian Traditional Medicinal Plant usage and the way that Ayurvedic, and Unani styles of medicine use this type of herb. Researchers have conducted research on these types of herbs for more than fifty years now. Many of the previously isolated active components from plants will be important for Use in Contemporary Healthcare. Researchers of India's Medicinal Flora have examined the three primary parts that make up the majority of the existing plant resources and have provided current from these plant resources to Use in Contemporary Healthcare. Organic product drug resources can consist of several types of items, including plant parts, base extracts and isolated active compounds, and are all of the drugs used in much of the current supply of medicinal products. All natural compound types have significance because they have medicinal properties or help improve pharmaceutical products [1]. Justicia is an extremely widespread plant, distributed from South East Asia to South Asia (as far as India could be concerned) and Southeast Asia. This plant will grow at elevations of up to 1300m of the Himalayas, depending on the region of origin. The total number of currently known species that exist is about 420 with very few being reported on scientifically to date. Of the 420 known species overall, 13 have been investigated on an Asian basis; 15 have been reported on North America and 8 from Africa. Of the 420 total known species, 23 species have been studied from a chemical standpoint, 31 species have had a reported pharmaceutical use, and 18 species have been investigated and had some sort of biological/chemical study done to them during the last 10 years only. The majority of these plants range in height from 50cm as the minimum to 90cm as the maximum in size. The leaves of Justicia adhatoda L. are lanceolate and broad in width, with a range of length from 10-16cm's and a range of width from 5-7cm's. The flower structure of these plants are almost always white in color with a dense arrangement of white flowers that are arranged in an axillary fashion in a pom-pom like structure. The fruit

of these flowering plants are young with club shaped capsules. The propagation of these flowering plants occurs through planting seed that have been scattered in areas where the seed want to be established or the plant material is grown and the plant has gone dormant at that time. Honey bees have been reported to show preference for pollen and nectar in flowers and form strong colonies [2].

1.1 General uses of Malabar nut plant-

- The Ayurvedic system of medicine regularly utilizes this plant leaves for assisting with respiratory issues such as colds, coughs, bronchitis, and asthma.The use of leaves in treatments for skin diseases, tuberculosis, dysentery, diarrhea, vomiting, and leprosy is customary[3].
- Over the past 2,000 years, this plant has been acknowledged as an important medicinal plant in traditional Indian medicine. In Sri Lanka, it has been used to manage heavy menstrual bleeding (menorrhagia), excessive phlegm/moistness, impotence, bleeding from piles (haemorrhoids), and sexual dysfunctions [4].
- The root extract is used by some of the rural population of South Asia to assist with coughs, liver conditions, and diabetes.Throughout Southeast Asia, Malabar has been used for conditions such as diphtheria, malarial fevers, leucorrhoea, tuberculosis, and various eye disorders in the form of root powder, paste or decoctions.
- Cough one of the many tribes in India and Nepal) eat the leaves and flowers from this plant as part of their everyday Diet. This plant leaves is presently available in various dietary supplements supporting respiratory health; supporting the immune system; and managing weight [5].

1.2 Vernacular name of Malabar nut plant-

- Hindi- Adosa, vaska
- Sanskrit- Shwetavasa, vasa, vaska
- English- Malabar nut
- Marathi - Vasuka
- Punjabi - Bansa
- Bengali- Basak
- Gujarati - Aradusi
- Tamil – Adatodai

Botanical classification of j.adhatoda	
Kingdom	Plantea
Division	Angiosperm
Class	Eudicots
Order	Lamiales
Family	Acanthaceae
Genus	*Justicia*
Species	*J.adhatoda*

Table 1. classification of j.adhatoda

Fig1. Leaves

The primary alkaloids found in the leaves and roots of this plant are Vasicine (0.85%) and Vasicinone (0.027%) respectively. In addition, there are several other alkaloids in the leaves including Hydroxypeganine, Adenosine, Anisotine and Vaccinone. Other trace elements found in this plant include Crystalline Acid, Betaine, Steroids, Alkanes and Essential Oil [6,7,8].

Fig 2. Flower

1.3 Extraction method

Ethanol was utilized as the solvent during Soxhlet extraction of the herb Malabar Nut to isolate bioactive phytochemicals. Ethanol is an effective solvent for extraction due to its ability to extract many different classes of phytochemicals that vary in polarity, as well as being a safe and environmentally friendly solvent to use in biological research [9].

Step 1- J. adhotoda plants were collected, cleaned entirely to remove dirt and other non-plant impurities, and kept in the shade for 6 - 10 days until completely dry. After drying, the plant material was crushed into a coarse powder using a clean mechanical grinder. Once powdered, the material was kept in sealed container (airtight) in a cool, dry place until extraction.

Step 2- Selection of Solvent Ethanol is the solvent of choice for extraction. Many phytochemicals, including alkaloids, flavonoids, tannins and phenolics, are found in ethanol and found throughout the plant J.adhotoda . Ethanol is less toxic than methanol and is also used as a universal solvent for biological assays. The extraction solvent was ethanol: water at a 100:100 ratio.

Step 3 -Soxhlet Extraction Procedure ten (10) grams of the dried powder material was weighed out and placed in a cellulose Soxhlet extraction thimble. This thimble was then placed into a Soxhlet extraction apparatus in the main extraction chamber. The extraction flask is a round bottom flask (500 mL) that was filled with approximately 200 mL of ethanol.

Step 4- Final extract of *J. adhotoda*

Fig 3. Dried Form of leaves

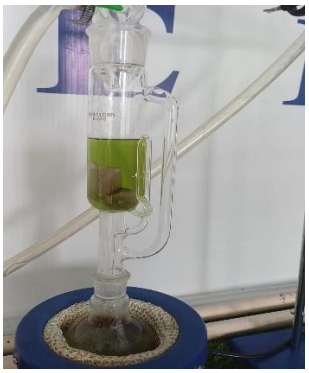

Fig 4. Soxhlet apparatus

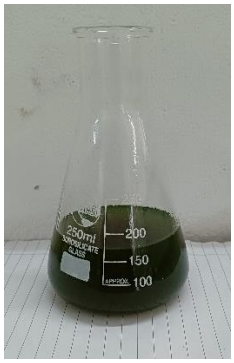

Fig 5. Plant extract

2. Material and method

2.1 **Phytochemical test**

s.no	Phytochemical test	Result
1.	Alkaloid test	+++
2.	Steroid test	-
3.	Tannin acid test	+++
4.	Terpenoids test	++
5.	Flavonoid detection test	+++
6.	Phenol test	+++
7.	Saponins test	+++
8.	Dragendroff test	+++
9.	Cardiac glycoside	+-

A) Alkaloid test [10]

Test	Procedure	Observation	Inference
1.Wagner test	1 ml sample + few drops of wagner's reagent	Brown reddish ppt	Alkaloid are present
2.Mayer's test	1 ml sample + 1-2ml mayer's reagent	White cream/ yellow ppt	Alkaloids are present

B) Steroid test

Test	Procedure	Observation	Inference
1.Liebermann's test	1ml sample + acetic anhydride heat and cool it + add sulfuric acid	Violet blue / green	Steroids are absent

C) Tannin acid test

Test	Procedure	Observation	Inference

1. Ferric chloride test	1 ml sample + 1ml $Fecl_3$	Blue green colour	Tannin are present
2.Lead acetate test	1ml sample + few drops lead acetate in sample	White or white brown ppt	Tannin are present
3.Gelatin test	1 ml sample + 1g gelatin	White ppt	Partially present

D) Terpenoid test [11]

Test	Procedure	Observation	Inference
1.Salkowski test	1ml sample + 2ml chloroform + few drop of H_2SO_4 shake it	Reddish brown colour will be appear it	Terpenoids are present

E) Flavonoid detection test [12]

Test	Procedure	Observation	Inference
1. Alkaline reagent test	1ml sample + few drop of NaOH solution + add dil. HCL.	Yellow colour will be appear	Flavonoids are present
2. Shinoda test	1ml sample+ add dilute HCL+ add magnesium piece (1 and 4) + add few drop of conc. HCL to dissolve mg^{2+}	Purple colour produced	Partially present

F) Phenol test

Test	Procedure	Observation	Inference
1.Ferric acid test	1ml sample + add gelatin	Blue black ppt	Phenols are present
2.Lead acetate test	1ml sample + alcoholic solution + diluted H_2SO_4 + add NaOH	White ppt	Partially present

G) Saponins test

Test	Procedure	Observation	Inference
1.Froth test	1ml sample + water shake it	Forth are observed	Saponins are present

H) Dragenodroff test

Test	Procedure	Observation	Inference
1.Krauts test	1ml sample + 1-2ml dragenodroff reagent	Reddish brown ppt	Brown precipitate appear Partially

I) Cardiac glycoside test

Test	Procedure	Observation	Inference
1.Keller killiani test	1ml sample + add 1.5 ml glacial acetic acid + few drop of $Fecl_3$ + add 0.5 ml conc. H_2SO_4	Reddish brown layer acquiring bluish green color after standing was observed	Partially present

2.2 Biochemical test

2.2.1 Gram staining-

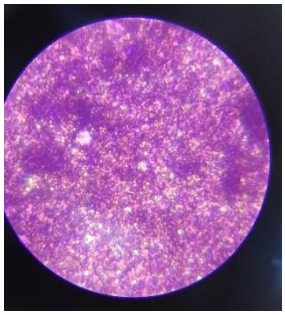

Fig 6. Gram positive bacteria- staphylococcus

- Gram staining technique is a standard method used by laboratories for identification of bacteria.
- It uses a series of four reagents in order to produce images of the types of bacteria that can be identified by their cell wall.
- This technique is based on two types of bacterial cell wall types: 1) Gram-positive with a thick layer of peptidoglycan and, therefore, retains the crystal violet color after being decolorized, producing purple in color; 2) Gram-negative, with a thin layer of peptidoglycan that does not retain the crystal violet color during decolorization, resulting in a pink appearance.
- The inspect includes preparation of the bacteria having a thin layer of bacteria, use of heat fixation, sequential staining, decolorization, counter staining and observation of the bacteria with an objective lens.
- In this experiment, there were rod-shaped Gram-negative bacteria that produced a pink color under the microscope. The bacteria identified were similar in morphology to the two pathogenic bacteria, Escherichia coli or Salmonella typhi.
- The Gram stain technique provides rapid and useful information for classification of bacteria and the subsequent identification of the type of antibiotic that should be used against the bacterial pathogens.
- In this experiment, the antimicrobial properties of Staphylococcus species were examined to evaluate whether a plant extract has antimicrobial of activity

2.2.2 Urease test-

Fig7.Urease positive test

- The urease test is a way to determine the presence of urease enzyme, which is made by many different types of bacteria. Urease catalyzes the hydrolysis (breaking down) of urea into carbon dioxide and ammonia. When ammonia is released, the pH of the medium goes up and becomes alkaline.
- In the medium phenol red is used as an indicator of pH:
- -At an acidic or neutral pH, it appears yellow or orange in color
- -At an alkaline pH, it turns bright pink or fuchsia in color.

- After streaking a heavy inoculum of the organism onto an agar slant, it is going to incubate for 37 °C for no more than 48 hours (many organisms can provide positive results within only a few hours).
- A positive urease test will produce a pink color, and will be observed in organisms including:
    - -Proteus vulgaris
    - -Klebsiella pneumoniae
    - -Helicobacter pylori
- The urease test is used to: Differentiate between urease positive Proteus species and other Enterobacteriaceae Identify Helicobacter pylori (including the urease breath test for clinical diagnosis) Provide part of the biochemical test panel used for classification of bacteria. Read the test results using fresh media, and record them before 48 hours, as spontaneous decomposition of urea will lead to false-positive results.

2.2.3 Amylase test-

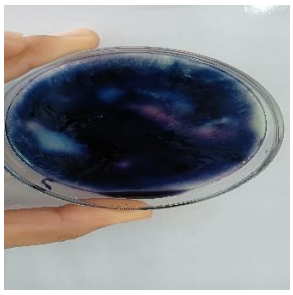

Fig 8. Partially positive

- The amylase or starch hydrolysis test is a procedure that assesses whether specific types of bacteria create amylase enzymes to break down starch into simple sugars such as maltose and glucose.
- To determine if amylase is produced, cultures of bacteria are spread onto starch agar plates (nutrient agar plates with 1% soluble starch). The bacteria will then be incubated at temperatures between 30 - 37 degrees Celsius for 24 - 48 hours.
- Upon completing the incubation, the agar surface will be stained with Gram's iodine solution. When iodine interacts with unbroken starch, it yields a bluish-black colour. The presence of a 'halo' or clear zone around the bacterial growth will indicate that the test result is positive and that the starch has been hydrolyzed.
- Based on this experiment, my results are also positive, since there were regions of clear starch hydrolysis surrounding the bacterial growth.

2.2.4 Nitrate test-

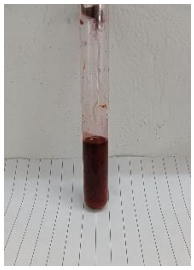

Fig 9. Positive test

- Growing Inoculated representative organisms from specimens in It is an important test for determining type organ and organism primarily uses that same replicable substrate as a type of "biological molecule".

- We use the Enzyme activity of nitrate utilizing bacteria to determine if they possess the enzyme activity for reducing a nitrate (by using the Nitrate Reductase).
- Organisms are grown in a Nitrate broth, which contains Potassium NO_3^- salt form of nitrate as the individual source of nitrogen while being incuibated with the following conditions:
- -Thermal incubate at 35 to 37 degrees Celcius
  -Thermal incubate for 24 to 48 hours.
- After incubation test is performed by adding nitrate test reagents A and B A=sulfanilic acid and B=α-naphthylamine; at the time to develop a red reagent indicating the reduction of nitrates to nitrites completed after reductive gas.
- If the test has shown no color after the second step, prior to adding soil and gas has formed, we then confirm this finding by adding zinc (Zinc adds NO_2^- to NO_3^-, meaning NO_2 only produced by nitrate-reductase activity) indicating the complete reduction of nitrate will produce no color with addition of Z n NO_2 number being produced.
- The use of the Nitrate Reduction Test is very important in the identification and differentiation of bacteria types and serving as a differential test, specifically for Staphylococcus species in clinical and research microbiology.

3.Antimicrobial activity against staphylococcus (gram positive bacteria)

Extracted *J.adhatoda* leaves with n-hexane, methanol, and water to test these extracts for antibacterial and antifungal activity against several strains of bacteria and fungi. They used ciprofloxacin to determine antibacterial activity and fluconazole for antifungal activity. the methanolic extracts had the best results of the three extraction solvents but that all solvents produced promising results. Used methanol, ethanol, acetone, chloroform or water to extract *J.adhotoda* from the same bacterial strains; they found all extracts performed well, but those extracted with diethyl were the most effective.

Phytochemicals like ombuin (isolated) displayed an excellent MIC range of 0.125 - 0.5 mg/mL, while hexane or ethyl acetate (semi purified) exhibited moderate activity (MIC range 0.25 - 1.0 mg/mL). The majority of the crude methanol and ethanol extracts, however, resulted in MBC (minimum bactericidal concentration) values that ranged from 0.78 - 50 mg/mL, indicating that they have less antibacterial activity than purified substances. This suggests that Malabar nut's antibacterial action on staphylococcus, is highly influenced by certain bioactive phytochemicals and therefore clearly reflects the need to isolate and identify bioactive phytochemicals for work on developing plant-based antibacterial compounds.

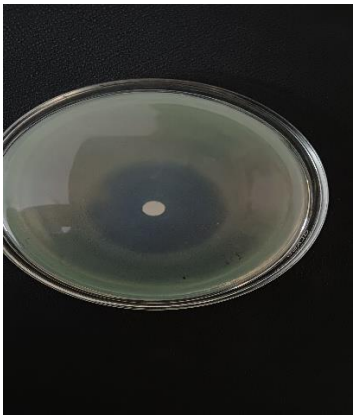

Fig 10. Zone of Inhibition (staphylococcus)

4. Conclusion

The present study confirms the traditional medicinal use of an important plant, Malabar Nut or Justicia adhatoda, by showing that this plant has extensive phytochemical compounds as well as an antibacterial capability. The

results show that there were bioactive compounds in the extract including; alkaloids, flavonoids, tannins, phenols, saponins, and glycosides, all of which have known antimicrobial effects. The majority of the findings suggest that Justicia adhatoda has the potential to be used as a source of natural antibacterial material for use in pharmaceuticals, clinics, or herbal products. However, additional studies are needed to verify and maximize the potential therapeutic efficacy by separating active compounds via purification, evaluating toxicity, and assessing any mechanism of action utilized by Justicia adhatoda.

5. Discussion

The current research supports traditional medicine's use of the herbal remedy, Justicia adhatoda, as a potential treatment for various bacterial infections. In Ayurveda, it is known as Vasaka and has been used for centuries to treat respiratory issues like coughs, asthma, bronchitis and tuberculosis due to its historical use as an expectorant and bronchodilator. Its past uses in treating fevers, inflammation, skin infections and wound healing indicate its wide range of applications in healthcare. The presence of antibacterial activity in this research provides evidence of the traditional use of the herb and suggests J. adhatoda extracts could lead to the formulation of new types of antibacterial agents from plant extracts based on their very strong antibacterial properties. More research needs to be conducted to isolate active ingredients found in J adhatoda, determine if they are toxic to humans, and to conduct animal tests to support this natural medicine for use in modern healthcare settings.

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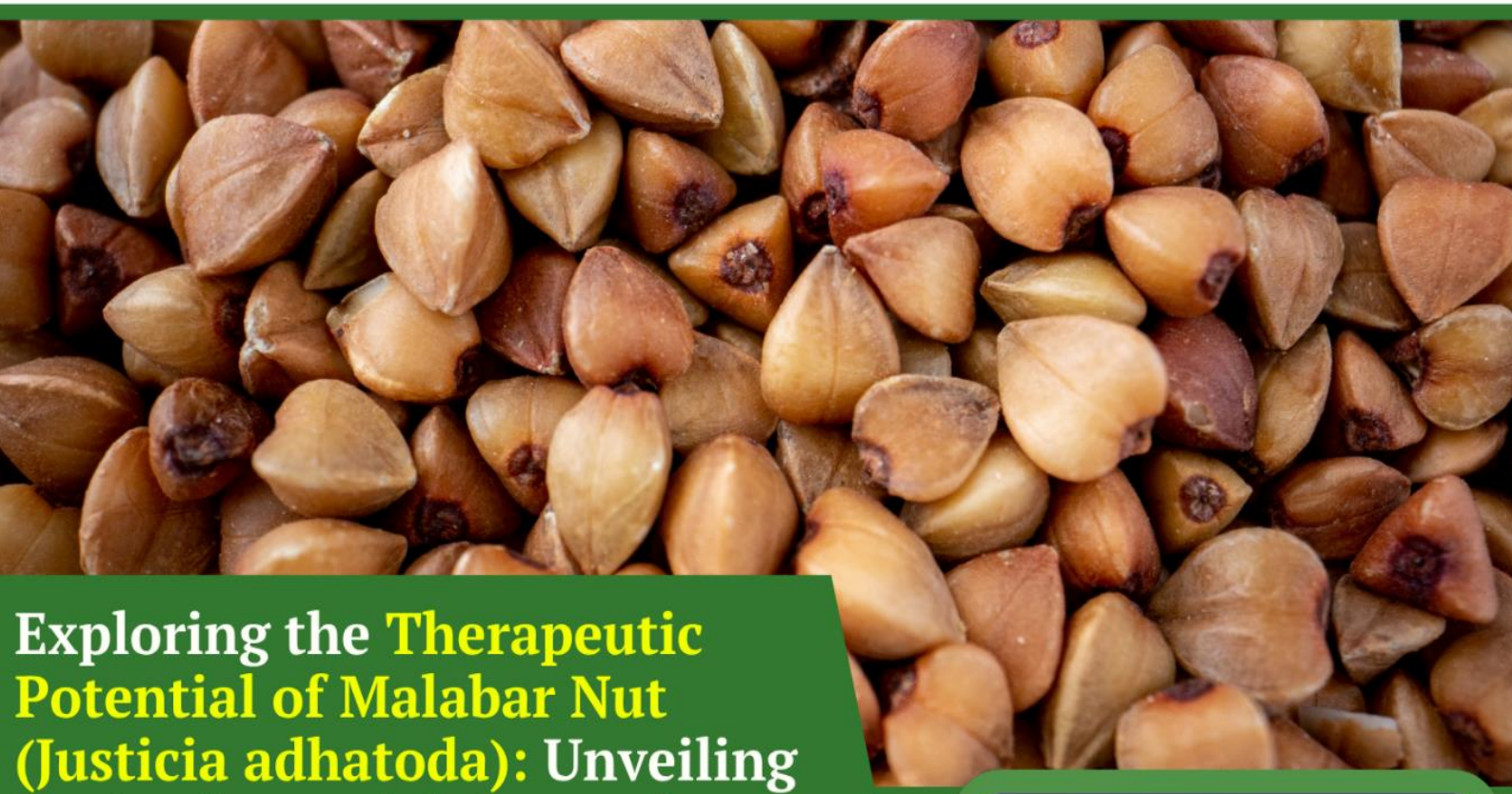

Exploring the Therapeutic Potential of Malabar Nut (Justicia adhatoda): Unveiling Its Antibacterial Properties

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