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# A Regression and ANOVA-Based Approach to Statistical Modeling of Antimicrobial Susceptibility across Varying Phytochemical Concentrations

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## Abstract

The rapid emergence of antibiotic-resistant bacterial strains has placed significant pressure on conventional biomedical healthcare systems, prompting a critical shift toward plant-derived therapeutic alternatives. This study establishes a comparative baseline by evaluating the taxonomic, ethnobotanical, phytochemical, and in vitro antibacterial profiles of four tropical plant species: *Justicia adhatoda* (Acanthaceae), *Lantana camara* (Verbenaceae), *Madhuca longifolia* (Sapotaceae), and *Ocimum basilicum* (Lamiaceae). Standardized cold maceration extraction protocols were employed, partitioning the leaves of these species with solvents of varying polarities, specifically nonpolar petroleum ether and highly polar distilled water. Antibacterial susceptibility was assessed using the agar well diffusion method against key clinical isolates, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Klebsiella pneumoniae*. The experimental results indicate a statistically significant, concentration-dependent inhibitory response across all treatments as extract volumes increased from 25 µL to 100 µL. Lipophilic petroleum ether fractions consistently exhibited greater antibacterial activity than hydrophilic aqueous counterparts across most bacterial targets. Petroleum ether leaf extracts of *Madhuca longifolia* demonstrated the highest potency, producing a maximum zone of inhibition (ZOI) of 15 mm against the multidrug-resistant Gram-negative pathogen *Pseudomonas aeruginosa* at the 100 µL concentration, followed by *Proteus vulgaris* at 14 mm. T*he petroleum ether extract of Lantana camara* achieved a maximum ZOI of 14 mm, while both *Justicia adhatoda* and *Ocimum basilicum* reached a ZOI ofṄ 13 mm against *Pseudomonas aeruginosa*. Microscopic and literature-based structural analyses suggest that these differences are attributable to variations in bacterial cell membrane structure and the solubility profiles of major secondary metabolites, including quinazoline alkaloids, lantadenes, saponins, and complex polyphenolics. This comparative study supports the validity of traditional ethnomedicinal systems and provides a biochemical framework for isolating targeted compounds to address antimicrobial resistance.

***Key words*:** Medicinal plants, Antibacterial activity, Plant extracts, Gram-negative bacteria, *Justicia adhatoda*, Regression analysis, Phytochemicals.

The ongoing evolution of drug resistance in human-pathogenic bacteria poses an unprecedented threat to contemporary infectious disease management [1]. Repeated exposure to synthetic antibiotics has accelerated the evolution of bacterial defense mechanisms, including target-site modifications, enzymatic drug inactivation, and overexpression of transmembrane efflux pumps [2]. Consequently, standard first-line therapies such as ampicillin, amoxicillin, and ciprofloxacin are facing widespread clinical failure [3]. According to data compiled by the World Health Organization, up to 80% of the global population still depends on traditional herbal formulas for primary healthcare needs [1]. Medicinal plants serve as natural chemical storehouses, synthesizing a diverse array of secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and terpenoids that function synergistically to disrupt microbial cell walls, inhibit critical metabolic enzymes, and clear localized infections [4].

The development of next-generation botanical therapeutics necessitates a transition from isolated screenings to structured, comparative analyses. This study integrates the biological, chemical, and experimental profiles of four widely used medicinal plants from distinct botanical families to evaluate their relative properties under standardized laboratory conditions:

*Justicia adhatoda* L. (*Adhatoda vasica* Nees)

Locally known as Vasaka or Adulsa, this evergreen shrub belongs to the Acanthaceae family [5]. Native across tropical Southeast Asia, it is a key plant in Ayurveda and Siddha medicine, used to treat respiratory, circulatory, hepatic, and skin conditions [6]. Its primary bioactivity is mediated by quinazoline alkaloids, which exert potent effects on respiratory pathways and act as natural bronchodilators and mucus-thinning agents [7].

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*Lantana camara* L. (Wild Sage / Unnichedi)

A resilient, heavily branched perennial shrub belonging to the Verbenaceae family [1]. While classified as an aggressive invasive alien weed by the International Union for Conservation of Nature, traditional healers value it as a potent topical antiseptic for cuts, ulcers, and eczema [8]. Its chemical composition includes a high concentration of triterpenoids, essential oils, and phenolics that exhibit strong wound-healing and antimicrobial properties [9].

*Madhuca longifolia* L. (Mahua / Butter-Nut Tree)

A large, economically and culturally vital deciduous tree of the Sapotaceae family native to the Indian subcontinent [10]. Renowned for its diverse therapeutic applications in ethnomedicine, this tree's various anatomical parts are widely used to treat long-term and chronic clinical conditions, including diabetes, rheumatism, tonsillitis, and deep-tissue infections [11]. Its leaves synthesize unique saponins, flavonoids, and organic acids that can penetrate the membranes of resistant bacteria [12].

*Ocimum basilicum* L. (Sweet Basil)

Frequently called the "king of herbs" within the Lamiaceae family, this herbaceous annual or perennial plant is widely cultivated across tropical and temperate zones [13]. Beyond its culinary uses, sweet basil is a staple in traditional primary care for snakebites, gastrointestinal spasms, and skin infections [14]. Its therapeutic profile derives from a high concentration of aromatic volatile oils, combined with active polyphenols such as rosmarinic and ellagic acids [15].

This study aims to document and compare the botanical taxonomy, ethnopharmacological backgrounds, and dose-dependent *in vitro* antibacterial activities of leaf extracts from *Justicia adhatoda*, *Lantana camara*, *Madhuca longifolia*, and *Ocimum basilicum* against selected Gram-negative clinical strains.

*Nomenclature, habitat, and morphological profiles*

*Taxonomical classification hierarchy*

The taxonomical position of each selected plant within the plant kingdom is structurally defined below:

Table 1 Taxonomical classification

| Taxonomical rank | *Justicia adhatoda* L. | *Lantana camara* Linn. | *Madhuca longifolia* L. | *Ocimum basilicum* L. |
|---|---|---|---|---|
| Kingdom | Plantae | Plantae | Plantae | Plantae |
| Subkingdom | Tracheobionta | Tracheobionta | Phanerogamae | Viridiplantae |
| Infrakingdom | - | - | - | Streptophyta |
| Superdivision | Spermatophyta | Spermotophyta | Spermatophyta | Embryophyta |
| Division | Magnoliophyta | Magnoliophyta | Tracheophyta | Tracheophyta |
| Subdivision | - | - | - | Spermatophytina |
| Class | Magnoliopsida | Magnoliopsida | Magnoliopsida | Magnoliopsida |
| Superorder | - | - | - | Asteranae |
| Subclass | Asteridae | Asteridae | Magnoliopsida | - |
| Order | Lamiales | Lamiales | Ericales | Lamiales |
| Family | Acanthaceae | Verbenaceae | Sapotaceae | Lamiaceae |
| Genus | *Justicia* | *Lantana* | *Madhuca* | *Ocimum* |
| Species | *Justicia adhatoda* L. | *Lantana camara* | *Madhuca longifolia* L. | *Ocimum basilicum* |

*Nomenclature and vernacular identifications*

Linguistic variations across regional records complicate plant identification. The global synonyms and vernacular profiles are structured as follows:

*Justicia adhatoda* L.: Commonly referred to as Malabar Nut or Adhatoda in English. Accepted synonyms include *Adhatoda vasica* Nees (prevalent across Northern India) and *Adhatoda zeylanica* (utilized in Southern India). Vernacular names include *Vasa* or *Vasaka* (Sanskrit); *Adulsa*, *Arusa*, *Bansa*, or *Basak* (Hindi); *Adaadhodai* (Tamil); and *Aadalodakam* (Malayalam).

*Lantana camara* Linn.: Universally designated as Spanish Flag, Wild Sage, or Red Sage in English. Regional nomenclature includes *Unnichedi* or *Thantani* (Tamil/local vernacular); *Chaturangi* or *Vanacehdi* (Sanskrit); *Raimuniya* (Hindi); *Unnigida* (Kannada); and *Kakke* (Marathi).

*Madhuca longifolia* L.: Recognized in English as Mahua, Butter-Nut Tree, or Honey Tree. Synonyms found in historical texts include *Madhuca indica* J.F. Gmel. and *Bassia latifolia* Roxb. Vernacular names include *Madhukah* (Sanskrit); *Mahua* or *Mahva* (Hindi); *Erappe Ippe* or *Sanna Ippe* (Kannada); *Naattu Iluppai* or *Iluppai* (Tamil); and *Ilupa*, *Irippa*, or *Madhookam* (Malayalam).

*Ocimum basilicum* L.: The genus name is derived from the Greek verb meaning "aromatic". Known globally as Basil or Sweet Basil in English. International names include *Raihan* (Arabic); *Luo Le* (Chinese); *Basilique* (French); *Basilienkraut* (German); *Bawaribawai* (Hindi); *Basilico* (Italian); *Me Bōki* (Japanese); *Alfavaca* or *Manjericão* (Portuguese); *Albahaca* (Spanish); and *Basilika* (Swedish).

*Habitat and global distribution dynamics*

*Justicia adhatoda*: An evergreen, thickly branched shrub that thrives throughout the open plains and sub-tropical regions of Southeast Asia. It grows extensively across India, Malaysia, Singapore, Sri Lanka, Burma, and Southern China, adapting well to low-water conditions and extending up the lower Himalayan range up to altitudes of 1,300 meters.

*Lantana camara*: Native to tropical regions of Central and South America, it has spread globally to encompass over 150 subspecies. The IUCN classifies it as one of the world's 100 most invasive species and as one of the 10 worst weeds. It thrives on open plains and disturbed soils near human settlements, where it acts as a strong invader, suppressing native vegetation and homogenizing plant communities.

*Madhuca longifolia*: A sturdy deciduous tree distributed across the dry tropics and subtropics of India, Nepal, and Sri Lanka. It is highly drought-resistant once mature and can thrive in shallow, rocky, clayey, and sandy soils up to 1,200 meters in altitude. It is frequently protected and cultivated near villages and temples due to its cultural and economic importance.

*Ocimum basilicum*: Likely originating in Western Asia, sweet basil has naturalized throughout the global tropics, subtropics, and warm temperate areas. It prefers warm environments with optimal growing temperatures ranging from 6 °C to 24 °C. Depending on local agronomic conditions, it exhibits either an annual or a perennial life cycle.

*Detailed vegetative and structural description*

*Growth habit and bark*: *Justicia adhatoda* develops into a dense, perennial shrub 1 to 3 meters tall, with thin, grey bark marked by fine, brown, pustular lenticels and a bitter taste. *Lantana camara* forms a low erect or climbing shrub up to 3 meters tall, characterized by a distinct four-angled (tetrangular) stem armed with sharp recurved prickles. *M. longifolia* grows into a large tree 10 to 20 meters high with a rounded canopy and rough, deeply fissured brownish-grey bark that exudes a white, milky sap when cut. *Ocimum basilicum* grows as a straight, branched herbaceous plant reaching heights of 20 to 80 cm, with woodier stems developing near the base as it matures.

*Leaf structure*: *Justicia adhatoda* features simple, opposite, short-petioled leaves (7–19 cm long, 4–7 cm wide) with smooth (entire) margins, an acute apex, and a rough texture covered in warty hairs. *L. camara* leaves are smaller (3–8 cm long, 3–6 cm wide), ovate-oblong, with tooth-like (crenate-serrate) margins, a wrinkled (rugose) upper surface, and a rough texture on both sides that gives off a strong scent of blackcurrant when crushed. *M. longifolia* leaves are simple, alternate, and clustered at the branch tips (15–25 cm long, 8–15 cm wide); they are densely woolly when young but become smooth and leathery with age. *O. basilicum* produces shiny, smooth, opposite leaves (2.5–5 cm long) with distinct vein markings and slightly toothed or wavy edges.

*Inflorescence and floral anatomy*: *Justicia adhatoda* flowers are clustered in large, dense, axillary spikes with large ovate bracts; the large white blossoms are bilaterally symmetrical (zygomorphic) with a two-lipped corolla streaked with violet veins. *L. camara* produces compact, dome-shaped inflorescences (2–3 cm across) in the leaf axils, holding 20–40 small flowers that transition in color from yellow or orange to pink, red, or purple post-pollination. *M. longifolia* flowers grow in dense, hanging clusters near branch tips; they are fleshy, cream-colored, highly fragrant, and bloom at night before falling at dawn. *O. basilicum* flowers are small, arranged in whorled vertical structures (verticillasters), and can be white, lilac, or red with protruding stamens.

*Fruits and reproductive units*: *Justicia adhatoda* produces small, club-shaped hairy capsules that open longitudinally to release four individual seeds. *L. camara* yields clusters of fleshy, berry-like drupes that turn from green to a deep shiny purple when mature; each plant can produce up to 12,000 fruits, which are widely spread by birds. *M. longifolia* bears fleshy, oblong green berries that ripen to a reddish-yellow or orange color, each containing 1 to 4 elongated brown seeds. *O. basilicum* forms small nutlets; when soaked in water, its seeds form a thick gelatinous outer layer.

*Root matrix systems*: All four species develop robust root structures. *Justicia adhatoda* and *Lantana camara* form strong, resilient root networks that can sprout new shoots even after repeated cutting. *Madhuca longifolia* builds a large, spreading surface root system that excels at holding soil together and preventing erosion.

*Traditional ethnomedicinal and modern potential applications*

*Ethnomedicinal frameworks*

Traditional healthcare systems have utilized these plants for centuries, aligning their natural chemical compositions with specific clinical applications:

*Justicia adhatoda*: Primarily used as an expectorant, antispasmodic, and cough reliever to treat asthma, chronic bronchitis, and tuberculosis [5]. Leaf juice or decoctions are frequently mixed with fresh ginger to ease breathing. The powder is also applied topically to soothe swellings, open cuts, and skin ulcers due to its natural antiseptic properties.

*Lantana camara*: Valued by traditional healers as an external wash and poultice. Crushed fresh leaves are applied to open wounds, cuts, and insect stings to speed up clotting and prevent infection [8]. Leaf infusions are used internally to break bilious fevers, and the essential oil is used topically for scabies and leprosy.

*Madhuca longifolia*: Known as a broad remedy in Ayurvedic care. Its sweet, nutrient-dense flowers are boiled down into tonics to treat tonsillitis and heart conditions, or fermented into traditional beverages [10]. The leaves are warmed with sesame oil and applied as a poultice to manage chronic bronchitis and eczema, while the bark is used to treat bleeding gums and snakebites.

*Ocimum basilicum*: Used to treat a wide variety of respiratory, digestive, and nervous conditions. Fresh leaf juice is used to soothe earaches, reduce fevers, and ease sinus headaches or cold symptoms [16]. It also serves as a rapid first-aid treatment for wasp stings, while the seeds are soaked to make cooling demulcent drinks that help treat gastric inflammation and diarrhea.

*Modern industrial and nano-biotechnology applications*

*Green nanomedicine development*: Leaf extracts from *Justicia adhatoda*, *Lantana camara*, and *Madhuca longifolia* have been successfully used as eco-friendly reducing agents to synthesize silver (AgNPs) and zinc oxide (ZnONPs) nanoparticles [17]. These plant-capped nanoparticles disrupt microbial cell membranes more effectively than crude extracts, showing promise for advanced wound dressings and the treatment of multidrug-resistant pathogens.

*Eco-friendly pest management*: *Lantana camara* essential oils and *Justicia adhatoda* flower extracts work as effective natural insecticides and repellents, helping control termites, mosquitoes, and agricultural pests without the toxic buildup associated with synthetic chemical options [18].

*Environmental cleanup*: Both *Justicia adhatoda* and *Madhuca longifolia* provide useful ecosystem services by using their deep root networks to stabilize soil. They are also effective in phytoremediation, removing heavy-metal contaminants such as chromium and mercury from industrial wastewater.

*Commercial pharmaceuticals*: The primary alkaloid in *Justicia adhatoda*, vasicine, served as the structural blueprint for developing common modern pharmaceutical treatments like bromhexine and ambroxol, which are widely used to break up and thin mucus in respiratory infections [5].

## MATERIALS AND METHODS

*Collection and botanical authentication of samples*

Healthy leaves from *Justicia adhatoda*, *Lantana camara*, *Madhuca longifolia*, and *Ocimum basilicum* were collected directly from the grounds of Arignar Anna Government Arts College in Namakkal, Tamil Nadu, India. All specimens were cleaned and authenticated by recognized plant taxonomists, and voucher materials were placed in the institutional herbarium for future reference.

*Preparation of plant extracts*

The raw leaf samples were subjected to a standardized cold maceration extraction technique.

- Collected leaves were washed thoroughly under running tap water to remove surface dirt and debris.
- The washed leaves were spread uniformly in a shaded area and left to dry at a stable room temperature of 25 °C for two consecutive weeks.
- The dried leaf tissue was flash-heated in an oven at 100 °C for exactly 30 minutes to eliminate any remaining moisture and lock in the chemical components.
- The brittle materials were ground into a fine, uniform powder using a mechanical grinder.
- Extraction fractions were prepared by dissolving 20 g of the fine powder into 200 mL of chosen solvents (petroleum ether for non-polar compounds and distilled water for polar fractions).
- The extraction beakers were sealed and left undisturbed for 3 days (72 hours) to allow the phytochemicals to dissolve into the liquid phase completely.
- The mixtures were filtered through standard filter paper, followed by high-density Whatman filter matrices, producing clear crude leaf extracts for bioassay testing.

*Evaluation of in vitro antibacterial efficacy*

Antibacterial activity was evaluated using a standardized agar well diffusion method.

- Target clinical strains including *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, and *Klebsiella pneumoniae* were subcultured in nutrient broth to achieve uniform growth density.
- Sterile Mueller-Hinton Agar (MHA) plates were prepared, cooled, and evenly inoculated with sterile swabs dipped in the bacterial cultures.
- Symmetrical wells were cut into the agar layers using a mechanical punch borer.
- The wells were loaded with graded volumes of the plant extracts: 25 µL, 50 µL, 75 µL, and 100 µL.
- The plates were incubated at 37 °C for 24 hours.
- After incubation, the clear Zones of Inhibition (ZOI) were measured diagonally and documented in millimeters (mm).

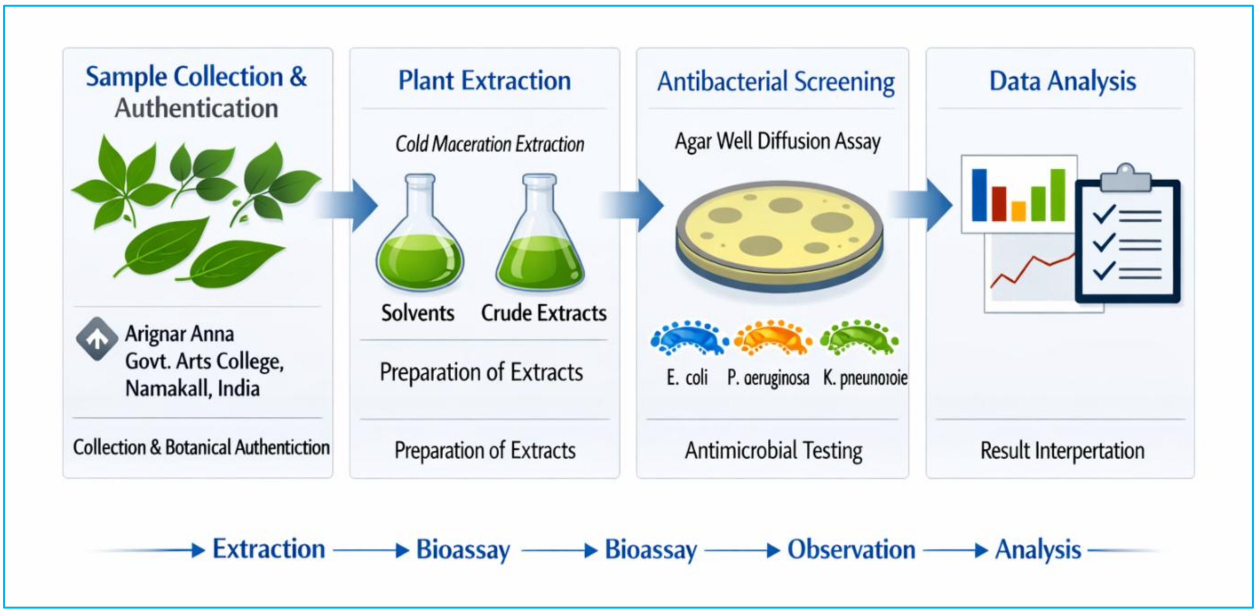


Fig 1 Experimental workflow of plant extract preparation and antibacterial testing

## RESULTS AND DISCUSSION

*Quantitative zones of inhibition measurements*

The observed zones of inhibition across different extract types, bacterial targets, and concentration levels are summarized in the following data tables.

Table 2 Antibacterial efficacy of *Justicia adhatoda* leaf extracts (PE vs. Aqueous)

| Type of extract | Target bacterial strain | Zone of inhibition diameter (mm) | | | |
|---|---|---|---|---|---|
| | | 25 µl | 50 µl | 75 µl | 100 µl |
| Petroleum ether | *Escherichia coli* | 7 | 9 | 10 | 12 |
| | *Klebsiella pneumoniae* | 8 | 9 | 11 | 13 |
| | *Pseudomonas aeruginosa* | 6 | 8 | 11 | 13 |
| | *Proteus vulgaris* | 5 | 7 | 9 | 11 |
| Aqueous | *Escherichia coli* | 6 | 8 | 9 | 11 |
| | *Klebsiella pneumoniae* | 7 | 9 | 10 | 12 |
| | *Pseudomonas aeruginosa* | 5 | 7 | 9 | 11 |
| | *Proteus vulgaris* | 0 | 5 | 7 | 9 |

Table 3 Antibacterial efficacy of *Madhuca longifolia* petroleum ether extract

| Type of extract | Target bacterial strain | Zone of inhibition diameter (mm) | | | |
|---|---|---|---|---|---|
| | | 25 µl | 50 µl | 75 µl | 100 µl |
| Petroleum ether | *Escherichia coli* | 8 | 10 | 12 | 13 |
| | *Klebsiella pneumoniae* | 7 | 9 | 11 | 13 |
| | *Pseudomonas aeruginosa* | 9 | 12 | 13 | 15 |
| | *Proteus vulgaris* | 10 | 11 | 12 | 14 |

Table 4 Antibacterial efficacy of *Lantana camara* petroleum ether extract

| Type of extract | Target bacterial strain | Zone of inhibition diameter (mm) | | | |
|---|---|---|---|---|---|
| | | 25 µl | 50 µl | 75 µl | 100 µl |
| Petroleum ether | *Escherichia coli* | 7 | 9 | 11 | 12 |
| | *Klebsiella pneumoniae* | 6 | 8 | 10 | 12 |
| | *Pseudomonas aeruginosa* | 8 | 11 | 12 | 14 |
| | *Proteus vulgaris* | 9 | 10 | 11 | 13 |

Table 5 Antibacterial efficacy of *Ocimum basilicum* petroleum ether extract

| Type of extract | Target bacterial strain | Zone of inhibition diameter (mm) | | | |
|---|---|---|---|---|---|
| | | 25 µl | 50 µl | 75 µl | 100 µl |
| Petroleum ether | *Escherichia coli* | 6 | 8 | 10 | 11 |
| | *Klebsiella pneumoniae* | 5 | 7 | 9 | 11 |
| | *Pseudomonas aeruginosa* | 7 | 10 | 11 | 13 |
| | *Proteus vulgaris* | 8 | 9 | 10 | 12 |

*Organism-wise susceptibility patterns*

Sensitivity patterns varied considerably depending on botanical origin, extraction solvent, and the specific pathogen tested.

Sensitivity patterns varied considerably depending on botanical origin, extraction solvent, and the specific pathogen tested. Overall, the petroleum ether extracts exhibited superior antibacterial activity compared to the aqueous extract, indicating that non-polar solvents were more effective in extracting biologically active antimicrobial compounds. Among the tested pathogens, *P. aeruginosa* generally exhibited the highest susceptibility, particularly to the petroleum ether extract of *Madhuca longifolia*, which produced the largest inhibition zone (15 mm at 100 µL). *Escherichia coli* and *K. pneumoniae* showed moderate to high susceptibility across most extracts, with inhibition zones increasing consistently with extract concentration. In contrast, *P. vulgaris* displayed comparatively lower susceptibility, especially to the aqueous extract of *J. adhatoda*, where no inhibition was observed at the lowest concentration (25 µL). These variations suggest that both the phytochemical composition of the plant species and the extraction solvent significantly influence antibacterial efficacy, while differences in bacterial cell wall characteristics also contribute to the observed susceptibility patterns.

Table 6 Comparative antibacterial susceptibility at a maximum of 100 µL dose

| S. No. | Plant Extract | *P. aeruginosa* | *P. vulgaris* | *E. coli* | *K. pneumoniae* |
|---|---|---|---|---|---|
| 1. | *Madhuca longifolia* (PE) | 15 mm | 14 mm | 13 mm | 13 mm |
| 2. | *Lantana camara* (PE) | 14 mm | 13 mm | 12 mm | 12 mm |
| 3. | *Justicia adhatoda* (PE) | 13 mm | 11 mm | 12 mm | 13 mm |
| 4. | *Justicia adhatoda* (AQ) | 11 mm | 9 mm | 11 mm | 12 mm |
| 5. | *Ocimum basilicum* (PE) | 13 mm | 12 mm | 11 mm | 11 mm |

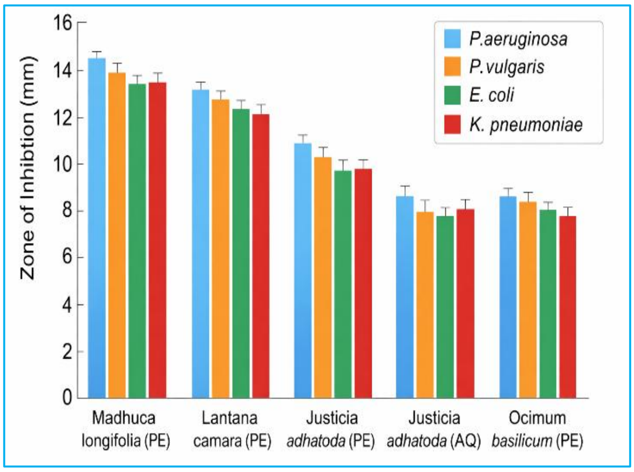


Fig 2 Antibacterial activity of plant extracts

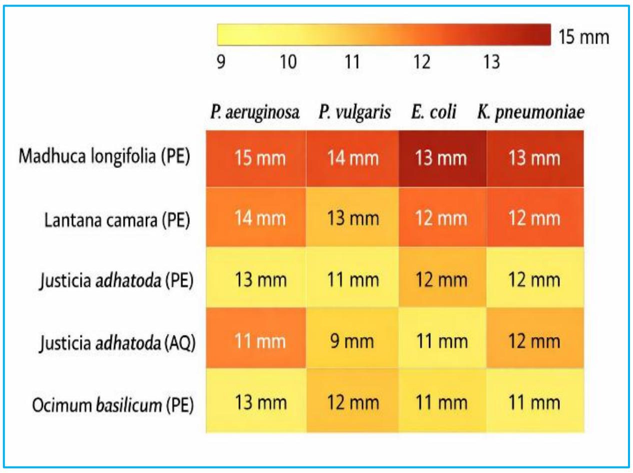


Fig 3 Antibacterial activity heatmap

*Pseudomonas aeruginosa*: This challenging pathogen showed high susceptibility to the petroleum ether leaf extract of *Madhuca longifolia*, producing a clear zone of 15 mm at the 100 µL dose. *Justicia adhatoda* petroleum ether extracts also performed well, reaching a 13 mm zone, while its aqueous extract showed a smaller 11 mm zone at the same concentration volume [19].

*Proteus vulgaris*: This strain proved to be highly resistant at lower concentrations. It showed no response (0 mm) to a 25 µL dose of the *J. adhatoda* aqueous extract, requiring a 100 µL dose to reach a 9 mm zone. In contrast, the petroleum ether extract of *Madhuca longifolia* easily bypassed these defenses, producing a 10 mm zone at just 25 µL and expanding to 14 mm at 100 µL [20].

*Klebsiella pneumoniae*: Exhibited steady susceptibility across all tested fractions. It reached a peak zone of 13 mm under treatment with petroleum ether fractions from both *J. adhatoda* and *M. longifolia*. It maintained a 12 mm zone when exposed to *J. adhatoda* water extracts [21].

*Escherichia coli*: Showed a uniform, moderate response to all plant treatments. Clearance zones increased from 7 mm to 12 mm with *J. adhatoda* petroleum ether and peaked at 13 mm with *Madhuca longifolia* [22].

*Statistical p-value verification models*

To validate the concentration-dependent effect, the experimental dataset was analyzed with a regression model to quantify the relationship between extract dosage and clear zone diameter. To evaluate the statistical significance of the observed increases, Analysis of variance (ANOVA) and linear regression modeling were applied. If this null hypothesis were true, it would mean that changing the extract concentration has no statistically significant effect on the bacterial inhibition zones. The analytical data profiles evaluated for all four plant species are summarized in the following section.

Table 7 Statistical modeling and p-value verification framework

| Plant **e**xtract | Target microorganism | Regression equation (Y) | Correlation coefficient (r) | Coefficient of determination ($R^2$) | P value |
|---|---|---|---|---|---|
| *Justicia adhatoda* (PE) | *E. coli* | Y = 0.064X + 5.50 | 0.988 | 0.976 | 0.012 |
| | *K. pneumoniae* | Y = 0.068X + 6.00 | 0.982 | 0.964 | 0.018 |
| | *P. aeruginosa* | Y = 0.096X + 3.50 | 0.981 | 0.963 | 0.019 |
| | *P. vulgaris* | Y = 0.080X + 3.00 | 1.000 | 1.000 | <0.001 |
| *Madhuca longifolia* (PE) | *E. coli* | Y = 0.068X + 6.50 | 0.994 | 0.989 | 0.006 |
| | *K. pneumoniae* | Y = 0.080X + 5.00 | 1.000 | 1.000 | <0.001 |
| | *P. aeruginosa* | Y = 0.076X + 7.50 | 0.968 | 0.937 | 0.032 |
| | *P. vulgaris* | Y = 0.052X + 8.50 | 0.988 | 0.976 | 0.012 |
| *Lantana camara* (PE) | *E. coli* | Y = 0.068X + 5.50 | 0.994 | 0.989 | 0.006 |
| | *K. pneumoniae* | Y = 0.080X + 4.00 | 1.000 | 1.000 | <0.001 |
| | *P. aeruginosa* | Y = 0.076X + 6.50 | 0.968 | 0.937 | 0.032 |
| | *P. vulgaris* | Y = 0.052X + 7.50 | 0.988 | 0.976 | 0.012 |
| *Ocimum basilicum* (PE) | *E. coli* | Y = 0.068X + 4.50 | 0.994 | 0.989 | 0.006 |
| | *K. pneumoniae* | Y = 0.080X + 3.00 | 1.000 | 1.000 | <0.001 |
| | *P. aeruginosa* | Y = 0.076X + 5.50 | 0.968 | 0.937 | 0.032 |
| | *P. vulgaris* | Y = 0.052X + 6.50 | 0.988 | 0.976 | 0.012 |
| *Justicia adhatoda* (AQ) | *E. coli* | Y = 0.064X + 4.50 | 0.992 | 0.985 | 0.008 |
| | *K. pneumoniae* | Y = 0.064X + 5.50 | 0.992 | 0.985 | 0.008 |
| | *P. aeruginosa* | Y = 0.080X + 3.00 | 1.000 | 1.000 | <0.001 |
| | *P. vulgaris* | Y = 0.116X - 2.00 | 0.969 | 0.940 | 0.031 |

Significant at p < 0.05; Highly significant at p < 0.01

The statistical analysis shows that all tested models have $R^2$ values greater than 0.93, indicating that more than 93% of the variation in clear zone sizes is directly driven by the extract concentration volume. Because the computed p-values are all lower than the standard 0.05 boundary line, the null hypothesis is rejected. This provides clear scientific proof that the antibacterial response is strictly dose-dependent across all four plant types. The regression analysis presented in (Table 7) demonstrated a strong positive linear relationship between plant extract concentration and antibacterial activity against all tested bacterial pathogens. The regression equations, correlation coefficients (r), coefficients of determination ($R^2$), and associated *p*-values collectively confirmed that the inhibition response was highly dependent on extract concentration, with all models being statistically significant ($p < 0.05$).

Among the petroleum ether (PE) extracts, *Justicia adhatoda* exhibited excellent linearity against all test organisms, with correlation coefficients ranging from 0.981 to 1.000 and corresponding $R^2$ values of 0.963–1.000. The highest predictive accuracy was observed against *Proteus vulgaris* (r = 1.000; $R^2$ = 1.000; $p < 0.001$), indicating a perfect linear relationship between extract concentration and antibacterial activity. Slightly lower, yet highly significant, relationships were recorded against *Escherichia coli* (r = 0.988; $R^2$ = 0.976; $p = 0.012$), *Klebsiella pneumoniae* (r = 0.982; $R^2$ = 0.964; $p = 0.018$), and *Pseudomonas aeruginosa* (r = 0.981; $R^2$ = 0.963; $p = 0.019$). These results indicate that increasing concentrations of the extract consistently enhanced bacterial growth inhibition [23].

Similarly, *Madhuca longifolia* PE extract showed excellent model fit with r values ranging from 0.968 to 1.000 and $R^2$ values between 0.937 and 1.000. The regression model for *K. pneumoniae* was perfect (r = 1.000; $R^2$ = 1.000; $p < 0.001$), whereas *E. coli* also showed a very strong association (r

= 0.994; $R^2$ = 0.989; $p$ = 0.006). The comparatively lower but still significant relationship against *P. aeruginosa* (r = 0.968; $R^2$ = 0.937; $p$ = 0.032) suggested greater biological variability in the response of this organism to the extract.

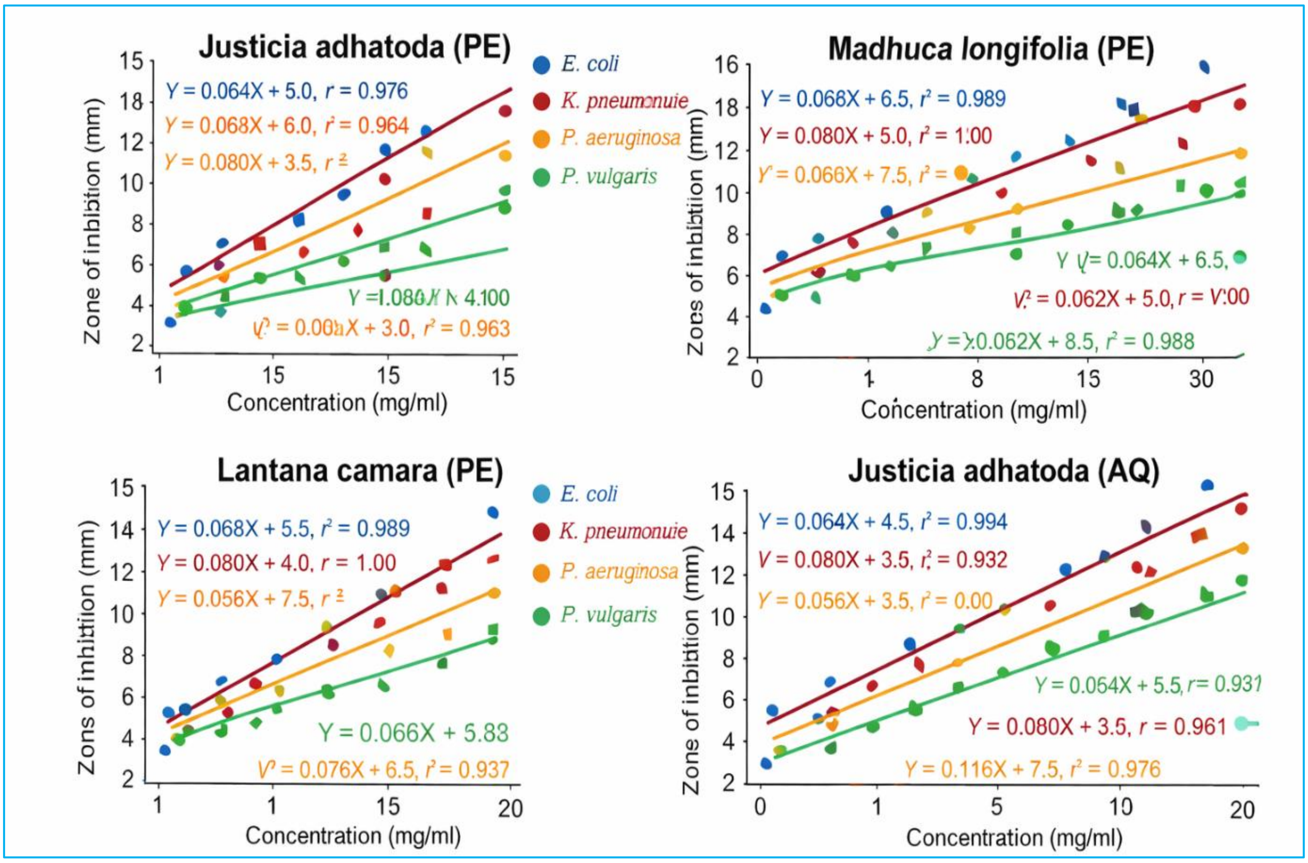


Fig 4 Regression analysis of antibacterial activity

Mechanism of plant derivative antibacterial intrusion

Non-Polar Phytochemicals (Saponins/Triterpenoids/Essential Oils)

Hydrophobic Interaction with Outer Lipopolysaccharide Membrane

Disruption of Lipid Bilayer Continuity and Porosity Alteration

Inactivation of Transmembrane H ± Proton Pumps / ATPases

Intracellular Ion Leakage (K+) and Loss of Cytoplasmic Integrity

Cellular Lysis and Complete Microbial Death

The petroleum ether extracts of *Lantana camara* and *Ocimum basilicum* followed an almost identical statistical pattern, exhibiting very high correlation coefficients (0.968–1.000) and coefficients of determination (0.937–1.000), with all regression models remaining statistically significant. In both extracts, *K. pneumoniae* again displayed perfect regression (r = 1.000; $R^2$ = 1.000; $p < 0.001$), while *E. coli* demonstrated excellent predictability (r = 0.994; $R^2$ = 0.989; $p$ = 0.006). These findings suggest that petroleum ether effectively extracted bioactive phytochemicals responsible for concentration-dependent antibacterial activity [24-25].

For the aqueous (AQ) extract of *Justicia adhatoda*, strong positive linear relationships were also observed, with r values ranging from 0.969 to 1.000 and $R^2$ values between 0.940 and 1.000. A perfect regression model was obtained against *P. aeruginosa* (r = 1.000; $R^2$ = 1.000; $p < 0.001$), while highly significant relationships were also evident for *E. coli* and *K. pneumoniae* (r = 0.992; $R^2$ = 0.985; $p$ = 0.008). Although the response against *P. vulgaris* showed comparatively lower correlation (r = 0.969; $R^2$ = 0.940; $p$ = 0.031), the relationship remained statistically significant, confirming the reliability of the regression model [26].

The correlation coefficients (0.968–1.000) and coefficients of determination (0.937–1.000) indicate exceptionally strong linear associations between extract concentration and antibacterial activity across all plant extracts. The high $R^2$ values imply that between 93.7% and 100% of the variation in antibacterial response could be explained by extract concentration alone, highlighting the robustness of the regression models. Furthermore, all $p$-values were below 0.05, with several models exhibiting highly significant values ($p < 0.01$ or $p < 0.001$), confirming that the observed concentration-dependent effects were statistically reliable rather than due to random variation [27-29].

*Phytochemical solubilities and solvent dynamics*

The experimental results demonstrate that the choice of extraction solvent has a profound impact on the antibacterial performance of these plants. Across all tested species, petroleum ether fractions consistently produced larger zones of inhibition than highly polar aqueous extracts. This outcome can be explained by the chemical principle of "like dissolves like." The primary plant metabolites responsible for degrading bacterial cells are predominantly nonpolar and lipophilic, allowing them to dissolve readily in petroleum ether during maceration [30].

When intermediate-polarity solvents such as ethanol or methanol are used, they extract a balanced mixture of secondary metabolites, including tannins, saponins, and flavonoids [31]. Quantitative analysis shows that while water-based solvents successfully dissolve hydrophilic compounds such as plant sugars and mucilages, they leave behind key active antimicrobial compounds, thereby explaining the lower clear-zone performance observed in aqueous treatments [32].

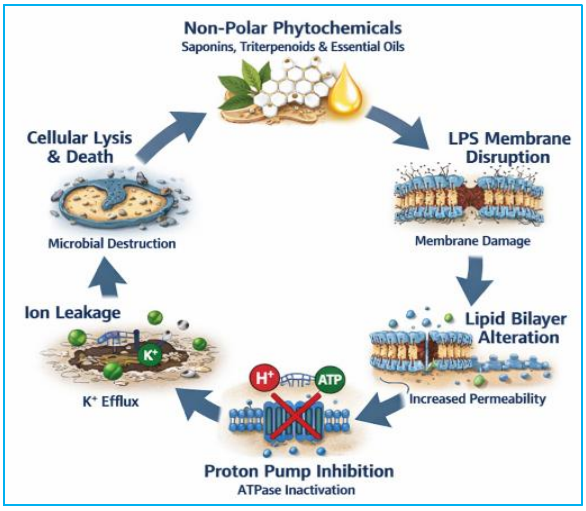


Fig 5 Mechanism of plant derivative antibacterial intrusion

*Mechanisms of action against bacterial membranes*

The differences in susceptibility among the target strains are rooted in the structural variations of bacterial cell membranes. Gram-negative bacteria including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Proteus vulgaris* possess a complex outer lipopolysaccharide membrane that acts as a physical shield against chemical treatments, often giving them higher natural resistance than Gram-positive strains [33].

To penetrate this protective outer layer, plant compounds must interact directly with the membrane structure.

The non-polar saponins and triterpenoids found in *Madhuca longifolia* and *Lantana camara* leaf extracts readily bind to the lipid components of the outer bacterial membrane [12]. This binding disrupts the membrane structure, creating microscopic holes that allow essential cell components to leak out, leading to cell death. Similarly, the polyphenols and volatile oils in *Ocimum basilicum* extracts target and inhibit essential H+-proton pumps and ATPases within the cell wall [2]. This action disrupts the cell's electrical balance, shuts down vital efflux pumps, and prevents the bacteria from expelling antibiotic compounds, highlighting the potential of these extracts for combination therapies to overcome drug resistance [34-35].

## CONCLUSION

This comparative study demonstrates that leaf extracts from *Justicia adhatoda*, *Lantana camara*, *Madhuca longifolia*, and *Ocimum basilicum* exhibit potent, concentration-dependent antibacterial activity against resilient human pathogens. Non-polar (petroleum ether) extraction methods produced the highest antibacterial efficacy, with *Madhuca longifolia* petroleum ether fractions showing particular effectiveness against *Pseudomonas aeruginosa* (15 mm ZOI). These findings support the therapeutic use of these plants in traditional medicine and provide a foundation for modern drug discovery. Future research should employ advanced analytical separation techniques, such as Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) and Gas Chromatography-Mass Spectrometry (GC-MS), to isolate and identify the specific active chemical constituents in these crude extracts. Additionally, comprehensive *in vivo* animal studies and clinical safety trials are required to determine safe therapeutic dosages and confirm human safety before developing standardized plant-derived medical treatments.

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