

## Antimicrobial Activity Of *Murraya Koenigii* And *Zingiber Officinale* Plants On Drug Resistant Pathogens Isolated From Clinical Specimens And Preliminary Phytochemical Analysis Of The Crude Extract Preparations.

Nagesh Malik<sup>1</sup>, Samreen Ahmed<sup>2</sup>

<sup>1,2</sup> Department of Microbiology, V.E.S. College of Arts, Science and Commerce, Chembur, Mumbai 400071, India

Corresponding Author: Nagesh Malik

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**Abstract:** The aim of the present study was to investigate the antimicrobial activity and phytochemical screening of *Murraya koenigii* and *Zingiber officinale* plant extracts. Ethanol and Methanol were used as the solvents for extraction. The antimicrobial activities of these plant extracts were tested against the drug resistant Gram-positive and Gram-negative bacterial strains. The bacteria used in the study were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Salmonella* spp., *Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecium* and *Neisseria* spp. The ethanol and methanol extracts of leaves of *Murraya koenigii* and rhizome extracts of *Zingiber officinale* showed promising results against the drug resistant pathogens with zone diameter of inhibition more than 15mm. The ethanol extracts gave larger zones of inhibition than methanol extracts, suggesting that ethanol is a better solvent in terms of extraction of phytochemicals from the plants. It was also observed from the results that the extracts worked better towards the inhibition of gram positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* than the gram negative bacteria used in the study. The Minimum Inhibitory Concentration was determined for the extracts which gave a zone diameter of inhibition of 15mm or more than 15mm to suggest the minimum therapeutic dose that can inhibit the test pathogens. The Preliminary Phytochemical Analysis revealed the presence of various phytochemicals like Alkaloids, Steroids, Flavanoids, Terpenoids, Reducing Sugars, Glycosides, Tannins, and Phenols in different extracts analyzed. Hence, it could be suggested the antibacterial activity observed in the different extracts tested could be due to the presence of these phytochemicals.

**Keywords:** Antibacterial activity, Drug resistant pathogens, ethanol, methanol, Phytochemical screening

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### I. Introduction

The use of various medicinal plants as a source of therapeutics can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as holly back, these plants are still widely used in ethnomedicine around the world. [1] Treatment of infections is compromised worldwide by the emergence of bacteria that are resistant to multiple antibiotics. New and emerging drug resistant bacterial strains, particularly methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), *Mycobacterium tuberculosis* (MTB), and multidrug resistance (MDR) Gram-negative bacteria, are increasing worldwide and add to the gravity of the situation. Development of resistant bacteria due to the chromosomal mutations is more commonly associated with the horizontal transfer of resistance determinants borne on mobile genetic elements [4]. Plants, particularly ethnomedicinal plants are important sources of natural products. They are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, and flavonoids and have been well-established to possess antimicrobial properties. Many plants have been evaluated not only for their inherent antimicrobial activity, but also for their action as a resistant modifying agent [5]. Ethnomedicine is concerned with the study of medical systems from the native's point of view. The ethnomedical approach proves particularly useful for the study of indigenous therapeutic agents because it allows the researcher to understand treatment patterns according to native explanatory models instead of only through the lens of biomedicine [3]. Plants are rich with a range of secondary metabolites such as tannins, alkaloids, flavonoids and a few more, which have been found in vitro to have antimicrobial properties. A number of phytotherapy manuals and research journals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity on humans [5]. Thereby, plants are now being considered as a source of antimicrobials not only to treat bacterial infections but

also certain parasitic and viral infections. Considering the potency of plants derived drugs requires the research to explore the varied aspects which would remain unexplored [4]. The isolation of bioactive compounds from medicinal plants, based on traditional use or ethnomedical data, is a highly promising potential approach for identifying new and effective antimalarial drug candidates. [7]. In this work antimicrobial activity of *Murraya koenigii* and *Zingiber officinale* plants on drug resistant pathogens isolated from clinical specimens is studied in depth.

## II. Material And Methods

### A. Sample Collection: The following samples of the plants were collected as shown in table 1

**Table No.1:** samples of plants and their sources

Sr No.	Common Name	Scientific Name	Part Used	Source of Collection
1	Curry Leaves	<i>Murraya koenigii</i>	Leaf	Chembur, Market
2	Ginger	<i>Zingiber officinale</i>	Rhizomes	Chembur, Market

### B. Screening of Clinical Pathogens for the drug resistance profile

- **Media used:-** St. Mueller's Hinton Agar  
St. Trypticase Soya Broth
- **Antibiotics:** - Standard Antibiotic Discs impregnated with the test antibiotics which were selected by referring standard CLSI chart.

### C. Plant Tissue Homogenization and Extract Preparation

- **Plant Materials:** - Dried plant leaves of *Murraya koenigii* and Rhizomes of *Zingiber officinale*
- **Reagents:-** 95% Ethanol, 95% Methanol

### D. Antibacterial Activity of the Plant Extract and Determination of Minimum Inhibitory Concentration

- **Media used:-** Muller Hinton Agar
- **Test Samples:-** Plant Extracts
- **Reagents:-** 95% Ethanol, 5% Methanol

### E. Preliminary Phytochemical Analysis of the Plant Extracts

#### Reagents: -

Mercuric chloride, Distilled water, Potassium iodide, 2% H<sub>2</sub>SO<sub>4</sub>, Conc. H<sub>2</sub>SO<sub>4</sub>, Diluted ammonia solution, Fehling's solution A, Fehling's solution B, Chloroform, 5% FeCl<sub>3</sub> in Distilled Water 5% w/v solution of FeCl<sub>3</sub> in 90% alcohol

#### Antibiotic Susceptibility Test (AST)

The clinical pathogens were subjected to Antibiotic Susceptibility Test to determine the antibiotic resistance profile of the isolates, by Kirby Bauer's method using Mueller-Hinton Agar plates. A set of 12 antibiotics were selected to be tested against each test culture. Test cultures which were found to be resistant to four or more than four selected antibiotics were considered as drug resistant pathogens and were selected for further studies.

#### Homogenization and Preparation of plant extracts

Fresh plants parts (leaves or rhizomes) were purchased from local markets or from the residential gardens. They were washed twice, once with tap water and later with distilled water. The washed samples were left to dry in incubator at 55°C for a 24 hours.

The dried samples were made into fine powder by crushing in the mortar and pestle.

50gm of the powdered sample was soaked in 50ml of the solvents methanol and ethanol separately. The extraction was carried out continuously for a period of seven days by placing flasks on the shaker at 150 rpm. The plants extracts was concentrated by reducing the extract volume to approximately 5ml by heating at 64.7 °C and 78.37 °C for methanol and ethanol extracts respectively. The homogenized plant extracts were filtered first through the muslin cloth and then by whatman filter paper. The extracts were stored in screw-cap bottles and stored in refrigerator at 4 °C.

#### Antibacterial Activity of the Plant Extract by Agar Cup Diffusion Method

This was carried out by using standard agar cup diffusion method in which St. Mueller's-Hinton Agar assay plates were used; growth inhibition was measured as diameters of inhibitory zone and compared with the control.

### III. Results And Discussion

#### Antibacterial Susceptibility Test

Amongst the test cultures *E Coli* was found to be resistant to only Nitrofurantoin and sensitive to remaining eleven antibiotics, *Enterococcus faecium* was found to be resistant to Vancomycin , Erythromycin, Tetracycline and Gentamycin, and sensitive to remaining eight antibiotics, Salmonella spp was found to be resistant to only Erythromycin. The remaining test cultures *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Acinetobacter baumannii*, *Proteus mirabilis* and *Neisseria* spp. were found to be resistant to more than four antibiotics amongst the twelve antibiotics tested. Antibiotics for Antibiotic Susceptibility Test for *Neisseria* were selected by referring to the work of Reza Khaltabadi Farahani.

Of the above pathogens tested for their drug resistance profile *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas*, *Proteus mirabilis*, *Neisseria*, *Acinetobacter baumannii* and *Enterococcus faecium* were found to be resistant to four or more antibiotics tested.

*Staphylococcus aureus* remains an important cause of nosocomial blood- borne infection. These organisms are more likely to cause infections on the wards than in intensive care units. In our study, the antibiotic susceptibility test results for *Enterococcus faecium* are in agreement with the study conducted by Lynette M. Johnston et al. Overall, *E. faecium* was found to have a higher prevalence of resistance among the panel antibiotics, particularly tetracycline, vancomycin and erythromycin. It was even found to be resistant to gentamycin. [3]

*Proteus mirabilis* was found to be resistant to trimethoprim, kanamycin, tetracycline, ciprofloxacin and ampicillin. The results of this study are in agreement with the study conducted by Thomas T. Yoshikawa on drug resistant strains prevalent in hospital environment. [8]

*Pseudomonas* spp. was found to be resistant to neticillin, piperacillin, sulphafurazone, trimethoprim and tetracycline antibiotics.

*Acinetobacter baumannii* was found to be resistant a wide spectrum of antibiotics which includes ticarcillin, piperacillin, oxacillin, cefalothin, ceftazidime, nallidixic acid, norfloxacin, erythromycin, clindamycin and nitrofurantoin. *Neisseria* spp. was found to be resistant to penicillin, rifampicin, tetracycline, cefazolin and cephalothin based on the Antibiotic Susceptibility Test results. From a once easily treatable infection, *Neisseria* spp. has evolved into a challenging disease.

#### Antibacterial activity of the plant extracts

In the present study, the antibacterial activity of the ethenolic and the metenolic extracts of the leaves and the rhizome of *Murraya koenigii* (curry leaves), *Zingiber officinale* (Ginger) respectively were tested against the eight drug resistant test cultures/clinical isolates by agar well diffusion method. The results of the antimicrobial activity of the two different extracts are as shown in table 2.

##### Ethanolic and Methanolic extracts of *Zingiber officinale*

The result in Table 2 revealed that, the antimicrobial activity of ethanolic and methanolic extracts of rhizomes of *Zingiber officinale* showed a significant amount of inhibition of the test organisms. Gram positive cultures were found to be inhibited to a greater extent than the gram negative organisms. The zone diameter of inhibition for *Staphylococcus aureus* was 26 mm, 15mm and for *Staphylococcus epidermidis* was 25mm, 12.5 mm for ethanol and methanol extracts. Amongst gram negative organisms *Pseudomonas* spp. was found to be inhibited maximum with zone diameter of inhibition corresponding to 22mm, 19mm for ethanol and methanol extracts. Ethanol was found to be a better solvent than methanol in terms of /with respect to antibacterial activity of the extracts.

**Table No. 2: Antimicrobial activity of Ethanolic and Methanolic extracts of *Zingiber officinale* in terms of Diametr of Zone of Inhibition**

Test Cultures	Rhizomes extracts of <i>Zingiber officinale</i>	
	Ethanol	Methanol
<i>Staphylococcus aureus</i>	26	15
<i>Staphylococcus epidermidis</i>	25	12.5
<i>Pseudomonas</i> spp.	22	19
<i>Acinetobacter baumannii</i>	13.5	12
<i>Enterococcus faecium</i>	13	14
<i>Proteus mirabilis</i>	-	-
<i>Neisseria</i> spp.	-	-

The ethanol and methanol extracts of rhizomes of *Zingiber officinale* demonstrated a substantial amount of activity towards the test pathogens. The gram positive bacteria *Staphylococcus aureus* and *Staphylococcus epidermidis* were inhibited to a greater extent as compared to gram negative bacteria. The gram negative bacteria *Pseudomonas* spp., *Acinetobacter baumannii* and *Enerococcus faecium* were also inhibited by

the extracts of *Zingiber officinale*. *Proteus mirabilis* was found to be resistant to the extract. The major reason for the difference in activity towards gram positive and gram negative bacteria might be due to the differences in the cell wall structure and composition.

According to David G White et al., [5] resistance of the Gram-negative bacteria could be attributed to its cell wall structure. Gram-negative bacteria have an effective permeability barrier, comprised of a thin lipopolysaccharide exterior membrane, which could restrict the penetration of the extruding the plant extract. It has been reported earlier that Gram-negative bacteria are usually more resistant to the plant-origin antimicrobials and even show no effect, compared to Gram-positive bacteria. Gram positive bacteria have a mesh-like peptidoglycan layer which is more accessible to permeation by the extracts. [5] The results of the antibacterial activity of the *Zingiber officinale* proves that ethanol serves as a better solvent over methanol with respect to extraction of bioactive compounds responsible for demonstrating antibacterial activity. The efficiency of ethanol as efficient solvent is evident by comparison of the zone diameter of inhibition for the two solvents. This is similar to the findings of Alo M N et al., who reported that ethanol extracts showed more antibacterial activity against test pathogens than methanol extracts. [1]

The *Zingiber officinale* extracts were found to contain various phytochemicals which includes Saponins, Terpenoid, Glycoside, Reducing sugar and Alkaloids and did not showed the presence of Steroids, Flavanoids and Tannins. The results of the preliminary phytochemical test are considerably in accordance with the test results of Shipra Bhargava et al., [10]

**Ethanolic and Methanolic extracts of *Murraya koenigii***

The result in Table No 3 revealed that, the antimicrobial activity of ethanol and methanol extracts of *Murraya koenigii* leaves has a good antibacterial activity against the test organisms. It had a varying effect against the different test organisms. *Staphylococcus epidermidis* was inhibited maximum with zone diameter of inhibition of 23 mm and 22.5 mm for ethanol and methanol extracts respectively. *Staphylococcus aureus* and *Pseudomonas* spp were unaffected / showed no zone of inhibition. While *Neisseria* spp., *Acinetobacter baumannii*, *Proteus mirabilis* were also inhibited to different extents. Here ethanol as solvent did not show any significant difference in antibacterial activity than methanol.

**Table No. 3:** Antibacterial Activity of Ethanolic and Methanolic extracts of *Murraya koenigii* in terms of Diameter of Zone of Inhibition

Test Cultures	Leaf extracts of <i>Murraya koenigii</i>	
	Ethanol	Methanol
<i>Staphylococcus aureus</i>	-	-
<i>Staphylococcus epidermidis</i>	23	22.5
<i>Pseudomonas</i> spp.	-	-
<i>Acinetobacter baumannii</i>	15	14.5
<i>Enterococcus faecium</i>	20	19.5
<i>Proteus mirabilis</i>	11	11
<i>Neisseria</i> spp.	-	-

The ethanol and methanol extracts of leaves of *Murraya koenigii* were subjected for screening to detect antibacterial activity against the test pathogens. Amongst the gram positive bacteria *Staphylococcus aureus* was found to be resistant to the extracts and *Staphylococcus epidermidis* was seen to be inhibited to a considerable amount with a zone diameter above 20mm. Amongst the group of gram negative bacteria used in the study the extracts showed varying degree of antibacterial activity towards *Enterococcus faecium*, *Proteus mirabilis* and *Acinetobacter baumannii*. The remaining gram negative bacteria *Pseudomonas* spp. and *Neisseria* spp. was found to be resistant to the *Zingiber officinale* rhizome extracts. [8]

The bacteria including *Staphylococcus aureus*, *Pseudomonas* spp. and *Neisseria* spp. might have developed resistance mechanisms to overcome the antibacterial activity of these plant extracts. Particularly, in case of gram negative bacteria the reason for the ineffectiveness of the extracts to inhibit them might be due to the effective permeability barrier comprised of a thin lipopolysaccharide exterior membrane, which could restrict the penetration of the plant extract. [2]

Comparison of the two solvents used in the study in terms of antibacterial activity reflects that ethanol is a better solvent for the phytochemical extraction from *Murraya koenigii* leaves than the methanol.

According to the study conducted by Prashant Tiwari ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material. Since nearly all of the identified components from plants active against microorganisms are aromatic or saturated organic compounds, they are most often obtained through ethanol. [9]

Successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Properties of a good solvent in plant extractions includes, low toxicity, ease of evaporation at low heat, promotion of rapid physiologic absorption of the extract, preservative action, inability to cause the extract to complex or dissociate. The factors affecting the choice of

solvent are quantity of phytochemicals to be extracted, rate of extraction, diversity of different compounds extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, toxicity of the solvent in the bioassay process, potential health hazard of the extractants. Hence, ethanol can be regarded as an optimum solvent for phytochemical extraction from plant parts. [9]

The ethanol and methanol extracts of *Murraya koenigii* were found to contain Reducing Sugars, Saponins, Caretenoids, Steroids and Alkaloids and did not showed the presence of Terpenoids, Phenols, Tannins and flavanoids.

**Table No.4: Preliminary Phytochemical Analysis of the Plant Extracts**

Plant under study Phytochemical Tests Solvents	<i>Zingiber officinale</i>		<i>Murraya koengii</i>	
	EtOH	MeOH	EtOH	MeOH
<b>Alkaloids</b>	+	+	+	+
<b>Steroids</b>	-	-	+	+
<b>Terpenoids</b>	+	+	-	-
<b>Glycosides</b>	+	+	+	+
<b>Phenols</b>	-	-	-	-
<b>Tannins</b>	-	-	-	-
<b>Flavanoids</b>	-	-	-	-
<b>Saponins</b>	-	-	+	+
<b>Reducing Sugars</b>	+	+	-	-
<b>Caretenoids</b>	-	-	+	+

TableNo.4 shows the Preliminary Phytochemical Analysis of the ethanolic and methanolic Plant Extracts *Zingiber officinale* and *Murraya koengii*

#### IV. Conclusion

The preliminary phytochemical analysis was carried out in this study for the plant extracts which unravel the presence of various phytochemicals. The literature on antibacterial activity of plant extracts suggest that these phytochemicals identified plays a key role in inhibiting the bacteria, thereby reflecting the importance of the bioactive phytochemicals, possessing the desired antibacterial activity.

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. In plants, these compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, and phenol compounds, which are synthesized and deposited in specific parts or in all parts of the plant. The plants secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites.

The results of preliminary phytochemical analysis of the plant extracts suggest that these phytochemicals are the major contributing factor towards the antibacterial activity. The Therapeutic value of medicinal plants lies in the various chemical constituents in it. Hence, suggesting an alternative source of therapeutic agent for the treatment of infections caused by the drug resistant pathogens in future. The potential for developing antimicrobials into medicines appears rewarding, from both the perspective of drug development and the perspective of phyto-medicines.

This study dealt with the screening of drug resistance profile of the pathogens obtained from the hospitals and testing the antibacterial activity of the ethanol and methanol extracts of the *Murraya koenigii*, and *Zingiber officinale*. The work also revealed that of the nine bacterial pathogens tested for their drug resistance profile seven of the bacteria were found to be drug resistant. The present work demonstrates the antimicrobial potential of plant extracts by using various solvents. The results of this study reveals that ethanol and methanol extracts of *Murraya koenigii* leaves and *Zingiber officinale* rhizomes posses a considerable amount of antibacterial activity, with ethanol as a solvent showed better activity when compared to methanol extracts. The results also indicate that the plant extracts have little or no antibacterial effect on the Gram-negative bacteria, showing that they do not contain active ingredients against the gram negative organisms. The observed inhibition of Gram-positive bacteria, *Staphylococcus epidermidis* and *Staphylococcus aureus*, suggests that the plant extracts possesses compounds containing antibacterial properties that can effectively suppress the growth when extracted using ethanol as the solvent. Preliminary phytochemical analysis of the extracts revealed the presence of Alkaloids, Terpenoids, Glycosides, Reducing Sugars, Steroids, Phenols, Flavanoids, Caretenoids, etc to varying extent in the extracts. Past work suggest that these phytochemicals are the major contributing factor towards the antibacterial activity of the extracts. Comparisons with related data from the literature

indicate that according to the different methodologies of studies on antibacterial activity, the most diverse outcomes can be obtained. This study provides scientific insight to further determine the antimicrobial principles and investigate other pharmacological properties of the extracts. On the basis of the present finding, *Murraya koenigii* leaves and *Zingiber officinale* rhizomes possess the capabilities of being a good candidate in the search for a natural antimicrobial agent against infections caused by the gram positive bacteria and against some gram negative bacteria.

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