

EVALUATION OF THE ANTIMICROBIAL AND PHYTOCHEMICAL POTENTIAL OF SOME ETHNOMEDICINAL PLANTS

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ABSTRACT

The current study aimed to evaluate the antimicrobial potency of some ethnomedicinal plants: *Cymbopogon martinii*, *Kalanchoe pinnata*, *Morinda pubescens*, and *Vitex negundo*. The plant material (leaves) were shade-dried, coarsely ground, and sequentially extracted using a Soxhlet apparatus with organic solvents such as petroleum ether, acetone, methanol, and water. *In vitro* evaluation of the antimicrobial activity of the leaf extracts was conducted using the disc diffusion method against various gram-positive bacteria, gram negative bacterial and fungal pathogens like *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Aspergillus niger*, and *Candida albicans*. A Vernier calliper was used to measure the resulting zone of inhibition in mm. Results indicated that the petroleum ether extract of *Cymbopogon martinii* exhibited strong antibacterial potential against the tested organisms with a maximum 32 mm zone of inhibition. However, none of the extracts demonstrated activity against *Aspergillus niger*. Further, preliminary phytochemical analysis of various extracts revealed the presence of various types of bioactive metabolites. The study validates the traditional medicinal use of the plants.

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INTRODUCTION

Since antiquity, plants have been used as a fundamental source of herbal medicines. Medicinal plants are the most important

biological resources used to treat various types of ailments. Rural and indigenous peoples persist in using herbal remedies. Plants are effective for healing common

ailments and injuries, supporting food and nutrition, and acting as medicines. Several households still use various medicinal plants for therapeutic purposes. A survey in the Satpuda Hills of Nandurbar district, Maharashtra, focused on the ethnomedical knowledge and practices of the local tribal communities regarding plant species¹.

Jain et al. documented household and medicinal plants in their survey regarding the utilisation of medicinal plants by tribal communities in the Satpuda division, which encompasses the Dhule and Jalgaon districts of Maharashtra. The indigenous tribal people use the reported medicinal herbs to treat a variety of illnesses².

H.G. Champion classified India's forest types for the first time in 1936. That classification was revised by Champion and Seth in 1968 when they published the "Revised Survey of Indian Forest Types"³.

Plants have consistently played an important role in treating infectious ailments due to their availability. The study of medicinal plants for their phytochemical content has been examined and shown to possess antimicrobial properties. Plant species possess both primary and secondary compounds, which contribute to their antimicrobial activity. Plants produce secondary metabolites to protect themselves, and many of these compounds have antimicrobial effects. Secondary

metabolites contributed to the medicinal plants' therapeutic effectiveness, particularly their antimicrobial properties.

The grass species *Cymbopogon martinii* (Roxb.) Wats. is commonly called Roisa or Rosha gavat in the Marathi language. The Poaceae family encompasses the fragrant grasses of the genus *Cymbopogon*, along with various other grasses within this family that yield essential oils beneficial to the pharmaceutical sector. Globally, there are approximately 180 species, subspecies, varieties, and subtypes identified within this group⁴. *Cymbopogon martinii* is a perennial herb characterised by its erect growth habit. The leaves are green, simple, arranged alternately, leaf blade linear, and possess an aromatic scent, measuring 0.5 to 1.0 centimetres in width and 9 to 15 centimetres in length. They are linear or lanceolate in shape, and the spikelets are sessile, exhibiting an elliptic-oblong form⁵⁻⁶. *C. martinii* is referred to as Rohiso in the Navapur region and is considered a rare species⁷. The aromatic and valuable oil-producing plant *Cymbopogon martinii* is located in the talukas of Taloda, Dhadgaon, and Akkalkuwa. In Dhadgaon, this plant is considered sacred during the Ashwattama fair. People here also use it to repel mosquitoes.

Kalanchoe pinnata (Lamk.) Pers. is an ethnomedicinal plant within the Crassulaceae family, referred to as "Panphuti." It is a succulent plant with a branching stem. Leaves smooth, decussate, simple or pinnately 2-5 lobed, fleshy, with crenate margins⁸. In the medical system of Ayurveda, *K. pinnata* is a common herb used as an astringent and carminative. It is useful for vomiting and diarrhoea. This species is found in India's humid and warm climate naturally⁹. Typhoid fever and other bacterial infections, especially those caused by *E. coli*, *B. subtilis*, *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, and *S. typhi*, can be effectively treated with the plant¹⁰.

Morinda pubescens J.E.Sm. holds significant value as a medicinal plant within traditional medicinal systems, including Ayurveda, Siddha, and Unani. *Morinda pubescens*, also referred to as "Aal," is a member of the Rubiaceae family. This is a deciduous tree with black-brown bark. Leaves simple, elliptic, ovate-oblong, acuminate⁶. The ethanolic extract from this plant can serve as a good source of antibiotics to combat different bacterial pathogens¹¹. The fruits of *Morinda pubescens* are edible.

Vitex negundo L. belongs to the family Verbenaceae. There are more than 250 different species of both shrubs and trees in the genus *Vitex*. In India, 14 plant species

have been used as traditional remedies to treat infectious diseases and various ailments¹². *Vitex negundo* is commonly known as "Nirgundi". This is a shrub with quadrangular branches. Leaves opposite, palmately compound with 3-5 foliolate leaflets, elliptic to lanceolate⁸. Different *Vitex* species were collected from various parts of Tamilnadu, India, and were analysed for their antibacterial properties, revealing a broad spectrum of antibacterial activity from the methanol extracts¹³. Patil and Biradar (2011) studied the plant folklore of tribal and rural communities in various parts of the Hingoli forest in Maharashtra. In the forest area, ethnomedicinal plants are employed by the Adanis and tribals, as well as the Andhs and Bhils, within these forested areas¹⁴. Information on plant species was taken from the reference floras, like the Flora of Dhule and Nandurbar Districts (2003); Flora of Nasik District (1991).

MATERIALS AND METHODS

Collection of plant materials:

Ethnomedicinal plants, including *Cymbopogon martinii* (Roxb.) Wats., *Kalanchoe pinnata* (Lamk.) Pers., *Morinda pubescens* J. E. Sm., and *Vitex negundo* L. were collected from the Taloda forested areas and nearby villages. The plant specimens were authenticated by Dr S.K.

Tayade, Dept of Botany at PSGVP Mandal's Science College, Tal. Shahada, District Nandurbar. The voucher specimens are stored in the departmental herbarium as a reference for the future.

Macroscopic evaluation of leaves:

Macroscopic evaluation of the leaves from *C. martinii*, *K. pinnata*, *M. pubescens*, and *V. negundo* was done on attributes such as colour, shape, size, and odour. The study plants are shown as A, B, C and D, respectively, in Fig.1.

Preparation of extracts

Fresh leaves are washed using running tap water, then dried in the laboratory shade. The shade-dried leaves have then been coarsely powdered and subjected to sequential extraction using an apparatus called Soxhlet with multiple solvents such as petroleum ether, acetone, methanol, and aqueous. Employing the maceration method, the extract was obtained. The resulting extracts were evaporated at their respective boiling points and stored in airtight containers for future applications¹⁵⁻¹⁷.

Preliminary phytochemical screening

The analysis of phytochemicals in the leaf extracts was conducted using the standard methods outlined by Harborne (1973, 1984). The leaf extracts underwent preliminary phytochemical screening to determine the presence of different phytoconstituents¹⁸⁻²¹.

Evaluation of Antimicrobial Activity

The antimicrobial activities of eight extracts (two each) from four species of leaves were evaluated using the disk diffusion method. The extracts included petroleum ether and acetone extracts of *Cymbopogon martinii*, petroleum ether and acetone extract of *Kalanchoe pinnata*, acetone and aqueous extracts of *Vitex negundo*, along with acetone and methanol extracts of *Morinda pubescens*. The extracts exhibited activity against both gram-positive bacteria and gram-negative bacteria, and fungi. This microbial-type culture collection was carried out in the lab and comprises gram positive - negative bacteria as well as fungi. Table 1 shows the culture used for antimicrobial activity.

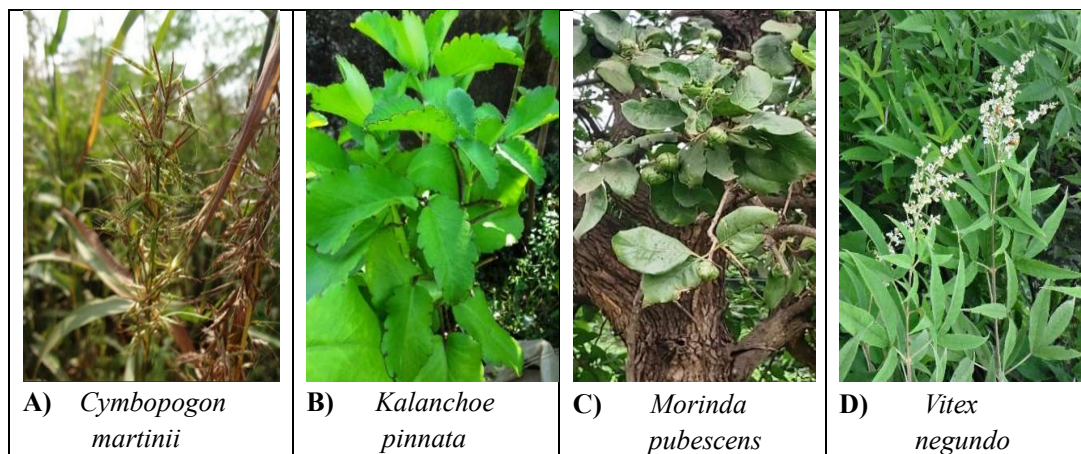


Fig.1 Morphology of ethnomedicinal plants

Table 1 shows the culture used for antimicrobial activity

Microorganisms	Strain Name	Strain reference
Gram-positive bacteria	<i>Bacillus subtilis</i>	NCIM 2063
	<i>Staphylococcus aureus</i>	NCIM 2079
Gram-negative bacteria	<i>Escherichia coli</i>	NCIM 2109
	<i>Proteus vulgaris</i>	NCIM 2172
Fungi	<i>Aspergillus niger</i>	NCIM 1028
	<i>Candida albicans</i>	NCIM 3471

Note: NCIM - National Collection of Industrial Microorganisms, NCL, Pune, Maharashtra

The disc diffusion method was applied to evaluate the antimicrobial activity of leaf extracts. The sterile disks (6 mm) were then placed on the surface of agar plates that contained the appropriate microbial strains after being submerged in a stock solution of extract (100 micrograms per millilitre). The plates were subsequently incubated for 48-72 hours at 27°C for mould, along with 24 hours at 37°C for both bacteria and yeast. A vernier calliper was used to measure the resulting zones of inhibition in millimetres. A standard disc containing 10 µg of chloramphenicol and 50 µg of amphotericin B served as the positive control, and Dimethyl sulfoxide (DMSO) served as the negative control. Nutrient agar is the microbiological medium used for bacteria, while potato dextrose agar is used for fungi²²⁻²³.

RESULTS AND DISCUSSION

In this study, the in vitro antimicrobial activity was performed with petroleum ether, methanol, acetone and aqueous extracts of the leaves of selected ethnomedicinal plants. The majority of the plant extracts showed antimicrobial potential against the various testing organisms.

Macroscopic evaluation: A comprehensive overview of the macroscopic characteristics of leaves, comprising size, colour, shape, and odour, is shown in Table 2.

Table 2: Macroscopic evaluation of leaves

Sr. No	Characters	Observation			
		<i>Cymbopogon martinii</i>	<i>Kalanchoe pinnata</i>	<i>Morinda pubescens</i>	<i>Vitex negundo</i>
1	Color	Dark green	Green	Green	Green
2	Shape	Leaf blade linear, lanceolate, tapering to a very fine point.	Elliptical, Curved, Crenate margins.	Simple, lamina elliptic-oblong, margin entire.	Palmately compound, apex acuminate.
3	Odor	Aromatic	Sweet and sour	Unpleasant	Astringent
4	Size	Varying in size up to 40 cm long	Varying in size, 8-20 cm long	10-12 cm long	2 to 6 cm long, 1 to 2 cm wide.

The *Cymbopogon martinii* is a fragrant, therapeutic plant with simple, green, aromatic leaves and linear, thin blades. The *Kalanchoe pinnata*, sometimes called Panphuti, has simple, green leaves that vary in size and have a sweet and sour odour. The leaves of *Morinda pubescens* are decussate, green, and taper to a point at the margins. The apex is acute to acuminate. The leaves of *Vitex negundo* are green, palmately compound, and have a pointed apex. They are 1-2 cm wide, 2-6 cm long, and narrowly oblong to lanceolate in shape.

Extraction of Yield

The leaves of four medicinal plants were extracted with a Soxhlet apparatus using petroleum ether, acetone, methanol, and water. The yield results of the leaf extract are shown in Table 3.

Table 3: Extraction yield of various leaves of medicinal plants

1) Extraction yield of *Cymbopogon martinii* leaves

Sr. No.	Extractives	Yield (%)
i	Petroleum ether extracts	3.19 %
ii	Acetone extracts	3.79 %

2) Extraction yield of *Kalanchoe pinnata* leaves

Sr. No.	Extractives	Yield (%)
i	Petroleum ether extracts	2.92 %
ii	Acetone extracts	3.80 %

3) Extraction yield of *Morinda pubescens* leaves

Sr. No.	Extractives	Yield (%)
i	Acetone extracts	3.4 %
ii	Methanol extracts	7.78 %

4) Extraction yield of *Vitex negundo* leaves

Sr. No.	Extractives	Yield (%)
i	Acetone extracts	3.19 %
ii	Aqueous extracts	3.79 %

Preliminary Phytochemical Analysis of Leaf Extracts

The reactivity exhibited by phytochemicals present in an extract serves as the foundation for chemical assays aimed at identifying various constituents. The leaf extract was subjected to a preliminary analysis to determine the presence of different phytochemicals. A qualitative phytochemical analysis of various leaf extracts from ethnomedicinal plants revealed the presence of a range of phytochemicals, with the maximum presence in phytosterols, followed by flavonoids, alkaloids, and tannins. The results of the qualitative phytochemical tests are presented in Table 4.

The leaf extracts derived from *Cymbopogon martinii* indicated that the acetone extract had a higher concentration of phytoconstituents compared to the petroleum ether extract. Similarly, leaf extracts of *Kalanchoe pinnata* had a higher concentration of phytoconstituents in the acetone extract than the petroleum ether extract. In contrast, the leaf extracts of *Morinda pubescens* indicated that more phytoconstituents were present in the aqueous extract than in the acetone extract. Lastly, leaf extracts of *Vitex negundo* also demonstrated a higher concentration of phytoconstituents in the acetone extract than in the other extracts. Overall, the four plant extracts exhibited a variety of phytochemicals, including alkaloids, flavonoids, tannins, carbohydrates, anthocyanins, proteins, reducing sugars, and phenolic compounds.

Table 4: Qualitative Phytochemical Analysis of Different Leaf Extracts

Sr. No	Plant Names and extracts of leaves	Phytochemicals
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		Alkaloids	Flavonoids	Tannins	Phytosterols	Anthocyanins	Carbohydrates	Protein	Amino acids	Reducing Sugar	Phenolic compound
1	<i>Cymbopogon martinii</i>										
i	Petroleum Ether extract	+	+	+	+	-	-	-	-	-	-
ii	Acetone extract	-	+	+	+	+	+	-	-	-	+
2	<i>Kalanchoe pinnata</i>										
i	Petroleum Ether extract	+	+	+	+	-	-	-	-	-	-
ii	Acetone extract	+	+	+	+	-	-	+	-	+	-
3	<i>Morinda pubescens</i>										
i	Acetone extract	-	+	+	+	-	-	-	-	-	+
ii	Methanol extract	+	+	-	+	-	+	+	-	+	-
4	<i>Vitex negundo</i>										
i	Acetone extract	+	-	+	+	-	-	-	-	-	+
ii	Aqueous extract	+	+	-	+	+	+	+	+	+	-

Note: (+) sign indicates presence and (-) sign indicates absence of phytoconstituent.

Antimicrobial Screening

The leaves of medicinal plants, *Cymbopogon martinii*, *Kalanchoe pinnata*, *Morinda pubescens*, and *Vitex negundo*, collected from the research area, were extracted using petroleum ether, acetone, methanol, and aqueous solvents to evaluate their antimicrobial activity. The microbes that were studied included the gram-positive bacteria *Bacillus subtilis* and *Staphylococcus aureus*, the gram-negative bacteria *Escherichia coli* and *Proteus vulgaris*, and the fungi *Aspergillus niger* and *Candida albicans*.

Antimicrobial activity was mainly focused on investigating the antibacterial and antifungal potentials of various extracts from different plant leaves. To evaluate the prepared solvent extract, the mentioned plant species were screened against the following organisms and compared with their +ve and -ve controls. To examine antimicrobial activity of leaf extracts, the selected microorganisms, such as *Bacillus subtilis* (NCIM 2063), *Staphylococcus aureus* (NCIM 2079), *Escherichia coli* (NCIM 2109), *Proteus vulgaris* (NCIM 2172), *Aspergillus niger* (NCIM 1028), and *Candida albicans* (NCIM 3471), were used. The study is mainly focused on the zone of inhibition of microbial growth. The different plant species of leaves

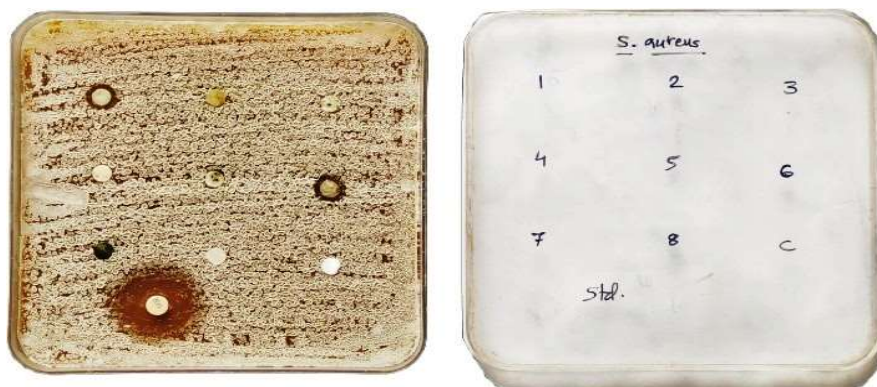
showed antimicrobial activities against these microorganisms, as presented in Table 5.

From the result, it is evident that the leaf extract of acetone shows the maximum activity of *Proteus vulgaris* against *V. negundo* leaves, and the aqueous extract shows the minimum activity of *S. aureus*. The measurement of the inhibition zones was conducted by excluding the diameter of the well. In all the extracts, the zone of inhibition expanded as the concentration increased. Various extracts obtained from selected ethnomedicinal plants were studied for their antimicrobial activity. The results from the antimicrobial evaluation of various leaf extracts are given in Table 5.

The extracts of various leaves in Figures 1 to 6 are labelled as follows: *C. martinii* leaf extract: No. 1 is labelled as petroleum ether extract, and No. 2 is labelled as acetone extract. *K. pinnata* leaf extract: No. 3 is labelled as petroleum ether extract, and No. 4 is labelled as acetone extract. *M. pubescens* leaf extract: No. 5 is labelled as acetone extract, and No. 6 is labelled as methanol extract. *V. negundo* leaf extract: No. 7 is labelled as acetone extract, and No. 8 is labelled as aqueous extract. Also, 'C' shows control and 'Std' indicates standard. This labelling helps identify the different extracts used in the figures.



Fig. 1 *Bacillus subtilis*



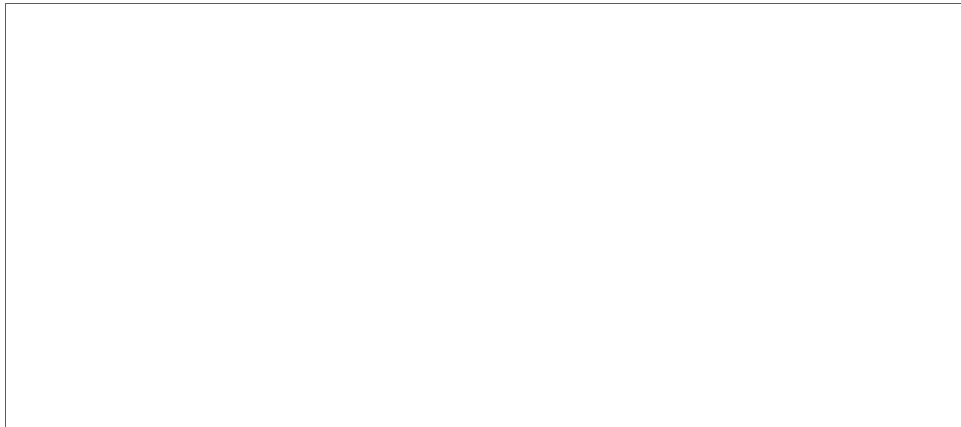


Fig. 2 *Staphylococcus aureus*



Fig. 3 *Escherichia coli*

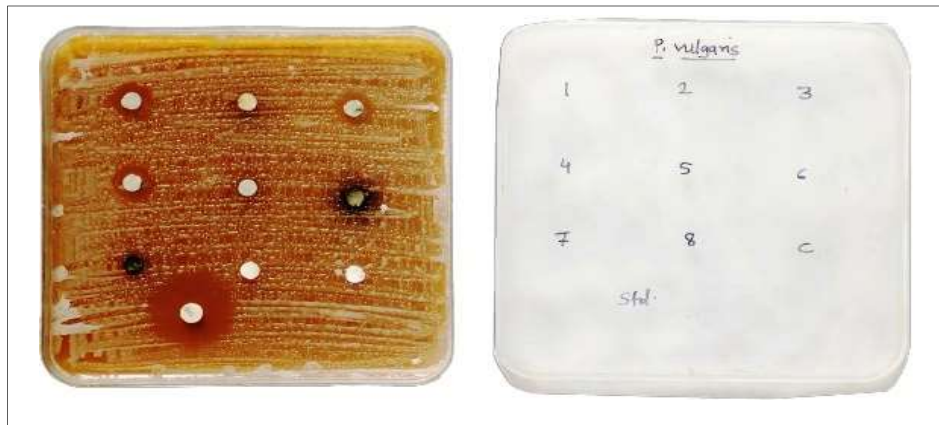


Fig. 4 *Proteus vulgaris*

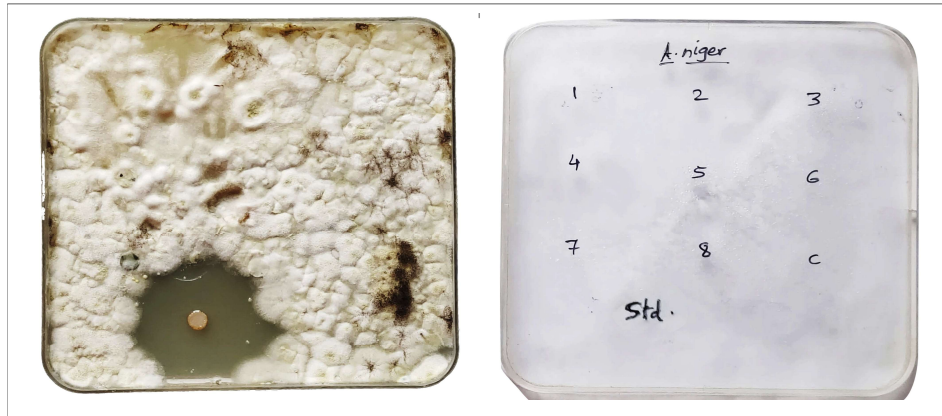


Fig. 5 *Aspergillus niger*



Fig. 6 *Candida albicans*

The petroleum ether extracts indicate greater activity compared to the other extracts against all the microorganisms. The most significant inhibition, such as the petroleum ether extract of *C. martinii*, exhibited a maximum inhibition zone, measuring 14.85 mm, against the microorganism *Proteus vulgaris* (Table 5, Fig. 4; labelled no.1). This was followed by the acetone extract of *V. negundo*, which had an inhibition zone measuring 14.84 mm against the microorganism *Proteus vulgaris* (Table 5, Fig. 4; labelled no.7). The acetone extract of *K. pinnata*, which exhibited an inhibition zone measuring 12.31 mm against the microorganism *Proteus vulgaris* (Table 5, Fig. 4; labelled no.4) was followed by the methanol extract of *M. pubescens*, which exhibited an inhibition zone measuring 9.63 mm against the microorganism *Bacillus subtilis* (Table 5, Fig. 1; .6), all at a concentration of 1 mg/g. The diameters of the

inhibition zones varied between 7 and 14.85 mm. The zone formations were not observed against *Aspergillus niger*.

Table 5: Antimicrobial Activities of Different Extracts of Leaves

Sr. No.	Compounds	Microorganisms					
		<i>B. subtilis</i>	<i>S. aureus</i>	<i>E. coli</i>	<i>P. vulgaris</i>	<i>A. niger</i>	<i>C. albicans</i>
1	Petroleum ether extract of <i>Cymbopogon martinii</i> leaves	7.06	-	8.04	14.85	-	-
2	Acetone extract of <i>Cymbopogon martinii</i> leaves	-	8.04	-	-	-	-
3	Petroleum ether extract of <i>Kalanchoe pinnata</i> leaves.	9.61	-	-	-	-	9.12
4	Acetone extract of <i>Kalanchoe pinnata</i> leaves.	-	-	-	12.31	-	-
5	Acetone extract of <i>Morinda pubescens</i> leaves.	7.05	-	-	-	-	-
6	Methanol extract of <i>Morinda pubescens</i> leaves.	9.63	-	9.16	-	-	-
7	Acetone extract of <i>Vitex negundo</i> leaves.	-	-	-	14.84	-	-
8	Aqueous extract of <i>Vitex negundo</i> leaves.	-	8.02	-	-	-	-
Control	DMSO	-	-	-	-	-	-
Standard	Chloramphenicol	26.21	20.74	28.68	27.22	-	-
Standard	Amphotericin - B	NA	NA	NA	NA	32.06	20.11

Note: Diameter of the mean zone of inhibition measured in 'mm' calculated by vernier calliper.

'-' means no zone of inhibition, and 'NA' means not applicable.

CONCLUSION

The study confirms the presence of biomolecules in selected medicinal plants,

supporting their traditional medicinal use. Scientific validation of the ethnomedicine would pave the way for the novel discovery of the molecules and may help to cater to the various human ailments. The study recommends that further purification of metabolites is needed to characterise the medicinally active molecules for drug development.

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