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Original Research Article

Screening plants for medicines against *Klebsiella* & *Pseudomonas* species infections

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ABSTRACT

Klebsiella & Pseudomonas species are multi drug resistant bacteria. These bacteria are resistant to number of antimicrobial agents. The research work was aimed at killing and inhibiting the growth of these multi drug resistant pathogens, by using phytochemicals. The phytochemicals are secondary metabolites produced by plants. According to WHO medicinal plants are best products for maintaining human health. Plants like *Terminalia bellerica* (Behada) and *Santalum album* (Chndan) found to be the most effective against all isolated multi drug resistant *Pseudomonas* species and *Klebsiella* species. 20 pathogens were isolated on MacConkeys agar plate. Out of these isolates, all isolates showed resistance to the more than two antibiotics. All of them studied for colony morphology, cell morphology, biochemical nature & 16srRNA sequencing. The 10 isolated multi drug resistant *Klebsiella species* were named as K1 to K10. The 10 isolated multi drug resistant *Pseudomonas species* were named as P1 to P10.

1. Introduction

The medicinal plants have the strong acceptance in the religious activities of north Indian native communities, who worship the plants in the form of various gods, goddesses and minor deities (Dhyani, 2000).[1] *Cynodon dactylon, Ocimum sanctum, Juniperus communis, Aegle marmelos, Ficus benghalensis, Ficus religiosa, Azadirachta indica, Musa paradissica* and *Nardostachys grandiflora* are the examples of medicinal plants highly used for the medicinal as well as religious purposes by the Hindus in southern and northern part of India. The Buddhist community in northern India regards *Terminalia chebula* as an important medicine as well as sacred fruit. It has been stated long ago that the therapeutic potency of the medicinal plants is more effective and superior suited to a person of particular region or the culture in which the plant is naturally growing (Nadkarni and Nadkarni, 1989).[2] This idea has given a way to the development of a new drug for the heart patients of specific ethnic groups in India.

Various phytotherapy manuals have specified different therapeutic plants for treating irresistible ailments because of their less side impacts and lessened poisonous quality (Lee *et al*., 2007).[3] There are a few reports on the antimicrobial movement of various natural separates (Islam *et al*.,[4] 2008; de Boer *et al*., 2005), Numerous plants have been found to cure gastrointestinal disarranges, respiratory sicknesses and cutaneous contaminations (Somchit *et al*., 2003; Santos *et al*., 1995).[5,6] As per WHO, restorative plants would be the best hotspot for acquiring assortment of medications (Santos *et al*., 2005).[6]

2. Materials and Methods

2.1. Collection of plant material

Fresh stem and seeds of total 16 medicinal plants were collected. The fruits, leaves, stem and seeds were separated, washed thoroughly with the tap water and shade dried, homogenized to fine powder and stored in air tight bottles which were used for solvent extraction.

Following plant materials had been selected for the purpose of study.

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2.2. Medicinal plant (Local name)

2.2.1. Stem

1. *Ficus benghalensis* (Wad)
2. *Terminalia arjuna* (Arjun)
3. *Santalum album* (Chndan)
4. *Boswella serrata* (Salaii)

2.2.2. Seeds

1. *Butea monosperma* (Palas)
2. *Terminalia bellerica* (Behada)
3. *Celosia argentea* (Kuradu)
4. *Cassia tora* (Takala)

2.3. Preparation of plant extracts

The Agar Well diffusion method was used to study antimicrobial activity of the collected medicinal plant extracts. The collected plant materials were washed thoroughly with distilled water before use. The crude extract of plant material was made by using mortar-pestle and different solvents and used for extraction. The plant extracts were prepared by using the solvents Viz. water and ethanol. 10g of the samples were then taken and homogenized with 100ml of respective solvents. This crude preparation was further left overnight in a shaker at room temperature and then centrifuged at 4000rpm for 20mins. The supernatant containing the plant extract was transferred to a pre-weighed beaker and then the extract was concentrated by evaporating the solvent at 60°C. The crude extract was then weighed up and dissolved in a 100ml of distilled water, and concentration obtained as 10%.

2.4. Collection of clinical samples from pathology labs

MDR positive clinical samples were collected from Jagtap lab. Barshi. And Ashwini Hospital Solapur. MacConkey's agar medium used for isolation and cultivation of MDR Pseudomonas and Klebsiella species.

2.5. Enrichment of samples

The samples were then enriched by inoculating in a Nutrient Broth for 24 hours. 1ml of these samples were added to 9 ml of Nutrient Broth for sufficient enrichment and incubated at 37°C.

2.6. Isolation and cultivation of multi drug resistant Pseudomonas and Klebsiella species from clinical sample

For the isolation of *Pseudomonas* and *Klebsiella species* strains, a loop full of clinical samples were streaked on MacConkey's agar.

The bacterial isolates were identified by using 16s rRNA sequencing of promising isolates, VITEK-2 and also compairing with the Bergey's Manual of Systematic Bacteriology.

2.7. Antimicrobial activity of plant extract against Klebsiella & Pseudomonas species

Inoculums containing 0.5ml of bacterial suspension was spread on the solid plates. Wells were made in the nutrient agar plate using the sterile cork borer. These wells were then filled with plant extracts at the same time different solvents were also used as a control. All these plates were incubated at 37°C for 24 hours and zone of growth inhibition around the wells was measured in mm (millimeter).

3. Results & Discussion

During isolation and identification of pure culture 20 pathogens were obtained on MacConkeys agar plate. Out of these isolates all organisms which showed resistance to the more than two antibiotics and all of them studied for colony morphology, cell morphology, biochemical nature & 16srRNA sequencing and named as K1 to K10 & P1 to P10. It was observed that all were Gram negative, motile, straight rods, no one them above isolates is capable of H2S production. P1 to P10 are oxidase positive, All 20 isolates are glucose & sucrose positive. By VITEK-2 study these organisms are identified *Klebsiella pneumonia* & *pseudomonas species.*

3.1. Evaluation of antibacterial activity of plant extracts

The results of antibacterial activity of Aquatic extracts and Ethanolic extracts of all the sixteen plants when tested individually for their antibacterial activity against the 20 species of *Klebsiella* and *Pseudomonas* isolated, which are known to cause infection in humans and are multi drug resistant species. The antibacterial activity was done by using Agar Well Diffusion Assay (Saeed et al., 2007).[7] It was expressed as the mean of zone of inhibition diameters (mm) produced by the medicinal plant extract. For the screening, the plates were prepared by using Nutrient Agar. The inoculum (90μl) of different bacterial isolates was spread evenly on the nutrient agar plate with the sterile spreader and sterile cork borer was used to cut the well. 20μl of different concentrations (10 mg/ml (200μg/well) and 25mg/ml (500μg/well)] of extract was poured in each well prepared in agar and incubated at 28°C for 48 hours. The Control (Negative control) well contained 20μl of 3% DMSO in place of the plant extract. The diameter of zone of inhibition was measured using distilled water and ethanolic extract and the mean was recorded Malar, J. J *et al* (2011).[8]

Table 1:

Culture Codes	Name of Medicinal Plant							
	Stem					Seeds		
	Ficus benghalensis (Wad)	Terminalia arjuna (Arjun)	Santalum album (Chandan)	Boswella serrata (Salaii)	Butea monosperma (Palas)	Terminalia bellerica (Behada)	Celosia argentea (Kuradu)	Cassia tora (Takala)
	Zone of Growth Inhibition (mm) using Distilled Water							
K1	00	14	10	10	24	12	8.0	21
K2	00	00	10	8.0	22	10	7.0	22
K3	00	9.0	12	8.0	20	8.0	8.0	20
K4	00	00	12	9.0	18	10	00	00
K5	00	00	14	12	00	14	00	00
K6	12	10	9.0	00	00	14	8.0	00
K7	12	00	8.0	00	00	8.0	9.0	00
K8	00	00	00	10	00	8.0	00	00
K9	00	8.0	00	10	00	12	00	18
K10	00	00	00	12	18	12	00	18
P1	00	12	12	12	10	10	00	10
P2	00	12	12	12	12	10	00	12
P3	00	12	14	12	16	8.0	00	00
P4	00	14	12	14	18	9.0	6.0	00
P5	00	10	12	10	13	12	8.0	00
P6	00	00	10	12	9.0	00	00	00
P7	12	00	00	00	00	00	10	10
P8	9.0	00	12	00	00	10	10	8.0
P9	8.0	9.0	12	00	8.0	12	12	9.0
P10	00	00	00	8.0	10	14	00	10

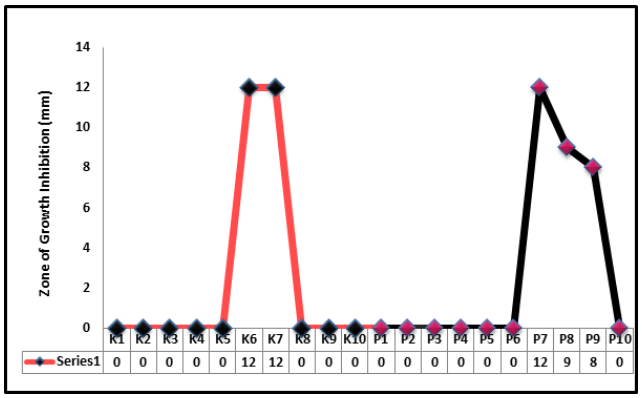

Fig. 1: Antibacterial Potential of *Ficus benghalensis* (Wad) against *Klebsiella species* and *Pseudomonas species*

3.2. Antibacterial Potential of Ficus benghalensis (Wad) against Klebsiella species and Pseudomonas species on nutrient agar incubated at 37°C in 24 hours

The results of antibacterial potential of *Ficus benghalensis (Nagchafa)* are given in figure. It is evident from that of the 10 promising strains of *Klebsiella Viz*. K1, K2, K3, K4, K5, K6, K7, K8, K9 and K10, shows zone of growth inhibition by K6 and K7 as 12mm, 12mm respectively in 24 hours of incubation. No zone of growth inhibition shown by K1, K2, K3, K4, K5, K8, K9 and K10 in 24 hours of incubation.

Whereas, that of the 10 promising strains of *Pseudomonas species Viz.* P1, P2, P3, P4, P5, P6, P7, P8, P9 and P10, the zone of growth inhibition shown by P7, P8 and P9 as 12mm, 9.0mm and 8.0mm respectively. No zone of growth inhibition shown by P1, P2, P3, P4, P5, P6 and P10 in 24 hours of incubation.

3.3. Antibacterial potential of terminalia Arjun (Arjun) against Klebsiella species and Pseudomonas species on nutrient agar incubated at 37°C in 24 hours

The results of antibacterial potential of *Terminalia arjuna (Arjun)* are given in figure. It is evident from that of the 10 promising strains of *Klebsiella Viz*. K1, K2, K3, K4, K5, K6, K7, K8, K9 and K10, shows zone of growth inhibition by K1, K3, K6 and K9 as 14mm, 9.0mm, 10mm and 8.0mm respectively in 24 hours of incubation. No zone of growth inhibition shown by K2, K4, K5, K7, K8 and K10 in 24 hours of incubation.

Whereas, that of the 10 promising strains of *Pseudomonas species Viz.* P1, P2, P3, P4, P5, P6, P7, P8, P9 and P10, the zone of growth inhibition shown by P1, P2, P3, P4, P5 and P9 as 12mm, 12mm, 12mm, 14mm, 10mm and 9.0mm respectively. No zone of growth inhibition shown by P6, P7, P8, and P10 after 24 hours of incubation.

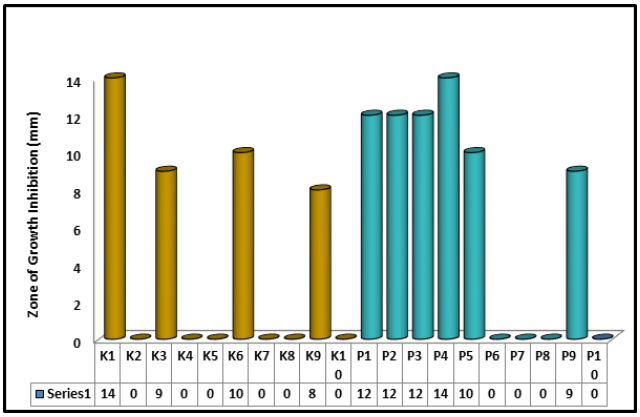

Fig. 2: Antibacterial Potential of *Terminalia arjuna* (Arjun)against *Klebsiella species* and *Pseudomonas species*

3.4. Antibacterial Potential of Santalum album (Chandan) against Klebsiella species and Pseudomonas species on nutrient agar incubated at 37°C in 24 hours

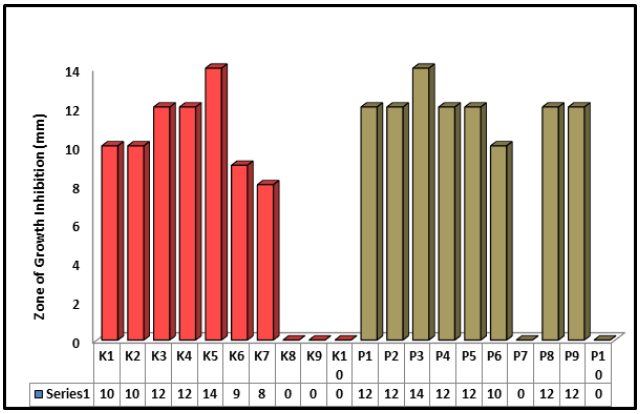

Fig. 3: Antibacterial Potential of *Santalum album* (*Chandan*) against *Klebsiella species* and *Pseudomonas species*

The results of antibacterial potential of *Santalum album (Chandan)* are given in figure. It is evident from that of the 10 promising strains of *Klebsiella Viz*. K1, K2, K3, K4, K5, K6, K7, K8, K9 and K10, shows zone of growth inhibition by K1, K2, K3, K4, K5, K6 and K7 as 10mm, 10mm, 12mm, 12mm, 14mm, 9.0mm and 8.0mm respectively in 24 hours of incubation. No zone of growth inhibition shown by K8, K9 and K10 in 24 hours of incubation.

Whereas, that of the 10 promising strains of *Pseudomonas species Viz.* P1, P2, P3, P4, P5, P6, P7, P8, P9 and P10, the zone of growth inhibition shown by P1, P2, P3, P4, P5, P6, P8 and P9 as 12mm, 12mm, 14mm, 12mm, 12mm, 10mm, 12mm and 12mm respectively. No zone of growth inhibition shown by P7 and P10 after 24 hours of incubation.

3.5. Antibacterial Potential of Boswella serrata (Salaii) against Klebsiella species and Pseudomonas species on nutrient agar incubated at 37°C in 24 hours

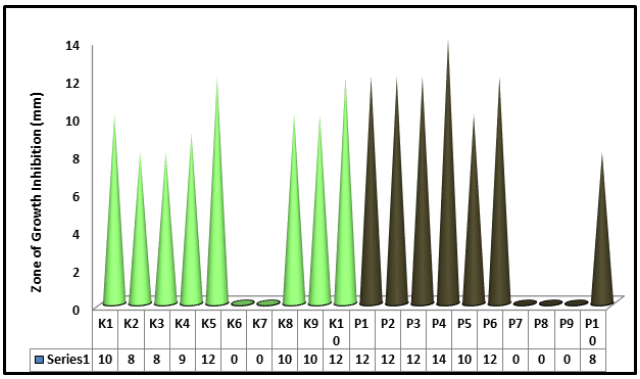

Fig. 4: Antibacterial Potential of *Boswella serrata* (Salaii) against *Klebsiella species* and *Pseudomonas species*

The results of antibacterial potential of *Boswella serrata (Salaii)* are given in figure. It is evident from that of the 10 promising strains of *Klebsiella Viz*. K1, K2, K3, K4, K5, K6, K7, K8, K9 and K10, shows zone of growth inhibition by K1, K2, K3, K4, K5, K8, K9 and K10 as 10mm, 8.0mm, 8.0mm, 9.0mm, 12mm, 10mm, 10mm and 12mm respectively in 24 hours of incubation. No zone of growth inhibition shown by K6 and K7 in 24 hours of incubation.

Whereas, that of the 10 promising strains of *Pseudomonas species Viz.* P1, P2, P3, P4, P5, P6, P7, P8, P9 and P10, the zone of growth inhibition shown by P1, P2, P3, P4, P5, P6 and P10 as 12mm, 12mm, 12mm, 14mm, 10mm, 12mm and 8.0mm respectively. No zone of growth inhibition shown by P7, P8 and P9 after 24 hours of incubation.

3.6. Antibacterial Potential of Butea monosperma (Palas) against Klebsiella species and Pseudomonas species on nutrient agar incubated at 37°C in 24 hours

The results of antibacterial potential of *Butea monosperma (Palas)* are given in figure. It is evident from that of the 10 promising strains of *Klebsiella Viz*. K1, K2, K3, K4, K5, K6, K7, K8, K9 and K10, shows zone of growth inhibition by K1, K2, K3, K4 and K10 as 24mm, 22mm, 20mm, 18mm and 18mm respectively in 24 hours of incubation. No zone of growth inhibition shown by K5, K6, K7, K8 and K9 in 24 hours of incubation.

Whereas, that of the 10 promising strains of *Pseudomonas species Viz.* P1, P2, P3, P4, P5, P6, P7, P8, P9 and P10, the zone of growth inhibition shown by P1, P2, P3, P4, P5, P6, P9 and P10 as 10mm, 12mm, 16mm, 18mm, 13mm, 9.0mm, 8.0mm and 10mm respectively. No zone of growth inhibition shown by P7 and P8 after 24 hours of incubation.

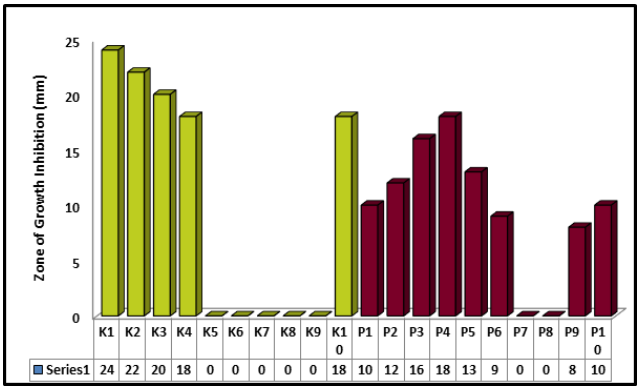

Fig. 5: Antibacterial Potential of *Butea monosperma (Palas)* against *Klebsiella species* and *Pseudomonas species*

3.7. Antibacterial Potential of Terminalia bellerica (Behada) against Klebsiella species and Pseudomonas species on nutrient agar incubated at 37°C in 24 hours

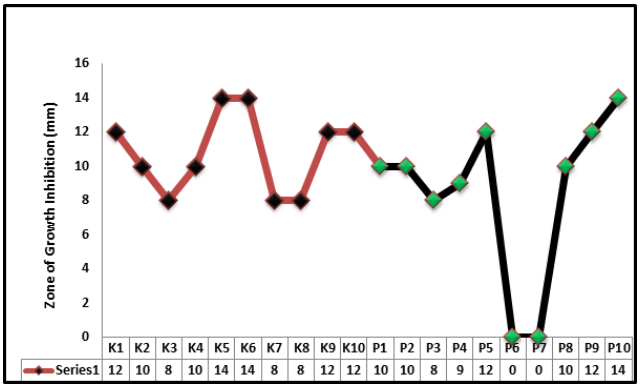

Fig. 6: Antibacterial Potential of T*erminalia bellerica (Behada)* against *Klebsiella species* and *Pseudomonas species*

The results of antibacterial potential of *Terminalia bellerica (Behada)* are given in figure. It is evident from that of the 10 promising strains of *Klebsiella Viz*. K1, K2, K3, K4, K5, K6, K7, K8, K9 and K10, shows zone of growth inhibition by all species of Klebsiella as K1, K2, K3, K4, K5, K6, K7, K8, K9 and K10 as 12mm, 10mm, 8.0mm, 10mm, 14mm, 14mm, 8.0mm, 8.0mm, 12mm and 12mm respectively in 24 hours of incubation.

Whereas, that of the 10 promising strains of *Pseudomonas species Viz.* P1, P2, P3, P4, P5, P6, P7, P8, P9 and P10, the zone of growth inhibition shown by P1, P2, P3, P4, P5, P8, P9 and P10 as 10mm, 10mm, 8.0mm, 9.0mm, 12mm, 10mm, 12mm and 14mm respectively. No zone of growth inhibition shown by P6 and P7 after 24 hours of incubation.

3.8. Antibacterial Potential of Celosia argentea (Kuradu) against Klebsiella species and Pseudomonas species on nutrient agar incubated at 37°C in 24 hours

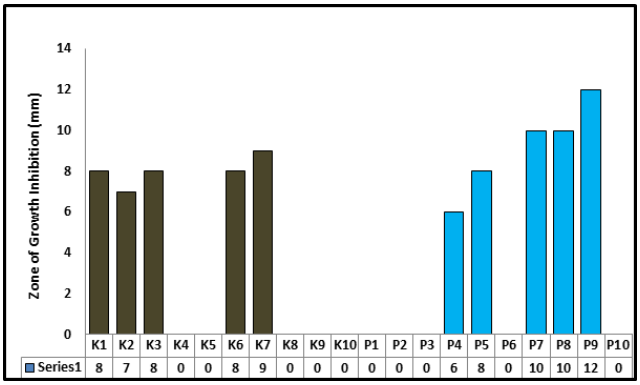

Fig. 7: Antibacterial Potential of *Celosia argentea (Kuradu)* against *Klebsiella species* and *Pseudomonas species*

The results of antibacterial potential of *Celosia argentea (Kuradu)* are given in figure. It is evident from that of the 10 promising strains of *Klebsiella Viz*. K1, K2, K3, K4, K5, K6, K7, K8, K9 and K10, shows zone of growth inhibition by K1, K2, K3, K6 and K7 as 8.0mm, 7.0mm, 8.0mm, 8.0mm and 9.0mm respectively in 24 hours of incubation. No zone of growth inhibition shown by K4, K5, K7, K8, K9 and K10 in 24 hours of incubation.

Whereas, that of the 10 promising strains of *Pseudomonas species Viz.* P1, P2, P3, P4, P5, P6, P7, P8, P9 and P10, the zone of growth inhibition shown by P4, P5, P7, P8 and P9 as 6.0mm, 8.0mm, 10mm, 10mm and 12mm, respectively. No zone of growth inhibition shown by P1, P2, P3, P6 and P10 after 24 hours of incubation.

3.9. Antibacterial Potential of Cassia tora (Takala) against Klebsiella species and Pseudomonas species on nutrient agar incubated at 37°C in 24 hours

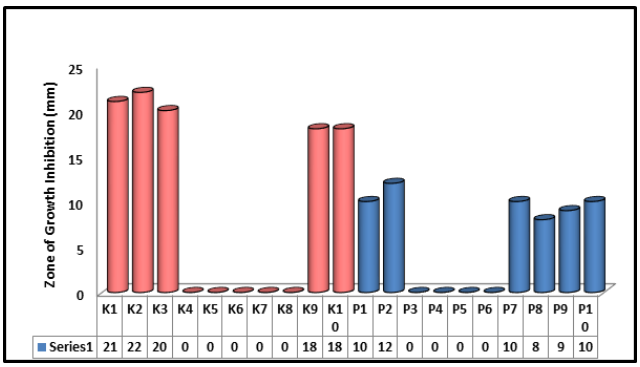

Fig. 8: Antibacterial Potential of *Cassia tora (Takala)* against *Klebsiella species and Pseudomonas species*

The results of antibacterial potential of *Cassia tora (Takala)* are given in figure. It is evident from that of the 10 promising strains of *Klebsiella Viz*. K1, K2, K3, K4, K5, K6, K7, K8, K9 and K10, shows zone of growth inhibition by K1, K2, K3, K9 and K10 as 21mm, 22mm, 20mm, 18mm and 18mm respectively in 24 hours of incubation. No zone of growth inhibition shown by K4, K5, K6, K7 and K8 in 24 hours of incubation.

The present study (Md. Al Nayem Chowdhury, 2013) was conducted with a view to evaluate the therapeutic potentials of twenty six plant extracts traditionally used in Bangladesh against human pathogenic bacteria *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis* by disc diffusion method. Out of twenty six plant extracts eight crude plant extracts namely *Allamanda cathartica* (leaf), *Allium sativum* (bulb), *Citrus limon* (fruit), *Tamarindus indica* (fruit), *Prunus domestica* (Fruit), *Averrhoa carambola* (fruit), Piper betle (leaf) and *Terminalia arjuna* (leaf) were found to exhibit potential antimicrobial properties against the isolated human clinical bacterial isolates whereas twelve plant extracts were failed to show any antibacterial activity against any of the isolates of *Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* followed by ten plant species in case of *Proteus mirabilis*.

4. Conclusion

Stem of *Ficus benghalensis* (Wad) and *Terminalia arjuna (*Arjun) showed highest activity against K6 isolate while seeds of *Butea monosperma (Palas), Terminalia bellerica (Behada*) showed highest activity K5, K6 and P10. All species K1 to K10 are sensitive to seed extract of *Terminalia bellerica (Behada).* Stem of *Ficus benghalensis* (Wad) has no activity against K1, K2, K3, K4, K5, P1, P2, P3 P4, P5, P6. It is highlighted that seeds of plants showed highest antimicrobial activity. The isolate P10 is more resistant to action phytochemicals.

5. Source of Funding

None.

6. Conflict of Interest

The authors declare that there is no conflict of interest.

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