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Research Article

Demonstration of *In Vitro* Anti-Bacterial Activity of Crucial Medicinal Plants in Bangladesh against Pathogens Isolated from Sputum Samples

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Abstract

Traditional medicinal plants have long been used in Bangladesh to treat human and livestock ailments. Although the use of medicinal plants is a well-documented tradition in the country, but their direct antimicrobial impacts are still poorly known. Now a day's scientists become interested to formulate the new drugs using medicinal plants due to its potential antimicrobial activity and less side effects against common cold. Present study designed to manifest the anti-bacterial properties of 10 categories of 50 (n = 50) medicinal plants including *Azadirachta indica, Ocimum tenuiflorum, Justicia adhatoda, Mentha spicata, Zingiber officinale, Terminalia chebula, Phyllanthus emblica, Bryophyllum pinnatum, Syzygium aromaticum* and *Terminalia belerica* through conventional culture techniques, methanol and ethanol extraction method and minimum inhibitory concentration (MIC) test. Pathogenic *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas* spp. was previously isolated from sputum samples. Current study revealed that *Staphylococcus* spp. was 80 - 100% resistant against almost all antibiotics and *Pseudomonas* spp. and *Klebsiella pneumoniae* was found to be highly resistant against amoxicillin whereas the significant antibacterial activity was noticed in plant extracts against all these isolates. Particularly, both ethanolic and methanolic extracts exhibits anti-bacterial activity ant the highest zone was recorded up to 25 mm and lowest zone was 12 mm in diameter. Overall, *Ocimum tenuiflorum* and *Justicia adhatoda* were evident their potency among all. However, this study also divulged the minimal inhibitory concentration (MIC) of all the samples, which inhibited the growth of all the tested bacteria. This contemporary study suggested consumer to reduce the indiscriminate uses of antibiotics and motivated farmer to cultivated more medicinal plant for overall public health.

Keywords: *Drug Resistance; Pathogenic Microbes; Common Cold; Medicinal Plant; Anti-Bacterial Activity; Extraction Methods*

Introduction

Bacterial diseases continued to be the greatest threats to the world regardless of efforts and progress in developing modern medicine [1]. The impact of bacterial diseases is particularly important in developing countries such as Bangladesh where there is limited access to modern drugs and prices are mostly unaffordable when the latter are available. Recently, the ever- expanding threat from drug-resistant bacteria calls for a global exertion to search for new solutions [2]. In Bangladesh, common cold is one of ordinary disease. It is the most recurrent self-limited infection of upper respiratory tract mainly caused by virus worldwide [3]. Although common cold is a virus mediated disease, but the normal flora of the upper respiratory tract including *Streptococcus pneumoniae* (Pneumococcus), *Haemophilus influenzae*, *Moraxella catarrhalis* and *Staphylococcus aureus* [4] which occasionally turn into pathogens causing infectious diseases.

At present complementary drugs, including herbal medicine is becoming an increasingly public health care practice, which has been applied for both general maintenance of health and for treatment of acute illnesses [5,6]. Medicinal plants become the essential sources of antimicrobial agents for its advantage over the chemical therapeutic agents [7-9]. An extensive range of clinical complications eradicated traditionally through a wide range of herbal medicines [10-14]. In developing countries, about two-thirds of the population uses medicinal plants as curing agents. In 2002 Food and Agriculture Organization estimated that, over 50,000 plants are used as a curing agent across the world. About 80% of the population in some African and Asian countries [8] and over 50% of the resident in Europe, North America, and other developed countries use plants and plant components for their medicinal needs at least once [15]. At present, medicinal plants play a vital role in Asia, both as preventive and curative measures, in spite of advances in modern medicine. Most of the rural people in Asian countries use plant-based traditional medicines for healthcare. In recent years, ineffectiveness of antibiotics notice because the number of drug resistance bacteria increases dramatically against the first, second and third generation of antibiotics and eventually the rapid emergence of resistance indicates that new generations of synthetic drug cannot withstand effectively against pathogenic bacteria [16-20]. Moreover, a huge number of reports revealed the adverse effects and toxicity that resulting from antibiotics which sometimes can be fatal. Various plants have been utilized due to their anti-microbial properties, which are because of phytochemicals synthesized in the secondary metabolism of the plant [21,22]. Plants are rich in a wide variety of secondary metabolites such as alkaloids, flavonoids, phenolic compounds and tannins, which have been found *in vitro* to have antimicrobial traits [23-27].

Beside the beneficial impacts of natural plant it has also some negative side, when large number of microbial contamination gets entry in the surface area of plants during the cultivation and unhygienic production procedure [28-30]. Consequently, implementation of modern technology to develop the herbal medicine is so important to minimize the growth of different microbial contamination as well as increase its acceptability towards the general individuals [31,32]. In addition, this sort of findings has enormous significance in developing countries perspective where the large number of population considers herbal medicine instead of synthetic drugs [33,34].

In the light of these realities, plant compounds which have been finest contributors to modern antibacterial treatments might be reconsidered as possible candidates. Since they have been utilized for long times with no documented obstruction, it appears to be sound to attempt less spectacular, however more liable products. Consequently, present study was aim to conduct the antimicrobial activity of commonly medicinal plant available in Bangladesh.

Materials and Methods

Collection and culture of clinical bacterial isolates

All bacterial isolates were collected form Microbiology Laboratory of Stamford University Bangladesh, which was previously isolated from clinical sputum samples of the patients suffer from common cold. And each isolates were previously identified with several biochemical tests. Three bacterial strains *Pseudomonas* spp., *Klebsiella pneumonia* and *Staphylococcus aureus* were included for this study. The stocks were stored and frozen at -20°C was and for resuscitated further subculture on MacConkey, Cetrimide agar and Mannitol salt agar medium.

Study area and sample collection

Ten categories of 50 (n = 50) medicinal plants specimen including *Ocimum tenuiflorum* (Tulsi), *Azadirachta indica* (Neem), *Justicia adhatoda* (Bashok), *Mentha spicata* (mint), *Zingiber officinale* (Ginger), *Terminalia chebula* (Hartaki), *Phyllanthus emblica* (Amlaki), *Bryophyllum pinnatum* (patharkuchi), *Syzygium aromaticum* (lobongo), *Terminalia belerica* (Bohera) were randomly collected from local forest, shop and herbarium during July 2019 to November 2019 following standard protocol. It is then immediately transferred to the laboratory to conduct *in-vitro* antimicrobial assay against germs isolated from the sputum samples.

Antimicrobial susceptibility test of clinical isolates

The antimicrobial susceptibility of *Klebsiella pneumonia*, *Pseudomonas* spp. and *Staphylococcus aureus* were carried out following Kirby Bauer Disk diffusion technique [35,36]. The antibiotic susceptibility pattern was examined by using common commercial antibiotics. They included Ciprofloxacin (5 μg), Cefpodoxime (30 μg), Amoxicillin (10 μg), Imipenem (30 μg), Ceftriaxone (10 μg), Chloramphenicol (10 μg), Tetracycline (30 μg), Polymyxin B (30 μg), Nalidixic acid (30 μg) Ofloxacin (5 μg). of the 24h broth culture of the strains were spread on Mueller Hinton Agar (Hi-Media, India) and antibiotic discs were subsequently placed on plates. Finally, the plates were incubated at 37°C for 24h to observe and measure the inhibition zone [37].

Solvent extraction

The plants were dried and powder form was prepared through grinding machine afterward the fine powders were added to 120 ml of ethanol and methanol in Durham's bottle. The samples were kept in shaking water bath at 130 rpm for 24h at 20°C. The dried residual extracts were dissolved in 10% dimethyl sulfoxide (DMSO) to a final concentration of 10 mg/ml [38]. Samples were stored overnight at -20°C until use.

Antimicrobial assay of different plant extracts by agar well diffusion methods

For the detection of the antibacterial activity of plant extract modified agar well diffusion method was followed [36,39,40]. At first the bacterial suspensions including *Pseudomonas* spp., *Klebsiella pneumoniae* and *Staphylococcus aureus* (turbidity compared with the McFarland standard) were prepared and 100 μl of the crude extract, ethanol and methanol extracts were introduced in the well. Beside the plant extract, one well was filled up with Buffer peptone water (BPW) as negative controls. Both positive (Gentamicin 10 μg) and negative (Ethanol and Methanol) control were used in case of each experiment. All the plates were incubated over night at 37°C to observe the clear zone of inhibitions around the well.

MIC determination by agar macro dilution method

The minimum inhibitory concentration (MIC) was examined using MHA media in Petri dishes seeded by a multiple inoculators [41]. The extracts was tested at seven final concentrations (32, 64, 128, 256, 512, 1024 $\mu l ml^{-1}$) on 3 microorganisms. The agar plates containing the extract and bacteria were incubated for 24h at 37°C. The activity was then evaluated by presence or absence of visible colonies. The positive control agent was Gentamicin 10 μg. MIC values were recorded as the lowest concentrations of components demonstrating no growth. Solvents were used to check for absence of antibacterial activity.

Results and Discussion

For the sake of consumers health safety it is very important to short out the new sources of diseases medication procedure which has activity against the microbes associated diseases and communicable diseases. One of our findings on different plant samples as well as herbal medicine successfully unveiled the anti-bacterial traits against the pathogenic strain which is in consistent to several other studies [42]. As we know that Bangladesh is a country of huge natural resources, plenty of green plants are available in everywhere those have medicinal activity, from this point of view we further focused to investigate the antibacterial potentiality of 10 commonly available plants.

Antibiogram studies of clinical isolates

Antimicrobial resistance is one of the most serious threatening issues for public health. Emergences of resistance among the most important bacterial pathogens are now frequently commencing, and surprisingly some pathogens have been identified as multi-drug resistant [43]. Based on the susceptibility to antibiotics, the bacterial resistance percentage was measured following [44]. Out of ten antibiotics used in this study, *Staphylococcus aureus* showed (60 - 100%) resistance against seven antibiotics, *Pseudomonas* spp. showed (60 - 99%) resistance against five and *Klebsiella pneumoniae* showed (40 - 80%) resistance against five antibiotics. That's mean *Klebsiella pneumoniae* was found to be more sensitive compare than other two pathogens (Figure 1).

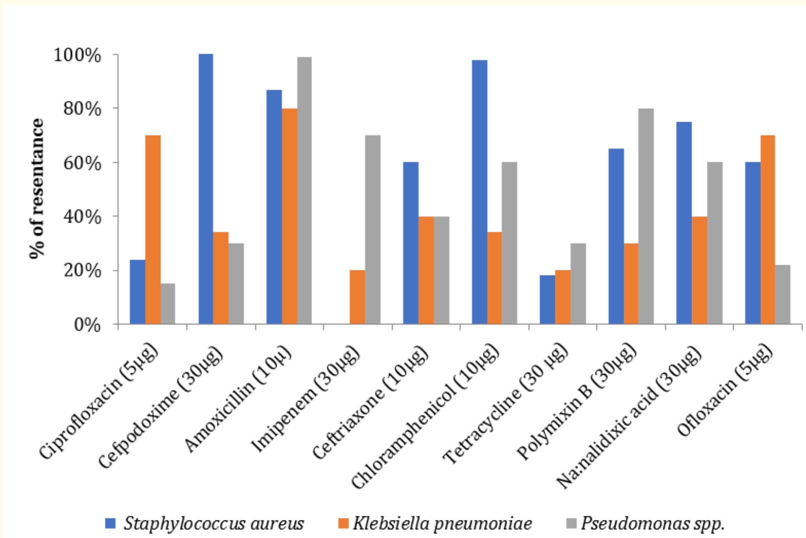

Figure 1: *Antimicrobial susceptibility pattern of different pathogenic isolates from sputum sample.*

Evaluation of *in-vitro* anti-bacterial activity of the plant samples

From the very ancient period plans has been using as an alternative medicine beside the synthetic drugs because of its huge ability to inhibit the growth of some diseases causing bacteria such as *E. coli, P. aeruginosa, S. aureus, Vibrio cholerae, B. subtilis, K. pneumoniae and Enterobacter aerogenes* [40,45]. A number of studies reported that the anti-bacterial features of natural herbs make them appropriate substitute of synthetic drugs which have already been resistance against pathogens [23,25]. According to our study, no antibacterial activity was found in case of crude fraction residual which supported a previous study [36] while the liquid extract of different plant like *Ocimum tenuiflorum* (Tulsi), *Azadirachta indica* (Neem), *Justicia adhatoda* (Bashok), *Mentha spicata* (mint), *Zingiber officinale* (Ginger), *Terminalia chebula* (Hartaki), *Phyllanthus emblica* (Amlaki), *Bryophyllum pinnatum* (patharkuchi), *Syzygium aromaticum* (lobongo) and *Terminalia belerica* were (Bohera) found to exhibit the bactericidal effects against *Pseudomonas* spp., *Klebsiella pneumonia* and *Staphylococcus aureus.* Against *Pseudomonas* spp. highest zone of inhibition was recorded for *Justicia adhatoda* ethanol extract 24 mm and lowest antimicrobial activity observed in *Syzygium aromaticum* ethanol extract 14 mm. On the other hand *Ocimum tenuiflorum* methanol extract the maximum zone was recorded 23 mm and 14 mm (lowest) zone was found in case of *Bryophyllum pinnatum* methanol extract (Table 1 and figure 2 and 3). Both ethanol and methanol extract of *Ocimum tenuiflorum* found to be effective against *Klebsiella pneumonia* and they produce a clear zone 23 mm (ethanol extract) and 24 mm (methanol extract). Lowest zone (12 mm) against *Klebsiella pneumonia* was recorded in *Bryophyllum pinnatum* ethanol extract (Table 2 and figure 4 and 5). *Ocimum tenuiflorum* methanol extract produced the maximum zone (25 mm) against *Staphylococcus* aureus and the lowest zone (12 mm) against *Staphylococcus aureus was* produced by ethanol extract of *Syzygium aromaticum* (Table 3 and figure 6 and 7). In this study almost all samples exhibited potential antimicrobial activity against pathogenic microorganisms collected from sputum samples.

Figure 2: *Antimicrobial activity (Methanol extraction) against Pseudomonas spp.*

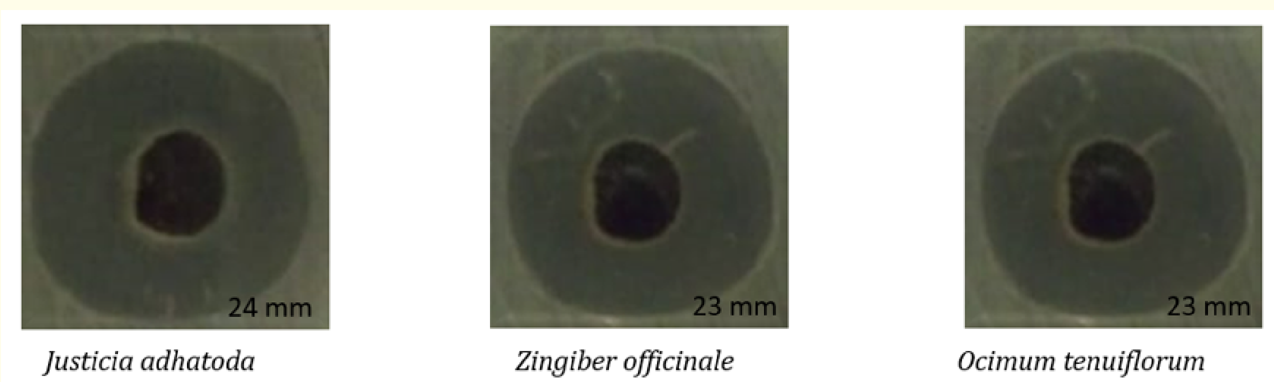

Figure 3: *Antimicrobial activity (ethanol extraction) against Pseudomonas spp.*

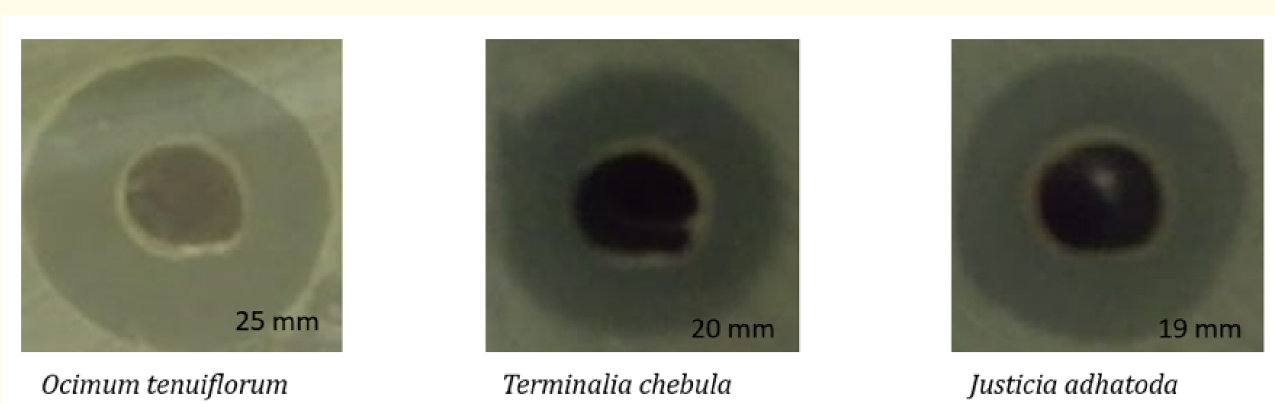

Figure 4: *Antimicrobial activity (Methanol extraction) against Staphylococcus aureus.*

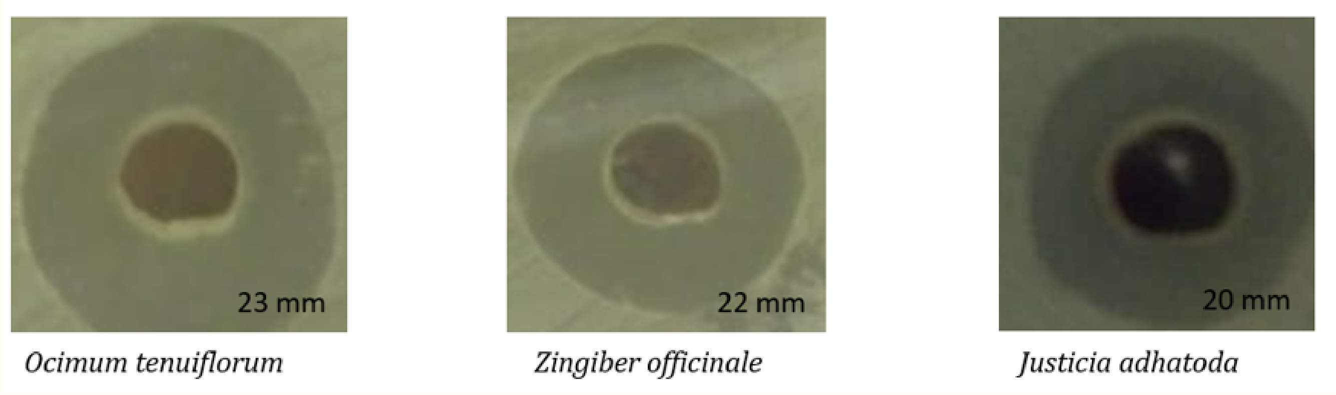

Figure 5: *Antimicrobial activity (Ethanol extraction) against Staphylococcus aureus.*

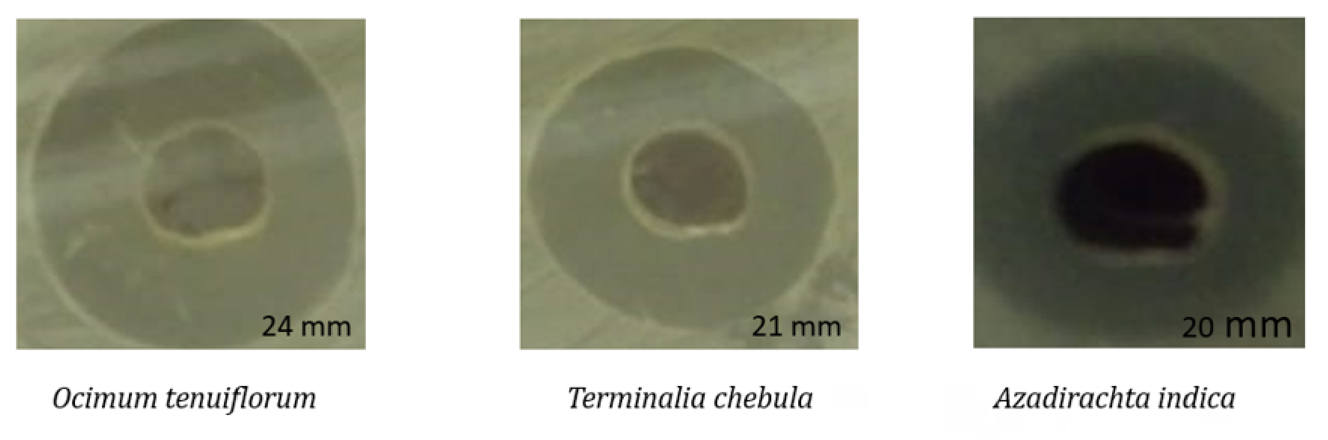

Figure 6: *Antimicrobial activity (Methanol extraction) against Klebsiella pneumoniae.*

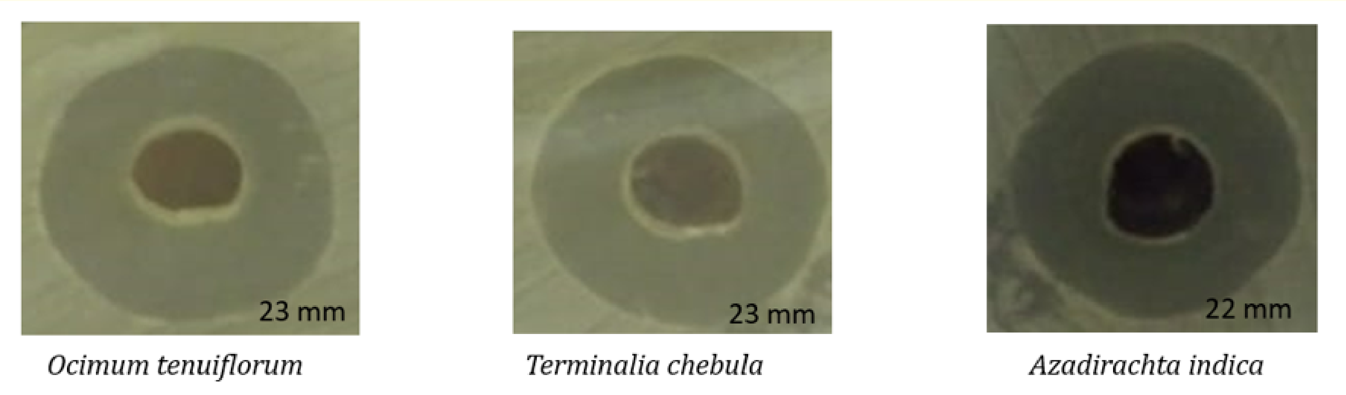

Figure 7: *Antimicrobial activity (ethanol extraction) against Klebsiella pneumoniae.*

Sample Name	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin 10 μg)
Justicia adhatoda	0	0	0	24	0	21	28
Mentha spicata	0	0	0	16	0	19	26
Zingiber officinale	0	0	0	23	0	20	22
Azadirachta indica	0	0	0	21	0	19	27
Ocimum tenuiflorum	0	0	0	22	0	23	22
Terminalia chebula	0	0	0	20	0	19	18
Phyllanthus emblica	0	0	0	17	0	21	19
Bryophyllum pinnatum	0	0	0	15	0	14	17
Syzygium aromaticum	0	0	0	14	0	15	23
Terminalia belerica	0	0	0	18	0	17	20

Table 1: *Antimicrobial activity against Pseudomonas spp.*

All the experiments have been done three times and the results were reproducible. One representative data have been shown.

Sample Name	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin 10 μg)
Justicia adhatoda	0	0	0	20	0	20	28
Mentha spicata	0	0	0	15	0	17	18
Zingiber officinale	0	0	0	20	0	19	22
Azadirachta indica	0	0	0	22	0	20	23
Ocimum tenuiflorum	0	0	0	23	0	24	28
Terminalia chebula	0	0	0	23	0	21	19
Phyllanthus emblica	0	0	0	20	0	19	23
Bryophyllum pinnatum	0	0	0	12	0	18	22
Syzygium aromaticum	0	0	0	13	0	14	26
Terminalia belerica	0	0	0	15	0	17	21

Table 2: *Antimicrobial activity against Klebsiella pneumoniae.*

All the experiments have been done three times and the results were reproducible. One representative data have been shown.

Sample Name	Zone of Inhibition in diameter (mm)						
	Crude fraction	Negative control (BPW)	Negative control (Ethanol)	Ethanol extract	Negative control (Methanol)	Methanol extract	Positive control (Gentamicin 10 μg)
Justicia adhatoda	0	0	0	20	0	19	28
Mentha spicata	0	0	0	20	0	18	18
Zingiber officinale	0	0	0	22	0	17	22
Azadirachta indica	0	0	0	20	0	21	24
Ocimum tenuiflorum	0	0	0	23	0	25	25
Terminalia chebula	0	0	0	19	0	20	21
Phyllanthus emblica	0	0	0	15	0	17	22
Bryophyllum pinnatum	0	0	0	14	0	18	26
Syzygium aromaticum	0	0	0	12	0	15	21
Terminalia belerica	0	0	0	20	0	19	23

Table 3: *Antimicrobial activity against Staphylococcus aureus.*

All the experiments have been done three times and the results were reproducible. One representative data have been shown.

Determination of MIC values of selected plant materials

According to collected data MIC values generally varied within the range from 32 µl to 1024 µl. The MIC values of *Syzygium aromaticum* was relatively higher (512 µl) against *Staphylococcus aureus* and *Klebsiella pneumonia.* The MIC values of *Mentha spicata* was also (512 µl) against *Klebsiella pneumonia.* The exceptions were *Ocimum tenuiflorum* which had lowest (64 µl) MIC values against *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas* spp. The MIC values of *Azadirachta indica*, *Terminalia chebula* and *Phyllanthus emblica* ware also (64 µl) against *Klebsiella pneumonia.* Moreover, the MIC values of *Justicia adhatoda*, *Zingiber officinale*, *Bryophyllum pinnatum* and *Terminalia belerica* were found (128 µl to 256 µl) against *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas* spp (Table 4).

Sample	Organisms		
	Staphylococcus aureus	***Klebsiella pneumoniae***	***Pseudomonas* spp.**
Justicia adhatoda	128 µL	128 µL	128 µL
Mentha spicata	256 µL	512 µL	256 µL
Zingiber officinale	128 µL	128 µL	128 µL
Azadirachta indica	64 µL	64 µL	128 µL
Ocimum tenuiflorum	64 µL	64 µL	64 µL
Terminalia chebula	128 µL	64 µL	64 µL
Phyllanthus emblica	128 µL	64 µL	128 µL
Bryophyllum pinnatum	256 µL	256 µL	256 µL
Syzygium aromaticum	512 µL	512 µL	256 µL
Terminalia belerica	128 µL	256 µL	256 µL

Table 4: *Minimum inhibitory concentration (MIC) of the sample.*

All the experiments have been done three times and the results were reproducible. One representative data have been shown.

Conclusion

This is now experimentally proved that the plant extracts have enormous amount of biologically active compound which act significant roles as a beneficial health tonic as well the potential candidate of future medicine. Our study also raveled that the plant extracts can inhibit the growth of many bacteria that promote to causes disease like common cold, such as *Pseudomonas* spp., *Klebsiella pneumoniae* and *Staphylococcus aureus*. To formulate the new medicine these samples could be the potential candidate towards the scientist. In compare of the synthetic drugs used as a positive control in this very study these plant samples showed suitable range of zone of inhibition. In future further investigation is needed to sort out the particular ingredients of the plants which would help us to standardize the concentration and proper doses of the samples against the pathogenic bacteria. The findings may mostly aid to design a model for the development of new herbal medicines which will be beneficial for the public health safety.

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Conflict of Interest

The authors have declared no conflict of interest.

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