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PHYTOCHEMICAL SCREENING AND *IN VITRO* EVALUATION OF ANTIBACTERIAL ACTIVITY OF SELECTED MEDICINAL PLANTS

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ABSTRACT

The present investigation was conducted for phytochemical screening of medicinal plant extracts as well as evaluation of their antibacterial activity against bacterial isolates. The phytochemical screening of methanolic extract of leaves of thornapple (*Datura metel*), eucalyptus (*Eucalyptus tereticornis*), Indian gooseberry (*Phyllanthus emblica*) and java plum (*Syzygium cumini*) was conducted in which flavonoids and alkaloids were detected in all of the tested plant extract. The antibacterial activity and minimum inhibitory concentration (MIC) of active plant extract were determined using agar well diffusion method against bacterial isolates including *Klebsiella sp.*, *Staphylococcus sp.* and *Pseudomonas sp.* Indian gooseberry exhibited maximum antibacterial activity against bacterial isolates of *Klebsiella sp.*, *Pseudomonas sp.* and *Staphylococcus sp*. Eucalyptus showed effective antibacterial activity against all of the tested bacterial isolates while thornapple was found effective against *Klebsiella sp.* and *Pseudomonas sp*. The MIC of Indian gooseberry extract was found lower against bacterial isolate of *Klebsiella sp.* and *Pseudomonas sp.* in comparison of eucalyptus extract while equal against *Staphylococcus sp*. The results of present study reveal that the medicinal plants are the potential source of antibacterial compounds that may be an alternative of antibiotics for the treatment of bacterial infections and also a solution of bacterial drug resistance.

Keywords: Medicinal plants, phytoconstituents, antibacterial activity, Minimum inhibitory concentration (MIC), antibacterial compounds

INTRODUCTION

Plant extracted components are utilized for the treatment of bacterial infections and for other human health benefits. In the reference of controlling continuously increasing microbial infections and drug resistance, phytoconstituents obtained from natural plants may be explored for the development of more effective, safe and less toxic drugs. Medicinal plants has been established as a rich sources of components such as flavonoids, alkaloids, phenolic compounds, terpenoids, tannins and steroids etc, that has to be evaluated for the discovery and development of new drugs. Thornapple (*Datura metel*) has been traditionally used for the treatment of skin diseases, wound healing, burn wound, fever, cough, asthma, diarrhoea, epilepsy, rheumatic pains and also as antimicrobial, antioxidant, anesthetic, anti-asthmatic, bronchodilator, and anti-tussive **[1, 2]**. Antibacterial nature of *D. metel* has been evaluated in previously reported studies **[2-5]**. Eucalyptus is traditional medicinal plants and exhibits anti-inflammatory effect, analgesic, antiviral, antifungal, antibacterial activity and antioxidant effect **[6-9]**. Indian gooseberry (*Phyllanthus emblica*) exhibits significant medicinal value and used for the treatment of different diseases including cancer, diabetes, liver tonic, ulcer, gastric problem, and inflammation **[10, 11]**. *P. emblica* exhibit antimicrobial activity **[12]**, antiviral activity **[13]** antifungal activity **[14]** and commonly used as immune modulator, analgesic, antioxidant, antitussive and antipyretic **[12, 15]**. Traditionally, Java plum (*Syzygium cumini*) has been used for the treatment of blood impurities, sore throat diabetes, jaundice, fever, bronchitis constipation, dysentery, ulcer, gastropathy and dermopathy **[16-20]**. *S. cumini* exhibit antimicrobial, antioxidant, antifungal, antiviral and anti-inflammatory activities **[20-22]**. Antibiotics are generally used for the treatment of bacterial infections but continuous, misuse or over consumption of antibiotics may lead lethal side effects in human and also increase the tendency of emergence of antibiotic resistance in microorganism. For safe and effective treatment of bacterial infections, medicinal plants should be focused to screen their phytoconstituents as alternative of synthetic drugs and antibiotics. Present study was focused on screening of phytochemicals in selected medicinal plants and for *in vitro* evaluation of their antibacterial activity against different foodborne bacterial isolates. In the previous step, different foodborne bacteria including *Klebsiella sp.*, *Pseudomonas sp.* and *Staphylococcus sp* have been isolated and biochemically characterized from spoiled fruits and vegetables **[23]**. The plant extracts having phytochemicals with potential antibacterial

properties must be explored for the development of new therapeutic drugs.

MATERIALS AND METHODS

Preparation of medicinal plant extract

The leaves of medicinal plants including java plum (*Syzygium cumini*), thornapple (*Datura metel*), eucalyptus (*Eucalyptus tereticornis*) and Indian gooseberry (*Phyllanthus emblica*) were collected locally and subjected for air drying at room temperature. Briefly dried leaves powder (10 g) was soaked in methanol (100%) and subjected for shaking continuously for three days at the room temperature. The crude extract was filtered through Whatman filter paper No.1 and filtrate was centrifuged (3000 rpm) for 10 minutes. The supernatant was evaporated and prepared crude methanolic plants extract were preserved at 4°C in sterile air tight bottle for further experiments.

Phytochemical screening

The phytochemicals **(Table 1)** screening of methanolic extract of plants was performed using standard protocols **[24-26]**. The different phytochemicals were screened such as alkaloids (Mayer's test), flavonoids (alkaline reagent test), glycosides (Keller-Killiani test), phenols and tannins (Ferric chloride test), steroids (Salkowski test) etc.

Bacterial isolates

The previously isolated and biochemically characterized foodborne bacterial isolates including *Staphylococcus spp. Klebsiella spp.* and *Pseudomonas spp.* were maintained on MacConkey agar (HiMedia), Cetrimide agar (HiMedia) and Eosin Methylene Blue agar (HiMedia) respectively.

Determination of preliminary antibacterial activity of plant extract

The preliminary antibacterial activity of plant extract against foodborne bacterial isolates was evaluated using agar well diffusion assay. The bacterial suspension (10^8 CFU/ml) was prepared as per the guidelines of Clinical and Laboratory Standards Institute (CLSI) **[27]**. Mueller Hinton agar (MHA) plate was prepared with standardized bacterial suspension. The plants extract (500 mg/ml) was reconstituted in DMSO (dimethyl sulfoxide). Further the reconstituted plants extract (50 µl) were poured aseptically in the wells and plates were incubated at 35 (±2°C) for 18–20 hours. The diameter of zone of inhibition (mm) was noted to determine the antibacterial activity of plant extracts. The chloramphenicol and DMSO were used as positive and negative control, respectively.

Determination of minimum inhibitory concentration (MIC) of plant extract

The Mueller Hinton Agar (MHA) plate was prepared using a standardized bacterial suspension according to the guidelines of CSLI 2012, as previously mentioned. The stock concentration (500mg/mL) of plants extract was serially diluted (i.e.: 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml,

15.625 mg/ml, and 7.81 mg/ml) and 50 µl of each diluted concentration transferred aseptically to the mentioned well respectively. The plates were incubated at 35 (±2°C) for 18-20 hours. The lowest concentration that inhibited bacterial growth was identified as the MIC.

Statistical analysis

Experiments for the determination of antibacterial activity (zone of inhibition) with methanolic extract of selected plants were performed in triplicate and mean ± standard deviations were calculated.

RESULTS

The prepared methanolic extract of medicinal plants (n=4) was subjected for screening of different phytochemicals **(Table 1)**. Flavonoids and alkaloids were detected in all of the tested plant extract while phlobatanins and gylcosides were not obtained in any of the tested plant extract. The antibacterial activity of plant extracts was detected using agar well diffusion method and MIC of most active plant extract was determined against foodborne bacterial isolates. The Indian gooseberry exhibited the maximum antibacterial activity against all of tested bacterial isolates including *Klebsiella sp.*, *Pseudomonas sp.* and *Staphylococcus sp*. Eucalyptus exhibited effective antibacterial activity against all of the tested bacterial isolates. Thornapple were found effective against *Klebsiella sp.* and *Pseudomonas sp.* isolate while Java plum was found effective against only bacterial isolate of *Klebsiella sp.* **(Figure 1)**. The MIC of Indian gooseberry and eucalyptus were determined using agar well diffusion method against bacterial isolate of *Klebsiella sp.*, *Pseudomonas sp.* and *Staphylococcus sp*. **(Figure 2)**. The MIC of Indian gooseberry was found lower in comparison of eucalyptus against bacterial isolate of *Klebsiella sp.* and *Pseudomonas sp.* while MIC of both the plants extract (Indian gooseberry and eucalyptus) was found similar against bacterial isolate of *Staphylococcus sp*.

Table 1: Phytochemical screening of selected medicinal plants

Sr. No.	Phytochemical	Methanolic extract of medicinal plants			
		Indian gooseberry	Eucalyptus	Thornapple	Java plum
1.	Alkaloids	+++	+++	+	+++
2.	Steroids	+++	++	-	+++
3.	Sterols	+++	+	-	+++
4.	Glycosides	-	-	-	-
5.	Phlobatannins	-	-	-	-
6.	Flavonoids	+++	+++	+	+++
7.	Tannins	+++	+++	-	+++
8.	Terpenoids	++	-	-	+++
9.	Phenolic	+++	+++	-	+++
10.	Coumarins	+++	+++	-	+++

(Note: +++: high presence, ++: moderate presence, +: low presence, - : absence)

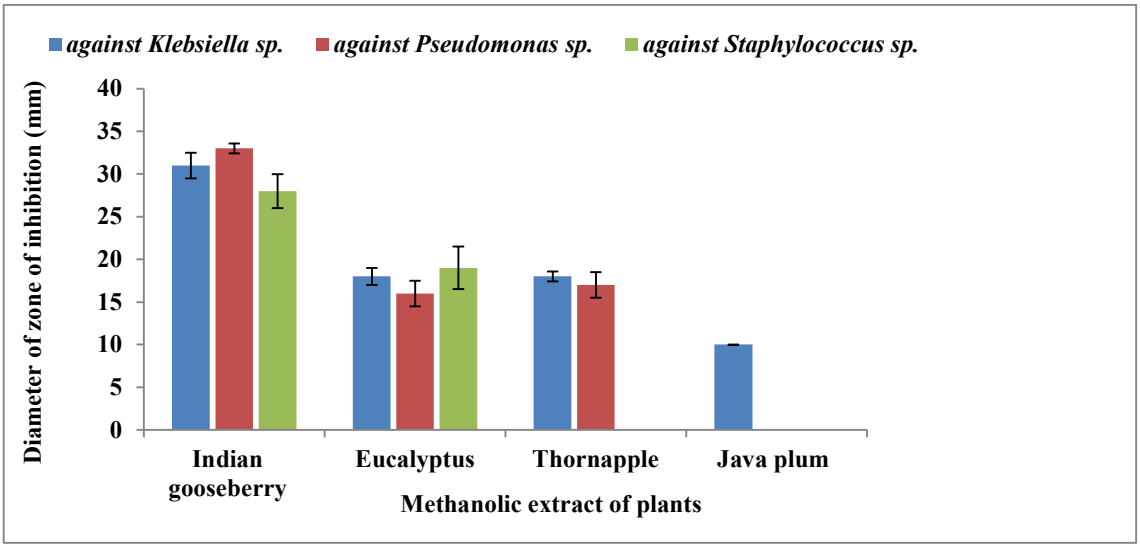


Figure 1: Antibacterial activity (zone of inhibition) of methanolic extract of plants against bacterial isolates

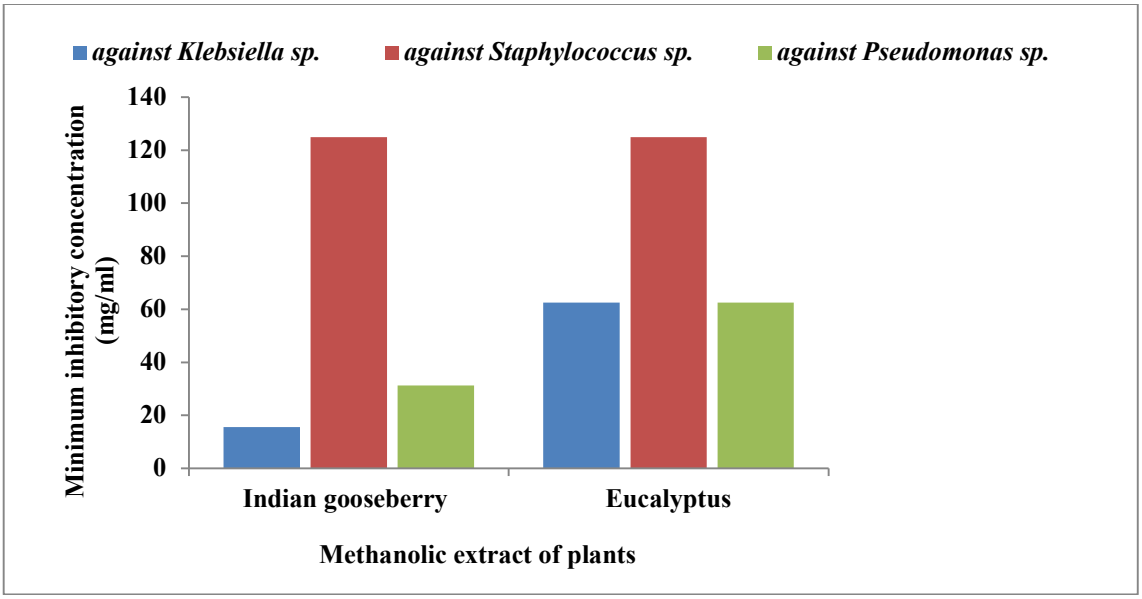


Figure 2: Minimum inhibitory concentration (MIC) of methanolic extract of plants against bacterial isolates

DISCUSSION

The antibiotics are generally used worldwide for the treatment of microbial infections but the increasing antibiotic resistance ability of microorganism has been established as a severe problem in treatment of infections. Different bacterial strains including *K. pneumonia*, *S. aureus*, and *P. aeruginosa* are the causative agents of various lethal human infections such as hospital acquired / nosocomial infections, respiratory infection, skin infection, urinary tract infections, hospital-acquired pneumonia etc. **[28-30]**. The microbial resistance problem indicates the need of alternative compounds that may be used for the development of safe and effective drugs for the treatment of infections as well as for controlling microbial resistance problem. The medicinal plants having antibacterial activity may be used as an

effective solution of microbial resistance and treatment of infections **[31-33]**. This study was designed for screening of phytochemicals as well as *in vitro* evaluation of antibacterial potential of selected medicinal plants. We determined the antibacterial activity of methonilic extracts of selected medicinal plants including java plum (*S. cumini*), thornapple (*D. metel*), eucalyptus (*E. tereticornis*) and Indian gooseberry (*P. emblica*) against previously characterized foodborne bacterial isolates. In the present study, flavonoids and alkaloids were detected in all of the plant extract while phlobatanins and glycosides were not obtained in any of the tested plant extract.

The different phytochemical was previously reported in crude extract of *D. metel* leaves using different solvents **[2, 34-36]**. In the present study, alkaloids and flavonoids were detected in methanolic extract as similar as previously reported studies by **Alabri *et al.*, 2014; Sangeetha *et al*., 2014. Mustapa *et al*., 2023 [2, 35, 36].** We found the effective antibacterial activity of methanolic extract of thornapple against *Klebsiella sp.* and *Pseudomonas sp.* isolate but thornapple did not show any antibacterial activity against *Staphylococcus* isolate. **Alabri *et al.*, 2014 [2]** reported antibacterial activity of different crude extracts of thornapple (*D. metel*) against *P. aeruginosa, K. pneumoniae and S. aureus*, *Escherischia coli*. In previously reported studies, different phytochemicals were also detected in extracts of *P. emblica* in different solvents **[15, 11, 37-39]**. In the present study, Indian gooseberry (*P. emblica*) exhibited effective antibacterial nature against all of the tested bacterial isolates including *Klebsiella sp.*, *Pseudomonas sp.* and *Staphylococcus sp*. **Farhana *et al*., 2022 [40]** reported the antibacterial activity of Indian gooseberry (*P. emblica*) extract against *P. aeruginosa*. **Sharma *et al*., 2023 [41]** reported in vitro antibacterial potential of methanolic extract of Indian gooseberry (*P. emblica*) leaves against different bacterial strains including S. *aureus*, *Bacillus subtilis*, *Micrococcus luteus*, *E. coli* and *K. pneumonia*. **Nath and Dhivya, 2019 [11]** reported antibacterial activity of Indian gooseberry (*P. emblica*) leaves extract against *E. coli* and *S. aureus*. In the present study, different phytochemicals including alkaloids, steroids, sterols, flavonoids, tannins, phenolic and coumarins were detected in eucalyptus methanolic extract. The presence of phytochemicals were also investigated in previous studies reported by **Jain *et al*., 2010; Ani *et al*., 2022; deCastro *et al*., 2022 [42-44]**. **Badrunnisa *et al.*, 2011 [45]** reported the potential antibacterial activity of ethanol and methanol extracts of *E. tereticornis* leaf against *P. aeruginosa*, *B. cereus* and B. *thurangenisis*. **Jain *et al*., 2010 [42]** reported the antimicrobial activity of bark and leaf methanolic extract of *E. tereticornis* against *S. mutans*, *S. aureus*, *E.*

coli and a *yeast C. albicans*. **Imran *et al*., 2017 [18]** reported the presence of different phytochemicals and antibacterial potential of java plum (*S. cumini*) leaf extract with different solvents. **Gowri and Vasanta, 2010 [46]** reported antibacterial activity of methanolic and aqueous leaf extracts of java plum (*S. cumini*) against different gram negative and gram-positive bacteria and also screened different phytochemicals. **Elfadil *et al*., 2015 [47]** reported antimicrobial activity of leaf extract of java plum (*S. cumini*) with different solvents against selected microorganisms such as *S. aureus*, *P. aeruginosa*, *E, coli*, *B. subtitles, Aspergillus niger* and *Candida albacans*. We found different phytochemicals in tested medicinal plants extract and also potential antibacterial nature against the bacterial isolates.

Further molecular investigations are required to identify and isolate the antibacterial compounds from medicinal plants that may be useful for treatment of bacterial infections and controlling emergence of drug resistance. Findings of this study indicate that the researchers should pay some more attention to investigate phytochemicals of medicinal plants to obtain the compounds which may be explored as alternatives of synthetic drugs or antibiotics.

CONCLUSION

The results of present study reveal the presence of different phytochemicals and also antibacterial potential of selected medicinal plants. The results suggest that plants may be a potential source of various antibacterial compounds and must be targeted for the development of new drugs as a solution of bacterial drug resistance as well as treatment of infections.

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Conflicts of interest

There are no conflicts of interest.

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