Antibacterial and antioxidant activities of Musa sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection

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ABSTRACT

Objective: To investigate different Musa sp. leave extracts of hexane, ethyl acetate and methanol were evaluated for antibacterial activity against multi–drug resistant pathogens causing nosocomial infection by agar well diffusion method and also antioxidant activities.

Methods: The four different Musa species leaves were extracted with hexane, ethyl acetate and methanol. Antibacterial susceptibility test, minimum inhibitory concentration and minimum inhibitory bacterial concentration were determined by agar well diffusion method. Total phenolic content and in vitro antioxidant activity was determined.

Results: All the Musa sp. extracts showed moderate antibacterial activities expect Musa paradisiaca with the inhibition zone ranging from 8.0 to 18.6 mm. Among four species ethyl acetate extracts of Musa paradisiaca showed highest activity against tested pathogens particularly E. coli, P. aeruginosa and Citrobacter sp. The minimum inhibitory concentrations were within the value of 15.63– 250 µg/mL and minimum bactericidal concentrations were ranging from 31.25– 250 µg/mL. Antioxidant activity of Musa acuminate exhibited maximum activity among other three Musa species.

Conclusions: The present study concluded that among the different Musa species, Musa paradisiaca displayed efficient antibacterial activity followed by Musa acuminate against multi–drug resistant nosocomial infection causing pathogens. Further, an extensive study is needed to identify the bioactive compounds, mode of action and toxic effect in vivo of Musa sp.

KEYWORDS
Musa, Multi–drug resistant, Nosocomial infection, Antioxidant activity

1. Introduction

A number of new antibiotics have produced by pharmacological industries in the last 30 years, resistance to these drugs by microorganisms has been increased day by day. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the medical world.
For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. This indigenous knowledge, passed down from generation to generation in various parts of the world, has significantly contributed to the development of different traditional systems of medicine\(^1\) as well as helped in exploration of different medicinal plants to find the scientific basis of their traditional uses. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency\(^2\). In the last few years, a number of studies have been conducted in different countries to prove such efficiency\(^3,4\). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils as well as in tannin\(^5,6\).

*Musa* sp. (*Musaceae*) also known as banana is a familiar tropical fruit and important source of food in the world. From its native South western Pacific home, the banana plant spread to India by about 600 BC and later on it spread all over the tropical world. It is possibly the world’s oldest cultivated crop\(^7\). It possesses efficient medicinal values such as stem juice is also used in nervous affectations like epilepsy, hysteria and in dysentery and diarrhoea. Several oligosaccharides comprising fructose, xylose, galactose, glucose and mannose occur naturally in banana\(^8\) making it an excellent prebiotic for the selective growth of beneficial bacteria in the intestine. It aids in combating diarrhoea and dysentery and promotes healing of intestinal lesions in ulcerative colitis. Roots of *Musa paradisiaca* (*M. paradisiaca*) are anthelmintic, flowers are astringent and fruits are mild laxative. It is also useful in celiac disease, constipation and peptic ulcer\(^9\). The present study included the *Musa* plant species used in traditional food and medicines, which were selected due to lack of scientific data especially on antibacterial and antioxidants activities.

2. **Materials and methods**

2.1. **Plant materials**

Fresh plant leaf materials of *Musa* sp. such as *Musa acuminate* Colla (*M. acuminate*), *Muse troglodytarum* L. (*M. troglodytarum*), *Muse sapientum* L. (*M. sapientum*) and *M. paradisiaca* L. were collected from different parts of Erode district, Tamil Nadu, India. Authentication was made by Dr. Rajarathinam at Botany Department, V.H.N.S.N. College, Virudhunagar, Tamil Nadu. Leaf materials were cleaned with distilled water and dried at room temperature under shade. It was ground to obtain coarse powder using an electric grinder.

2.2. **Preparation of plant leaves extracts**

Shade dried leaves (200 g) were coarsely powdered and subjected to successive solvent extraction by continuous Soxhlet extraction. The extraction was done with different solvents in their increasing order of polarity such as hexane, ethyl acetate and methanol (55–80%). Each time the marc was air dried and later extracted with other solvents. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator. The yield was found to be 2.04%, 1.06%, and 3.37% w/w with reference to the air dried plant. The dried extracts were dissolved in dimethylsulphoxide and subjected to antibacterial activity.

2.3. **Test organisms**

The bacterial sp. used for the study were *Escherichia coli* (*E.coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Enterobacter aerogenes* (*E. aerogenes*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Proteus mirabilis* (*P. mirabilis*), *Shigella flexneri* (*S. flexneri*), *Citrobacter sp.*, *Staphylococcus aureus* (*S. aureus*) MRSA and *Enterococcus faecalis* (*E. faecalis*) (VRE). All the stock cultures were obtained from Sun Micro Laboratory, Erode, Tamil Nadu, India. The organisms were periodically subcultured and maintained in nutrient agar slant at 4 °C.

2.4. **Culture media and inoculum preparation**

The test organisms were grown in 5 mL Brain Heart Infusion broth at 37 °C during antibacterial susceptibility test. Twenty–four hour old pure cultured bacteria were used to prepare a density of 10\(^7\) cells/mL of 0.5 McFarland standards during each test. Muller–Hinton agar was prepared according to the manufacturer’s instruction, autoclaved and dispensed at sterile plate. All the culture media were purchased from HiMedia Pvt. Ltd., Mumbai, India.

2.5. **Antibacterial susceptibility tests**

2.5.1. **Agar well diffusion**

Bacterial broth culture was prepared to a density of 10\(^8\) cells/mL of 0.5 McFarland standards. The aliquot was spread evenly onto Muller Hinton agar by sterile cotton swab. Then, the plated medium was allowed to dry at room temperature for 30 min\(^10\). On each plate, equidistant wells were made with a 6 mm diameter sterilized, cork borer, 2 mm from the edge of the plate. Fifty microliter of each leaves extract (1 mg) was aseptically introduced into an agar well. Chloroamphenicol (30 µg/mL) were used as positive controls and the extraction solvents hexane, ethyl acetate and methanol were included as negative controls. This was followed by allowing the agar plate on the bench for 40 min pre diffusion followed by incubation at 37 °C for 24 h. The formation of clear inhibition zone of ≥7 mm diameters around the wells was regarded as significant susceptibility of the organisms to the extract\(^11\). The experiment was
performed in triplicate. Experiments that gave contradicting results were done for the fourth time for an easy decision.

2.6. Determination of minimum inhibitory concentration (MIC)

MIC was determined for extracts that showed ≥7 mm diameter growth inhibition zone. The test was performed using agar well diffusion methods. In agar well diffusion, the extracts (500 µg/mL) was serially diluted as 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128 and 1:256 to bring 250 µg/mL, 125 µg/mL, 62.5 µg/mL, 31.25 µg/mL, 15.63 µg/mL, 7.81 µg/mL, 3.95 µg/mL, and 1.95 µg/mL concentrations, respectively. The extract was then aseptically introduced into an agar well. The inhibition zone was measured after 24 h incubation at 37°C and the minimum concentration that inhibited growth was considered as MIC value of the extracts.

2.7. Determination of minimum bactericidal concentration (MBC)

MBC was determined by sub-culturing the samples having a value of lesser or equal to MIC value. The highest dilution (lesser concentration) that yielded no single bacterial colony was taken as MBC.

2.8. Antioxidant activity

2.8.1. Total phenolic content

Phenolic contents of crude methanolic extract and fractions were estimated by the method of Taka et al[12]. 100 µL aliquot of sample was mixed with 0.5 mL of Folin–Ciocalteau’s phenol reagent and allowed to stand for 5 minutes at room temperature. After incubation, 1 mL of 5% Na₂CO₃ was added, and the reaction mixture was mixed thoroughly and allowed to stand for 60 min at room temperature in the dark. Absorbance of all the sample solutions was measured at 765 nm using spectrophotometer. Phenolic contents are expressed as gallic acid equivalents per gram (GAE/g) of methanolic extract.

2.8.2. Total antioxidant activity

Total antioxidant activities of crude methanolic extract were determined according to the method of Prieto et al[13]. 0.3 mL of sample was mixed with 3.0 mL reagent solution (0.6 mol/L sulfuric acid, 28 mmol/L sodium phosphate and 4 mM ammonium molybdate). Reaction mixture was incubated at 95°C for 90 min under water bath. Absorbance of all the sample mixtures was measured at 695 nm. Total antioxidant activity is expressed as the number of equivalents of ascorbic acid in milligram per gram of extract.

3. Results

In the present study, the evaluation of antibacterial activity of non–polar (hexane) and polar (ethyl acetate, methanol) extracts of the leaves of Musa sp. against seven gram–negative and two gram–positive bacteria were studied using agar well diffusion method. The data pertaining to the antimicrobial potential extract of the leaves of Musa sp. were presented in Table 1. The results revealed variability in the inhibitory concentration of each extracts against a panel of pathogenic bacteria. The diameters of growth inhibition zone were in the range maximum of 18.6±0.5 mm and minimum of 8.0±0.5 mm whereas the standard drug, chloramphenicol had higher zones of inhibition ranging from 11.1±0.3 mm to 22.4±0.20 mm (Table 1). The extracts showed antibacterial activity against all tested bacterial pathogens except hexane extract against E. aerogenes and P. mirabilis. The highest inhibition zone was observed on ethyl acetate extract of M. paradisiaca against E. coli (18.6±0.5 mm) and P. aeruginosa (16.4±0.6 mm). Moderate biological activity was demonstrated by hexane extract of M. paradisiaca and M. acuminate was found to be

### Table 1

Antibacterial activity of crude extracts of Musa sp. (1 mg/disc) against antibiotics resistant clinical pathogens causing nosocomial infection.

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<th>Cultivars of Musa spp.</th>
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effective against *S. aureus*, *Citrobacter* sp., *P. aeruginosa*, *P. mirabilis* and *E. aerogenes*. While *M. tropolodytarum* and *M. sapientum* were inhibited the growth only three gram extracts of *Musa* sp., *M. tropolodytarum* exhibited very low significant activity against tested bacterial pathogens.

The MIC tests of *Musa* sp. organic solvent extracts against 9 bacterial pathogens were carried out using the micro dilution technique. The MIC results are shown in Table 2. The most of tested pathogens were showed resistant to *M. tropolodytarum* leave extracts, later it was excluded from MIC study. The MIC ranged from 15.63 to 250.00 mg/mL. The most sensitive bacterial pathogen was *E. coli* (MICs ranging from 15.63 to 250.00 µg/mL), followed by *P. aeruginosa* (MICs ranging from 31.25 to 250 µg/mL), whereas the other pathogenic isolates (MICs ranging from 62.50 to 250.00 µg/mL) was less sensitive. Results from the MBC assays supported the MIC determination. These results further confirmed that ethyl acetate extract of *M. paradiisica* and *M. acuminata* were the most potent extracts. These extracts exhibited bactericidal nature as observed from their MBC values ranged from 31.25 to 250.00 mm (Table 3). However, plant extracts seemed to be species specific as they did not show the same trend of bactericidal activity.

Phenolic compounds are important fruit constituents because they exhibit antioxidant activity by inactivating lipid free radicals or preventing decomposition of hydroperoxides into free radicals. The TPC determined in methanolic extracts of *Musa* species are shown in Figure 1. The TPC ranged widely from 3.72 to 5.55 mg GAE/g. Among the four *Musa* species, the highest TPC was obtained from methanol extract of *M. paradisica* leaves (5.55 mg GAE/g), whereas low TPC was observed in methanol extract of *M. tropolodytarum* (2.09 mg GAE/g). The yield of the methanol extract of the four *Musa* species and its total antioxidant capacity are given in Figure 1. Total antioxidant capacity of *Musa* species are expressed as the number of equivalents of ascorbic acid. The phosphomolybdenum method was based on the reduction of Mo(VI) to Mo(V) by the antioxidant compound and the formation of a green phosphate/Mo(V) complex with a maximal absorption at 695 nm. Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract. *M. paradiisica* seems to be having

### Table 2

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### Table 3

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a higher capacity than the other three species. The antioxidant activity of the *Musa* plant species were in the order: *M. paradisiaca* > *M. acuminata* > *M. sapientum* > *M. troglodytarum*.

![Figure 1. Total phenolic and antioxidant activities of leaf extract of different *Musa* sp. Values are the average of duplicate experiments and represented as mean± standard deviation.](image)

### 4. Discussion

The continuous evolution of bacterial resistance to currently available antibiotics has necessitated the search for novel and effective antimicrobial compounds. Medicinal plants are have pharmacological effects can be natural composite sources act as new anti-infectious agent. So, today foremost demand is searching of effective, cheapest and improved antibacterial compounds. The present work more concern with such kind of search from the different species of traditional *Musa* plants. The antibacterial activity was more prominent on the Gram negative bacteria than the Gram positive bacteria. Gram negative bacteria were the most susceptible to inhibition by ethyl acetate and methanol extracts. However this study corresponds with the results of Karadi et al.[14] and Subrata Kumar et al.[15] who observed that banana have antimicrobial activities against pathogenic bacteria. The results presented that all the extracts had variable degree of antibacterial activity and the inhibition of bacteria growth was dose dependent as inhibitory action the extracts was found to increase with the increase of concentration against all bacterial pathogens as evidenced by the higher zone of inhibitions at higher concentration of each extract.

While considering bacterial pathogens, *M. paradisiaca* ethyl acetate extract showed maximum activity with MIC 15.63 µg/mL for *E. coli*. *M. acuminata* and *M. sapientum* ethyl acetate extracts showed 31.25 µg/mL MIC for *E. coli*, *P. aeruginosa*, followed by *P. mirabilis*, *Citrobacter* sp. and *E. faecalis*. In general, all the extracts were showed less than 250.00 µg/mL MIC against tested bacterial pathogens. The MIC analysis of bacterial pathogens showed that the ethyl extract was highly active in comparison to other extracts, which inhibited the series of tested organisms at a low concentration (15.63 µg/mL) and (250.00 µg/mL). Hence, we conclude that among four *Musa* species, ethyl acetate of *M. paradisiaca* possess effective antibacterial activities against tested nosocomial disease causing bacterial pathogens.

In general particular *Musa* species, the TPC was generally higher in the leaves than in the other parts, and the green had higher TPC than the dried materials. The results of TPC of *Musa* leaves extract of present work were slightly higher. Our findings are supported by the reported results of Loganayaki et al.[16]. The TPC of banana extracts have been reported in the literatures as high as 1.4 g GAE/100 g when extracted with methanol[17-19]. The total phenolic content measures the total amount of phenolic, which include flavonoids. Total flavonoids content is designed to quantify the amount of flavonoids. The total phenol and total flavonoid content of extracts have been reported as promising medicinal and nutritional ingredients[20]. The total phenol content of the plantain products is higher than some commonly consumed tropical plants[21]. Furthermore, the trends of the total phenol content and total flavonoid content results are in differ to the earlier study which revealed that there is a direct relationship between the total phenol content and the antioxidant activity in plant foods[22].

The results of present work were concluded that, *Musa* species leaf extracts possess effect antibacterial and antioxidant activities. The obtained results thus give the experimental basis to understand the use of *Musa* species in traditional medicine as an antibacterial and antioxidant agent. Further, studies are required to identify the active principles of the extract and its mode of action.

### Conflict of interest statement

The authors declare that there are no conflicts of interest

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### Comments

#### Background

Folkloric medicinal plants constitute the base of health care systems in many societies. *Musa* sp. belongs to family Musaceae. In the traditional system of medicines, *Musa* plants are used to treat different microbial diseases such as diarrhea, skin infections, etc. The literature reported that in different parts of the world system if medicine, the leaf, seed, stem of this plant have been used for the treatment of fire inflammation. Hence, there was a limited study; the aims of this investigation were to evaluate the antibacterial and *in vitro* antioxidant activities of *Musa* sp. leaf extract. The findings are an attempted and the paper shows some hopeful results.
Research frontiers

The present work was showed that *Musa* sp. extract exhibited effective antibacterial and antioxidant activities.

Related reports

Antibacterial and antioxidant activities of *Musa* plant extracts were reported with some promising results (Loganayaki et al., 2010; Karadi et al., 2011; Subrata Kumar et al., 2011). The antibacterial activity of *M. pudica* was studied but limited reports of antibacterial and antioxidant activities.

Innovations and breakthroughs

*In vitro* antioxidant and antibacterial activities of four different varieties of *Musa* sp. leaves extract were studied. The results were comparatively effective and more promising with earlier reports.

Applications

*Musa* sp. leaf extract has antioxidant and antibacterial properties. This extract may be used for formulation skin related cream and drug against emerging drug resistant pathogens.

Peer review

In this work the antibacterial effect of three different leaf–extract of *Musa* sp. against on clinical pathogens and antioxidant activity were determined by *in vitro* studies. Different species of *Musa* were used and among *M. paradisiaca* and *M. sapientum* exhibited effective antibacterial activity and total antioxidant activity. However, the data and the conclusion of this work are interesting against nosocomial infection causing bacterial pathogens.

References


