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Research Article

PHYTOCHEMICALS SCREENING, ANTIOXIDANT AND ANTIBACTERIAL POTENTIAL OF *MUKIA SCABRELLA* (MUSUMUSUKKAI) AGAINST NOSOCOMIAL BACTERIAL PATHOGENS

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ABSTRACT

In the present study phytochemical screening of the leaf extract of *M. scabrella* revealed the presence of antibacterial and antioxidant bioactive components. Phytochemical tests showed the existence of flavonoids, carbohydrates, steroids, tannins, alkaloids and phenolic compounds in the leaf powder of *M. scabrell*. Methanolic and ethyl acetate extracts of leaves, stem and root parts indicated the antibacterial activity against nosocomial bacterial pathogens. GC – MS analysis revealed the presence of bioactive molecules ascribed to the antibacterial activity. Thus, the results clearly indicated that *M. scabrella* could be used to explore the new drugs against nosocomial bacterial pathogens.

Keywords: *Mukia scabrella*, nosocomial bacterial pathogens, GC-MS, antibacterial and antioxidant bioactive components.

INTRODUCTION

Because of bacterial infections continue to be one of the major concerns, the necessity for the identification of novel classes of drugs augmented paramount importance in the natural drug discovery research. In particular, nosocomial bacterial infections have recently increased markedly and emerging as most difficult pathogens to eradicate with existing antibiotics. This has prompted discovery of novel agents from herbal plants that can selectively work on bacterial targets to prevent the future infections. In this milieu, medicinal plants considered as naturally important sources since they are the major source of life – saving drugs for human medicine. Due to this reason many laboratories are aiming at exploration of medicinal plants for their bioactive natural products. Increasing therapeutic benefits continuously attract the attention of pharmacologists for biomedical investigations on plant extracts and isolated phytochemicals (Bani *et al*., 2006; Chang *et al*., 2007).

Flavonoids have been reported to possess potent anti-MRSA activity (Hironori *et al*., 1996). Lignans has been reported to have antibacterial activity (Valsaraj *et al*., 1997). Cyclotides has also been to known to have antibacterial activity (Tam *et al*., 1999). Further, Alkaloids are also among the metabolites reported to have antibacterial activities (Newton *et al*. 2000). Terpenoids is also reported to have antibacterial activity (Reddy *et al*., 2001). Recently, Mbosso *et al.*, 2012 reported that anticancerous activity of Ficusamide against the human A549 lung cancer cell line. Thus, the isolation of valuable phytochemicals from medicinal plants towards to develop various natural drug to prevent the bacterial diseases in humans continues over the years. *M. scabrella* (Musumusukkai) is a climbing herbal plant inhabiting predominantly southern part of India and is well known for its curative effect on cough, cold and as expectorant and astringent in respiratory tract ailments in traditional medicine. In the present study we investigate phytochemicals of *M. scabrella* and evaluated their antibacterial activity against *Acinetobacter* sp, *pseudomonas aeruginosa*, *klebisella pneumoniae*, *Staphylococcous aureus*, and Methicillin resistant *Staphylococcous aureus* (MRSA).

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MATERIALS AND METHODS

Collection of plant material

Healthy plant (stem, leaf and root parts) of *M. scabrella* was collected from foothills of Kolli Mountains, Tamil Nadu, India (Latitude: 10°12′ - 11°7′ N, longitude: 76°-77°17′ E) and transported to the laboratory. The plant was according to the pertinent taxonomic procedures. The leaves of *M. scabrella* was subjected to shed drying and crushed to powder. The powder was then filtered using mesh 40 for further analysis.

Test bacterial strains

Clinical isolates of bacterial strains were obtained from Doctors Diagnostic Center, Trichy, Tamil Nadu, India. Test strains of *Acinetobacter* sp, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and Methicillin resistant *Staphylococcus aureus* were isolated from hospitalised Intensive care unit patients. *Acinetobacter* sp showed resistance to all fluroquinolone groups of antibiotics, cephalosporins, penicillin group, and aminoglycosides. *Pseudomonas aeruginosa* showed resistance to all fluroquinolones, Cephalosporins, Gentamicin, and Tobramycin. *Klebsiella pneumoniae* had resistance to all cephalosporins, fluroquinolones. They were grown in Luria–Bertani (LB) broth at 37° C.

Phytochemical analysis

Phytochemicals screening was performed using according to the previously described methods (Harborne (1984), Brunton (1995), Wagner *et al*. (1984). The tests were carried out to find out the presence of tannin, flavonoids, alkaloids, carbohydrates, terpenoids and steroids.

Test for tannins

Five gram of leaf, root and stem powdered sample was mixed with 100 ml of sterile double distilled water and then heated at 60° C for 10 minutes. The aqueous extract was then filtered with Whatmann No.1 filter paper. 1% ferric chloride solution was added to 5 ml of extract in a test tube. Formation of a blue-black, green or blue-green precipitate in the reaction mixture indicates the presence of tannins (Trease and Evans, 2002).

Test for flavonoids

One gram of leaf, root and stem powder sample was dissolved in 10 ml of 50% methanol and slightly warmed. After that the solution was filtered and added with metal magnesium and followed by few drops of conc. HCl. Formation of red, orange, or red to purple color indicates the presence of flavonoids (Trease and Evans, 2002).

Test for alkaloids

1 g of leaf, root and stem powder sample was dissolved in 10 ml of methanol and filtered through Whatmann No.1 paper. After that 3 ml of filtrate was mixed with 10 ml of 1% aqueous HCl. Mayer's reagent was then added with the reaction mixture. Appearance of buff-colored precipitate indicates the presence of alkaloids (Sofowora, 1993).

Test for carbohydrates

Two gram of leaf, root and stem powdered sample was dissolved in 20 ml of distilled water and then filtered through Whatmann No.1 paper. Filtrate was then added with few drops of Molisch's reagent. After that 1 ml of conc. H_2SO_4 added slowly. The reaction mixture was kept at room temperature for two minutes and then 5 ml of sterile water was added. Formation of Red or dull violet color at the interphase of the two layers indicates the presence of carbohydrates (Sofowora, 1993).

Test for terpenoids

One gram of leaf, root and stem powder was dissolved in 10 ml of chloroform and added with conc. H_2S0_4. Appearance of brown color at the interface indicates the presence of terpenoids.

Test for steroids

One gram of leaf powder was dissolved in 10 ml of ethanol and added with 2 ml of acetic anhydride. Formation of blue or green color indicates the presence of steroids.

GC-MS analysis

GC-MS analysis of the aqueous extract of *M. scabrella* was carried out using a Perkin–Elmer GC Clarus 500 system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with a Elite-5MS (5% diphenyl, 95% dimethyl poly siloxane) fused a capillary column (30 × 0.25 µm ID × 0.25 µm df). Ionization energy of 70 eV was employed for the detection of GC-MS peaks. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 3 µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250 °C, the ion-source temperature

was 200° C, the oven temperature was programmed from 110° C (isothermal for 2 min), with an increase of 10° C/min to 200°C, then 5° C/min to 280° C, ending with a 9 min isothermal at 280° C. The total GC/MS running time was 36 min. Turbo-Mass ver-5.2.software was used to analyze the mass spectra and chromatograms. NIST (National Institute Standard and Technology) Libraries was used to interpretate the mass spectrum of GC-MS.

DPPH Assay

The antioxidant activity of the aqueous extract of *M. scabrella* was determined on the basis of their free radical scavenging activity of the stable 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical. 1 g leaf powder was dissolved in 10 ml of distilled water and further diluted in water to obtain 1000 µg/ ml working solution. 1000 µg/ml solutions were series diluted into 10 µg/ml, 20 µg/ml, 30 µg/ml, 40µg/ml, 50 µg/ml, 60 µg/ml, 70 µg/ml, 80 µg/ml, 80 µg/ml and 100 µg/ml with water. In each reaction, the solutions were mixed with 1 ml of 0.1 mM 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 0.45 ml of 50mM Tris-HCl buffer (pH 7.4), and 0.05 ml samples were incubated at room temperature for 30 min. The reduction of the DPPH free radical was measured on UV–visible spectrometric measurements was performed on Hitachi double beam equipment (Model Lambda 35) at 517 nm. Extract concentration providing 50% inhibition (IC_{50}) was calculated using the graph by plotting inhibition percentage against extract concentration [10- 100 µg/ml]. Ascorbic acid (AA) was used as positive controls and tests were carried out in triplicates. The free radical scavenging activity (FRSA) was calculated by using the following equation: % inhibition = [(absorbance of control – absorbance of test sample)/absorbance of control] x 100%. The antioxidant activity of each sample was expressed in terms of IC50 (micromolar concentration required to inhibit DPPH radical formation by 50%) calculated from the inhibition curve.

Extraction of material

Aqueous extraction: 5 g of powdered samples of leaf, root and stem were dissolved in 100 ml of distilled water and boiled for 1 h at 60°C. After that the extract was filtered through Whatmann No.1 paper and centrifuged at 10,000 rpm at room temperature for 10 minutes. The resulting supernatant was further boiled and one portion in total volume left out in the beaker. The sample was then filtered through 0.2 µm Polyethersulfone (PES) syringe filters (Sartorius Minisart® high flow Syringe Filters).

Methanol extraction: 10 g of powdered samples of leaf, root and stem were dissolved in 200 ml of methanol in a one liter conical flask, plugged with cotton wool. The solution was then kept on an orbital shaker at 160 rpm for one day. The supernatant was collected by centrifugation at 10,000 rpm for 15 minutes. The solution was evaporated using rotary evaporator until complete removal of solvent. The final concentration of extract was made onto 500 mg/ ml in Dimetyl sulfoxide (DMSO) for further analysis and stored at 4°C in airtight bottles.

Ethyl acetate extraction: 10 g of powdered samples of leaf, root and stem were dissolved in 200 ml of Ethyl acetate in a one liter conical flask, plugged with cotton wool. The solution was then kept on an orbital shaker at 160 rpm for one day. The supernatant was collected by centrifugation at 10,000 rpm for 15 minutes. The solution was evaporated using rotary evaporator until complete removal of solvent. The final concentration of extract was made onto 500 mg/ ml in DMSO for further analysis and stored at 4° C in airtight bottles.

Antibacterial activity

Disc diffusion assay: Antimicrobial activity was tested for all extracts against drug resistant *test* bacterial strains on Luria - Bertani (LB) agar plate using a disc diffusion method. Zone of inhibition (ZoI) was determined to swot up the growth inhibition of test strains measured by a vernier calliper. Sterile standard antibiotic discs with diameter of 6 mm were purchased from HIMEDIA Laboratories, India. 50 µl (50 µg/disc) of test samples were loaded on the sterile disc and air dried completely. LB agar was spread plated about 10^6 CFU/ml of test pathogens, impregnated with the sample loaded disks incubated at 37° C for 18 h and ZoI was measured after incubation. Each extract was analyzed in triplicate and the mean values are presented.

Minimal Inhibitory concentration (MIC) test: The experiment was performed using serial dilution method. The stock solution of each test extracts was prepared at the concentration of 50

µg/ml in sterile Luria Bertani broth and serially diluted up to five times. Six assay tubes were taken to test the MIC for each strain. In the first tube 1 ml of the sterilized Luria Bertani broth was inoculated and then 1 ml of the test compound solution was added and thoroughly mixed to a concentration of 25 µg/ml. This solution was further diluted by inoculating 1ml from the prior test tube into subsequent tubes. 100 µl of each test bacterial strains was added in each tube. This was done in duplicate. All test procedures were performed under aseptic conditions. The inoculated tubes were kept at 37° C ± 1° C at 24 hrs. After the incubation period, tubes were observed for any deposits or turbidity to determine the MIC.

RESULTS AND DISCUSSION

Phytochemical analysis

In the present study we have tested phytochemicals from aqueous leaf extract. The results indicated the presence of phytochemical constituents and they listed in table 1.

Table 1. Phytochemical constituents of aqueous leaf extract of *M. scabrella.*

S. No.	Tests	Result
1	Tannins	+
2	Flavonoids	+
3	Alkaloids	+
4	Carbohydrates	+
5	Terpenoids	+
6	Steroids	+

Key: + = present; - = absent.

The presence of these phytochemicals might have responsible for the various bioactive potential of *M. scabrella* including antimicrobial and antioxidant potentials. In line with the results showed in this study, previous studies have also been reported the presence of various phytochemicals in *M. maderaspatna* (Petrus, 2012, Gomathy *et al.,* 2012). It is evident that tannin has used in pharmacologically as antidiarrheal. It has also been reported that tannins known to have antimicrobial properties (Akiyama *et al.*, 2001; Funatogawa *et al.*, 2004). Petrus, 2012 reported that flavonoids have been known to have potent antioxidant activity. In similar, our study suggests that leaf extract of *M. scabrella* has significant antioxidant activity and might be useful for the free radical scavenging activity. Further, Alkaloids are also among the metabolites reported to have antibacterial activities (Newton *et al*. 2000). Terpenoids is also reported to have antibacterial activity (Reddy *et al.,* 2001). It is evident that hydrolysable tannins (corilagin and tellimagrandin I) and a tea polyphenol epicatechin gallate have antimicrobial activity against antibiotic resistance of MRSA (Hatano *et al*., 2005). Isopimarane diterpenoids, the flavone cirsimaritin and the sterols b-sitosterol and stigmasterol were isolated from the aerial parts of *Aeollanthus rydingianus* showed antimicrobial activity against Gram-positive and Gram-negative bacteria and a yeast strain (Rijo *et al*., 2009).In addition, Cyclic alkyl polyol derivatives have recently been reported to have bactericidal properties (Roumy *et al*., 2009). It also has been well documented that endiandric acid derivatives of beilschmiedic acids is known to have antibacterial activity (Chouna *et al.,* 2009). Martinez *et al.,* 2010 reported that oligosaccharide is also known to have antimicrobial activity against multi drug resistant *Staphylococcus aureus.* Thus, the results of the phytochemical constituents *M. scabrella* are line with previous reports and could be explored for the biomedical values of human medicine.

GC-MS analysis: GC-MS analysis revealed the presence of various phytochemical compounds with different retention time as shown in figure 1. The compounds are including Benzo [1,2,4] triazine, 3-(3-nitrophenyl)-5,6,7,8-tetrahydro, Cephaloridine, Pyrazine, 1H-Imidazole, Pyrazine, Benzeneethanamine, 1,2,5-Trimethylpyrrole, 2- (Cyclopenten-1-yl) acetic acid, Norbornane, 5-Chlorovaleric acid, 3,5-dimethylphenyl ester, 3- Cyclopentylpropionic acid, N-acetyl-3-fluoroamphetamine, Psicofuranine, 10-oxotricyclo[5.4.0.0(2,9)] undecane-8-carboxylic acid-3,3,7-tr, Propanoic acid, Pentanoic acid, Methylmalonic acid, Benzene, 2-Chloroethyl methyl sulfone, Methylenecyclopropanecarboxylic acid, Levoglucosenone, Methyl salicylate, 1,2-benzenedicarboxylic, Benzoic acid, 2-(2-methylbutyl)oxy-, methyl ester, Aspirin methyl ester, Cyclohexanone, 3-Hexenoic acid, Aziridine, 1-methyl, Trifluoroacetyl-di-t-butylphosphine, 2,2-Dichloroethyl methyl ether, Carbonic acid, butyl 2-methoxyethyl ester, and Diethyl Phthalate. Thus, results of GC-MS analysis suggested the presence of antimicrobial, antifungal and antioxidant compounds in *M. scabrella*.

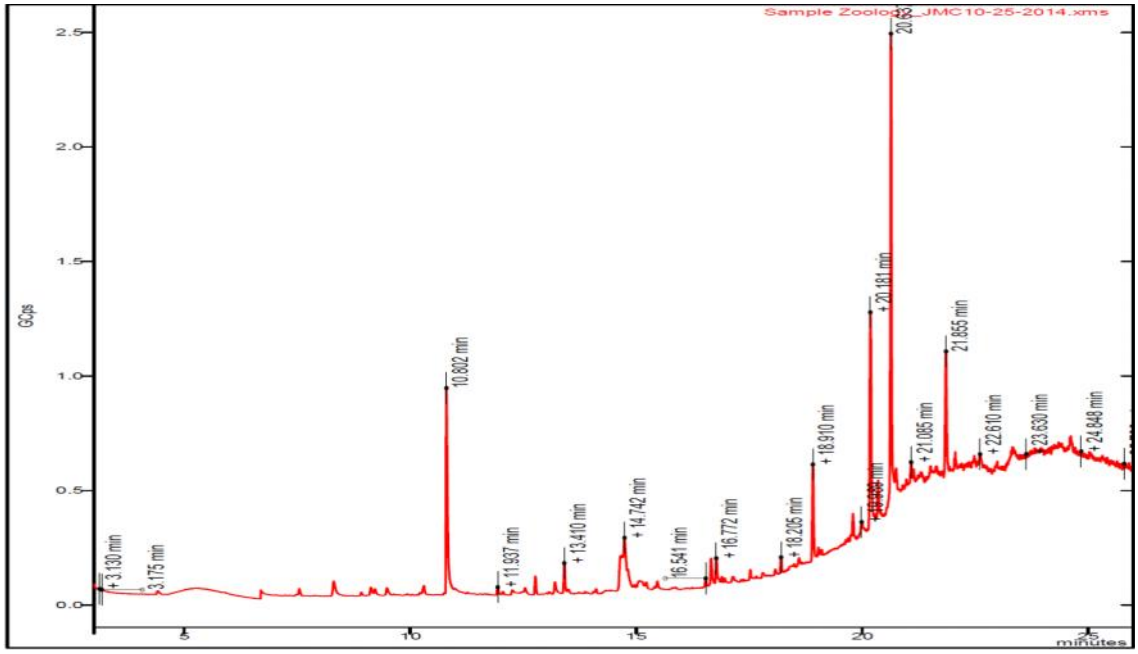

Figure 1. GC-MS analysis of leaf extract of *M. scabrella.*

DPPH activity

DPPH activity of leaf extract was visually noticed by color change from purple to yellow. It was observed that the scavenging activity increased with the increasing concentrations of test samples of leaf extract. The IC50 value for aqueous leaf extract was 40.01 µg/ml. These results suggested that aqueous extract of *M. scabrella* leaves exhibited the ability to quench the DPPH radical which indicated that extract has antioxidant properties with radical scavenging activity.

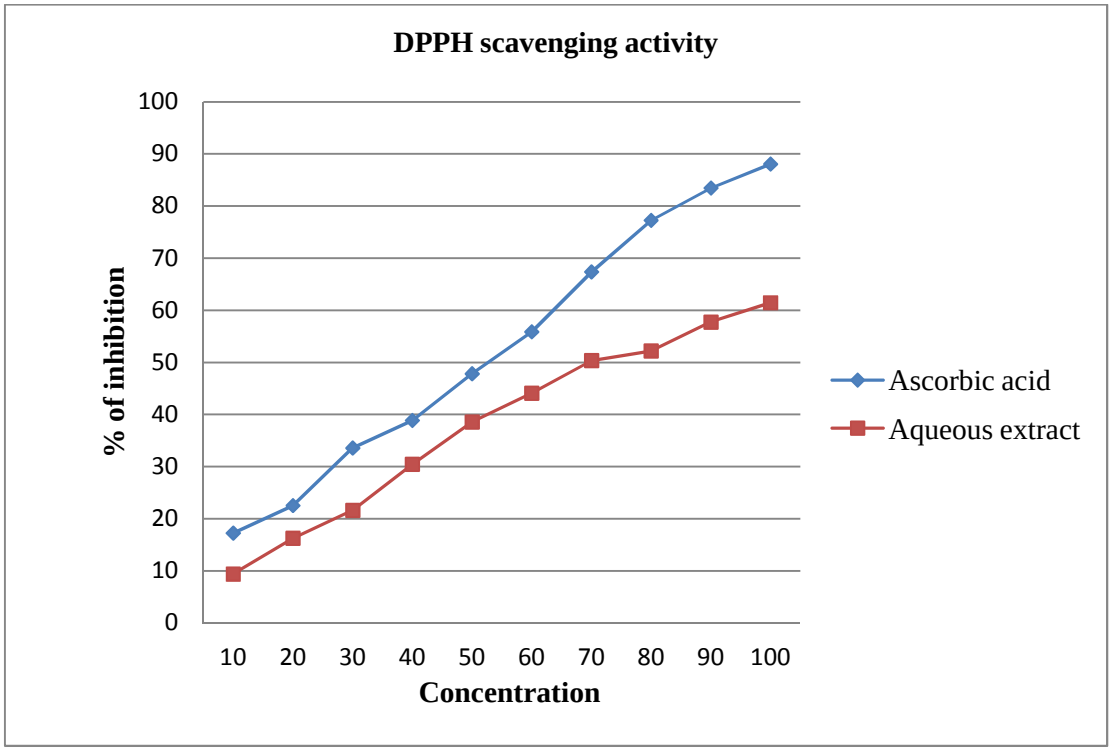

Figure 2. DPPH free radical scavenging activity of *M. scabrella* leaf extract.

Antibacterial activity

The outcomes of antibacterial activity of *M. scabrella* extracts are shown in table 1. Leaf, root and stem extracts had exhibited significant antagonistic activity against tested bacterial pathogens. In addition, we also observed bactericidal effect against MRSA, *Acenitobacter sp*. and *K. pneumoniae*. All extracts did not shown antibacterial activity against drug resistant microorganisms. Ethyl acetate extracts of root and leaf showed bactericidal activity against MRSA. Methanol extract of root showed bactericidal activity against multidrug resistant *Acenitobacter sp*. It has also been noted that Methanol extract of root showed bactericidal activity against multidrug resistant *K. pneumoniae*.

Table 1. Antibacterial activity of aqueous, methanol and ethyl acetate extracts of *M. scabrella* against different microorganisms.

Plant part used	Zone of Inhibition	AQ (50mg/ml)	ME (50mg/ml)	EA (50mg/ml)	KA (10µg/ml)
	Microorganisms				
Leaf	*S. aureus*	9± 0.21	15± 0.11	12± 0.24	24± 0.35
root		8± 0.24	16± 0.13	11± 0.21	24± 0.21
stem		8± 0.21	16± 0.12	10± 0.23	24± 0.34
	Acenitobacter sp				
Leaf		9± 0.18	14± 0.12	17± 0.21	27± 0.41
root		10±0.24	15± 0.26	20± 0.33	27± 0.21
stem		9± 0.22	14± 0.24	20± 0.24	27± 0.71
	K. pneumoniae				
Leaf		9± 0.19	13± 0.25	17± 0.11	24± 0.31
root		8± 0.25	15± 0.31	17± 0.26	24± 0.25
stem		9± 0.11	14± 0.24	20± 0.18	24± 0.21
	P. aeruginosa				
Leaf		8± 0.27	18± 0.24	14± 0.10	25± 0.31
root		8± 0.31	15± 0.18	15± 0.26	25± 0.40
stem		8± 0.21	20± 0.30	17± 0.11	25± 0.11
	MRSA				
Leaf		0	0	**15± 0.24**	0
root		0	0	**16± 0.19**	0
stem		0	0	0	0
	Acenitobacter sp (MDR)				
Leaf		0	0	0	0
root		0	**12± 0.26**	0	0
stem		0	0	0	0
	K. pneumoniae (MDR)				
Leaf		0	0	0	0
root		0	**11± 0.18**	0	0
stem		0	0	0	0
	P. aeruginosa (MDR)				
Leaf		0	0	0	0
root		0	0	0	0
stem		0	0	0	0

Values are mean inhibition zone (mm) S.D of three replicates.

(AQ-aqueous extract, ME-methanol extract, EA: Ethyl acetate extract and KA-Kanamycin (10 µg/ml); 0 = no inhibition).

All the parts of *M. scabrella* when extracted with methanol and ethyl acetate showed antibacterial activity against different nosocomial pathogens. Aqueous extract did not exhibit clear zone and the less activity might be due to the presence of thiocyanate, nitrate, chlorides and sulfates besides other water soluble components (Darout *et al.,* 2000). Whereas the activity was differed against MDR species of nosocomial bacterial pathogens. Methanol extract showed highest antibacterial activity against *P.aeruginosa*. Ethyl acetate extract exhibited highest antibacterial against *Acenitobacter sp, K. pneumoniae* and *P. aeruginosa*. The Minimal inhibitory concentration of aqueous extract against *S.aureus, Acenitobacter sp, K. pneumoniae,* and *P.aeruginosa* 10 µg/ml. The MIC of methanol extract against *S. aureus, Acenitobacter sp, K. pneumoniae,* and *P. aeruginosa* 6.2 µg/ml. The MIC of ethyl extracts against *S. aureus, Acenitobacter sp, K. pneumoniae,* and *P. aeruginosa* 8.4 µg/ml. Thus the results of the present study clearly indicated that *M. scabrella* extracts possess potential antibacterial activity against both non drug resistant and drug sensitive nosocomial bacterial pathogens.

CONCLUSION

At present, many parts of Europe, Japan and USA exploring phytochemicals from herbal plants for generating new drugs against emerging pathogens. The results from this study prospects to isolate new antimicrobial entities from *M. scabrella* for therapeutic applications in human medicine.

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