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Antimicrobial Activity of Bioactive Constituents Isolated from the Leaves of *Naravelia zeylanica* (L.) DC.

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ABSTRACT

Two sterols compounds were isolated from the petroleum ether extract of *Naravelia zeylanica* leaves. The antimicrobial activity of this extract and the isolated constituents taraxerol and β-sitosterol, were screened against twenty-seven clinical isolates from different infectious sources belonging to Gram-negative *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, and Gram-positive *Staphylococcus aureus* and five dermatitis fungi, *Trichophyton rubrum*, *Microsporum gypseum*, *Tricophyton tonsurans*, *Microsporum audouini*, and *Candida albicans*. The minimum inhibitory concentration of the isolated constituents was evaluated against the American Type Cell Culture (ATCC) and Microbial Type Culture Collection (MTCC) strains of the corresponding pathogenic microorganisms. The antimicrobial activity of the crude petroleum ether extract, and their isolated constituents taraxerol and β-sitosterol were 150 µg/µl, 100 µg/µl and 80 µg/µl respectively. The agar wells loaded with taraxerol, β-sitosterol and the petroleum ether extract exhibited a significant zone of inhibition against the clinical strains of *S. aureus* (23.63 ± 0.09 mm). A moderate zone of inhibition was observed on the clinical strains of *K. pneumoniae* (21.30 ± 0.15 mm) and *P. aeruginosa* (21.50 ± 0.29 mm). The results indicated that taraxerol exhibited significant antimicrobial activity against the clinical strains of pathogenic bacteria and fungi. Taraxerol can be used as a broad-spectrum antimicrobial agent against human pathogenic bacteria and fungi. The present work was accordingly performed to characterize more potent antimicrobial metabolites from an endemic medicinal climber *N. zeylanica*.

Keywords: antimicrobial, bioactive compounds, clinical isolates, dermatophytes, Ranunculaceae
Abbreviations: ATCC, American Type Cell Culture; **ANOVA**, Analysis of Variance **BHI**, Brain-Heart Infusion agar; **DMSO**, dimethyl sulfoxide; **LB**, Luria-Bertani; **MIC**, minimal inhibition concentration; **MTCC**, Microbial Type Culture Collection; **PBS**, phosphate buffer saline

INTRODUCTION

The use of higher plants and their extracts to treat infections is an age-old practice in traditional medicine, and medicinal plants have been used for centuries as remedies for human diseases because they contain components of therapeutic value. The acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics has led researchers to investigate the antimicrobial activity of medicinal plants (Bisignano *et al*. 1996; Lis-Balchin and Deans 1996; Maoz and Neeman 1998; Hammer *et al*. 1999). The increasing use of plant extracts in the food, cosmetics and pharmaceutical industries suggests that, in order to find active compounds, a systematic study of medicinal plants is very important. Prevalence of antibiotic-resistant bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control the bacterial diseases (Cown 1999; Bax *et al*. 2002). Due to improper medication and diagnosis many of the pathogenic clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* exhibited multi drug resistant characteristics and are highly disruptive to the intestinal epithelial barriers (Zaborina *et al*. 2006). Many investigators have evaluated the bioactivity of plant extracts and the isolated constituents against these infectious organisms (Parekh and Sumitra 2006).

Naravelia zeylanica (L.) DC. (Ranunculaceae) is a woody climber (**Fig. 1**) distributed in the Western Ghats of India (Saldanha 1984). *N. zeylanica* is useful on vitiated

Fig. 1 A twig of *Naravelia zeylanica* showing leaves with flowers.

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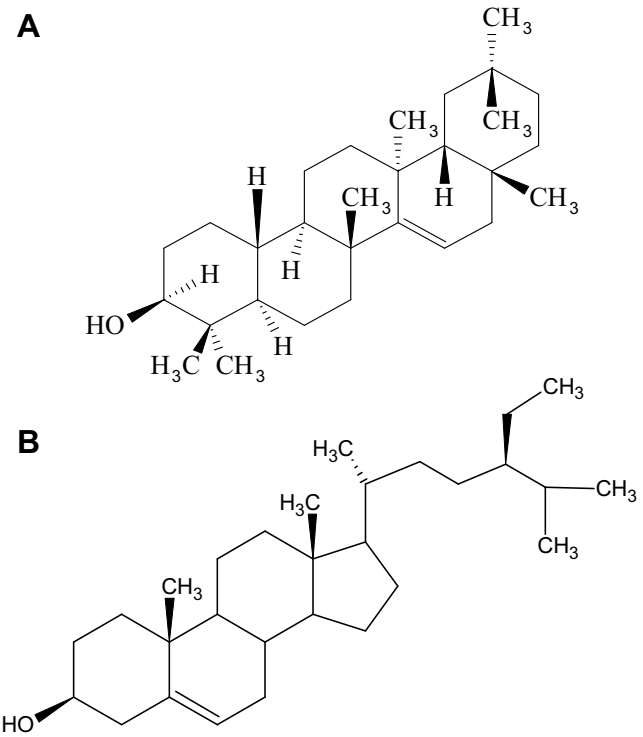

Fig. 2 Structure of (A) taraxerol, (B) β-sitosterol.

condition of pitta, helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, colic inflammation, wounds and ulcers (Praveendhar and Ashalatha 2003). The roots and stems have a strong, penetrating smell (Warrier *et al*. 1995). In Kerala, *N. zeylanica* is used as a source of drug for intestinal worms, skin disease, leprosy, toothache (Sivarajan and Balachandran 1958). In the Indian system of medicine 'Ayurveda' the plant is used to relive malarial fever, and headache, while root and stem paste is applied externally for psoriosis, itches and skin allergy (Harsha *et al*. 2002). The traditional medicine practitioners residing in the vicinity of the Bhadra Wild Life Sanctuary, Karnataka, India are using the leaf and stem juices for treating psoriasis and dermatitis. Many pharmaceutical industries in India (Hindustan Liver Ltd., Mumbai; Himalayan Drug House, Bangalore) are engaged in the production of skin ointments from this plant. Biosystematically also this species reveals much importance because only two stove climbing species are reported in the genus *Naravelia* (Saldanha 1976).

The present investigation reports the isolation of two compounds namely taraxerol (**Fig. 2A**) and β-sitosterol (**Fig. 2B**) from the petroleum ether extract of the leaves of *N. zeylanica* for the first time and the antimicrobial activities of these constituents were screened against clinical strains of *S. aureus*, *P. aeruginosa* and *K. pneumoniae*. The antifungal activity was evaluated against the strains of dermatitis fungi *Trichophyton rubrum*, *Microsporum gypseum*, *Tricophyton tonsurans*, *Microsporum audouini*, and *Candida albicans*.

MATERIALS AND METHODS

Plant materials

Leaves of *N. zeylanica* were collected from the Lakkavalli Reserve Forest Range of the Western Ghats region of Karnataka, India and identified by comparing with the authenticated specimen deposited at the Kuvempu University herbaria (Voucher specimen KUDB/Ang/278). The leaves were washed in running tap water, shade dried, powdered mechanically and sieved (Sieve No. 10/44) and subjected to Soxhlet extraction using the solvent petroleum ether 60-80 AR (SD Fine Chem Ltd., Mumbai, India). The extract was concentrated under reduced pressure at 40 ± 5°C using a rotary flash evaporator (Büchi, Flawil, Switzerland). From the 500 g of leaf powder, the yield of petroleum ether extract was 12.40 g, i.e. 0.025% (v/w).

Phytochemical studies

The extract was subjected to qualitative phytochemical tests for the screening of various secondary metabolites (Trease 1983). The crude petroleum ether extract was chromatographed on silica gel 60-120 using the mobile phase benzene and ethyl acetate in the ratio 9:1. The eluted fractions were collected at 5 min intervals. Two fractions labeled as N_2 and N_3 were collected in large quantities, and subjected to chemical group test, these compounds showed positive test for Salkowaski test (to the extract in chloroform a few drops of concentrated sulphuric acid were added, shaken and allowed to stand, red color produced in the chloroform layer) and Liebermann-Burchard test (the test solution in chloroform was treated with few drops of acetic anhydride with few drops of concentrated sulphuric acid was added from the sides of test tubes a brown ring at the junction of the two layers appeared also the upper layer turns green) for the presence of steroids (Kokate *et al*. 1990). The purity of all the eluted fractions was monitored by TLC and was subjected to spectral studies. The IR Spectra were recorded with KBr pellets on a Perkin- Elmer 1710 FT-IR spectrophotometer. The ^{1}H NMR spectra were obtained on a Bruker DR X (200 MHz) instrument and the FAB mass spectra were recorded on a JEOL SX102 Mass Spectrometer (Central Drug Research Institute, Lucknow, India).

Preparation of plant extracts

100 µg of crude extracts of petroleum ether was dissolved in 100 µl of 10% DMSO. For isolated constituents 50 µg of each were dissolved in 100 µl of 10% DMSO. The standard antibacterial drug Ciprofloxacin and antifungal drug Fluconozole were also tested at a concentration 50 µg/100 µl of each.

Table 1 Profile of the clinical strains used for antimicrobial activity.

Clinical strains	Clinical condition	Source
P. aeruginosa		
Ps-1	Bronchitis	Wounds
Ps-2	Otitis media	Pus
Ps-3	Burns	Sputum
Ps-4 and *Ps-5*	Upper UTI	Stool
Ps-6	Food poisoning	Hospital effluent
Ps-7	Cross infections in UTI	Hospital effluent
Ps-8	Septicemia	Old wounds
Ps-9	Unknown	Ear swab
K. pneumoniae		
Kp-1	Pneumonia	Mucus
Kp-2	Gram negative	Follicullitis stipules
Kp-3	Burns	Pus
Kp-4	UTI	Urine
Kp-5	Septicemia	Sputum
Kp-6	Cross infections in UTI	Urine
Kp-7	Abscess in immunodeficiency	Wounds
Kp-8	Upper UTI	Urine
Kp-9	Unknown	Hospital effluent
S. aureus		
Sa-1	Abscess in immunodeficiency	Wounds
Sa-2	Burns	Pus
Sa-3	Septicemia	Old wounds
Sa-4	Food poisoning	Stool
Sa-5	Burns	Pus
Sa-6 and *Sa-7*	Unknown	Hospital effluent
Sa-8	Abscess in immunodeficiency	Sputum
Sa-9	Otitis media	Ear swab
Fungal strains		
T. rubrum	Cutaneous mycoses	Skin
T. tonsurans	scaring of the scalp	Scalp ringworm
M. gypseum	ringworm infections	Skin
M. audouini	Cutaneous mycoses	Skin and hairs
C. albicans	Opportunistic mycoses candidosis	Lungs

Ps = clinical strains of *pseudomonas aeruginosa*, *Kp* = clinical strains of *Klebsiella pneumoniae*, *Sa* = clinical strains of *Staphylococcus aureus*.

Microorganisms

Twenty seven clinical strains of three of the most common bacterial pathogens, *S. aureus*, *P. aeruginosa* and *K. pneumoniae*, the ATCC (*Pseudomonas aeruginosa* - ATCC-20852; *Staphylococcus aureus* – ATCC-29737) and MTCC (*Klebsiella pneumoniae* - MTCC-618) strains of the corresponding bacteria and five clinically isolated pathogenic fungi, *T. rubrum*, *M. gypseum*, *T. tonsurans*, *M. audouini*, and *C. albicans* were used as test organisms. The different pathogenic microorganisms and their serotype were isolated from infected patients in the district health centre of Gulberga, and identified in the Department of Microbiology, University of Gulberga, India with the help of the National Chemical Laboratory, Pune, India. The profile of bacterial species and their strains of different clinical origin are shown in **Table 1**. All the bacterial microorganisms were maintained at -30°C in Brain Heart Infusion (BHI) containing 17% (v/v) glycerol. Before testing, the suspensions were transferred to LB broth and cultured overnight at 37°C. Inocula were prepared by adjusting the turbidity of the medium to match the 0.5 McFarland standards. Dilutions of this suspension in 0.1% peptone (w/v) solution in sterile water were inoculated on LB agar, to check the viability of the preparations. In case of fungal stocks cultures were stored on Brain Heart Infusion (BHI, Merck) culture media (pH 6.5).

Antimicrobial assay

The agar well diffusion method (Mukherjee *et al*. 1995) was used for the assessment of antimicrobial activity of the test samples. Medium (tryptone 10 g/l, yeast extract 5 g/l, sodium chloride 10 g/l, agar-agar 15 g/l, pH 7.2) was poured into sterilized Petri dishes (90 mm diameter). LB broth containing 100 µl of 24 h incubated cultures of the respective clinical isolates and the ATCC and MTCC strains were spread separately on the agar medium. Wells were created using a sterilized cork borer under aseptic conditions.

In order to identify antifungal activity of total extracts and fractions against fungal pathogens the agar diffusion assay was performed in BHI culture media (pH 6.5). Fungal cells were obtained by centrifugation at 1500 × *g*/4°C for 15 min and diluted in phosphate buffer saline (PBS), pH 7.2. Cell count was taken using heamocytometer after loading 10 µl of the cell suspension in PBS and No. of cells/ml was calculated, the final concentration of each strain was 106 cells/ml. Cultures were grown for 3 days at 37°C. In BHI agar plates 100 µl of fungi were spread and wells were made using cork borer and 50 µl of test compounds were loaded to each wells. The plates were refrigerated for 2 h in order to stop fungal growth and facilitate diffusion of the substances. The reference antibacterial agent Ciprofloxacin and antifungal agent Fluconozole were loaded in the corresponding wells. Plates were then incubated at 37°C for 48 h. At the end of the incubation period, inhibition zones formed on the medium were evaluated in mm.

The Minimal Inhibitory Concentrations (MIC) of the crude extracts and the constituents taraxerol and β-sitosterol were determined by micro dilution techniques in LB broth, according to Clinical and Laboratory Standards Institute (CLSI), USA guidelines. The bacterial inoculates were prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard colony forming units and diluted 1:10 for the broth micro dilution procedure. The micro titer plates were incubated at 37°C and MIC was determined after 24 h of incubation. The highest activity of the isolated compounds compared to those of the crude extracts indicates that those compounds alone are solely responsible for antimicrobial activity.

Stastical analysis

The results of these experiments are expressed as mean ± SE of three replicates in each test. The data were evaluated by one-way Analysis of Variance (ANOVA) and mean separations were carried out using Duncan's Multiple Range Test (DMRT, Gomez and Gomez 1984). Followed by Tukey's multiple comparison tests to assess the stastical significance. $P \leq 0.05$ was considered as statistically significant, using statistical software SPSS ver 11. (SPSS Inc., Chicago, USA).

RESULTS

The compounds N_2 and N_3 eluted from petroleum ether extract gave red color in Salkowaski test and green color in Liebermann-Burchard test indicated the presence of sterols. IR, ^{1}H NMR, and MASS spectroscopic studies confirmed the structures of the compounds. The IR spectrum of compound N_2 showed a broad absorption peak at 3300-3430 cm^{-1} corresponding to the –OH group. The ^{1}H NMR spectrum exhibited a broad peak at δ 5.2 ppm indicating the presence of an –OH proton. The $–CH_2$ and $–CH_3$ protons (**Fig. 3A**) were appeared as multiplet at δ 0.6- 2.3 ppm respectively. The mass spectrum gave a molecular ion peak at 426 *m/z* indicating the molecular weight (**Fig. 3B**). Based on the above compound N_2 was identified as taraxerol.

The IR spectrum of compound N_3 showed an -OH absorption stretching frequency at 3300-3430 cm^{-1} and a narrow band at 1594-1629 cm^{-1} corresponding to an olefinic group. In the ^{1}H NMR spectra two prominent singlets were noticed at δ 1.01 and 0.68 and other peaks at 0.5-2.5 ppm corresponding to methyl and methylene protons (**Fig. 4A**). Further investigation on mass spectral studies showed a molecular ion peak at 400 *m/z* corresponding to its molecular weight and fragmentation peak at 383 due to the loss of an –OH group (**Fig. 4B**). From the above spectroscopic data compound N_3 was identified as β-sitosterol.

The Minimal Inhibitory Concentrations (MIC) of the crude petroleum ether extract, and their constituents taraxerol and β-sitosterol were determined as 150 µg/µl, 100 µg/µl and 80 µg/µl respectively. The results of antibacterial activity between crude extract and the pure compounds showed a synchronizing effect on clinical strains of pathogenic bacteria and dermatitis fungi. The zones of inhibition of the microbial colony is depicted in **Tables 2-5**. The petroleum ether extract demonstrated antibacterial activity against all the clinical strains of bacteria. But it is more significant on *S. aureus* (19.43 mm), and *T. tonsurans* (11.60 mm). Among the purified bioactive components, taraxerol, a major sterol component, proved to be a potent bactericidal agent against *S. aureus* (23.60 mm). The other compound β-sitosterol showed a meager antimicrobial property against all the pathogenic strains of bacteria and fungi tested. Its inhibitory effect was more on the growth of *T. tonsurans* (16.23 mm) and *M. gypseum* (15.37 mm). Among the five dermatitis fungi cultured for antifungal assay, the zone of inhibition of the colony was found to be very less in *C. albicans* and *M. audouini*.

DISCUSSION

The results obtained in this study indicate a considerable difference in antimicrobial activity among the pure compounds. Taraxerol, the major component of petroleum ether extract exhibited significant antimicrobial activity against pathogenic bacteria and dermatitis fungi strains. Its bio-controlling potency is par with that of the standard antibiotic Ciproflaxin and Fluconozole. Generally the Gram-positive bacteria should be more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer and Gerhardt 1971) whereas the Gram-negative bacteria possess an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to drug constituents (Betoni *et al*. 2006). So the maximum inhibitory activity was observed in Gram-positive bacteria *S. aureus*. In case of Gram-negative *P. aeruginosa* and *K. pneumoniae* the zone of inhibitory activity was less significant because of a multilayered phospholipidic membrane carrying the structural lipopolysaccharide components (Nikaido and Vaara 1985). In spite of these barriers the compound taraxerol is effective in controlling the growth of pathogenic strains to a considerable extent.

The highest activity of taraxerol (0.096% v/w), when compared to that of the other constituent β-sitosterol and with the crude petroleum ether extract, indicated that it was

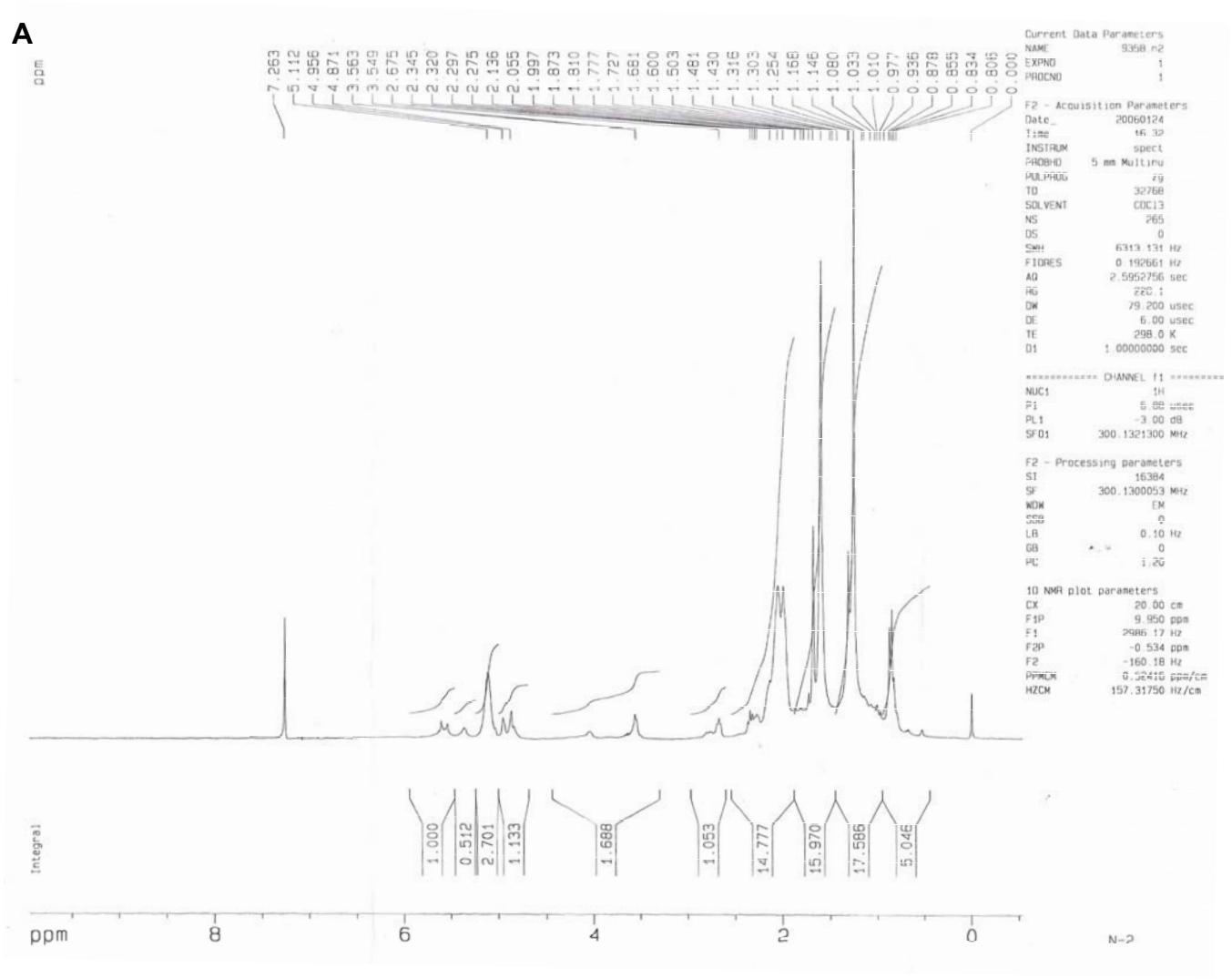

Fig. 3 (**A**) ^{1}H NMR Spectrum of taraxerol showing a broad peak at δ 5.2 ppm indicating the presence of an –OH proton. The $–CH_2$ and $–CH_3$ protons appeared as a multiplet at δ 0.6- 2.3 ppm. (**B**) MASS spectrum of taraxerol shows molecular ion peak at 426 *m/z* indicating the molecular weight.

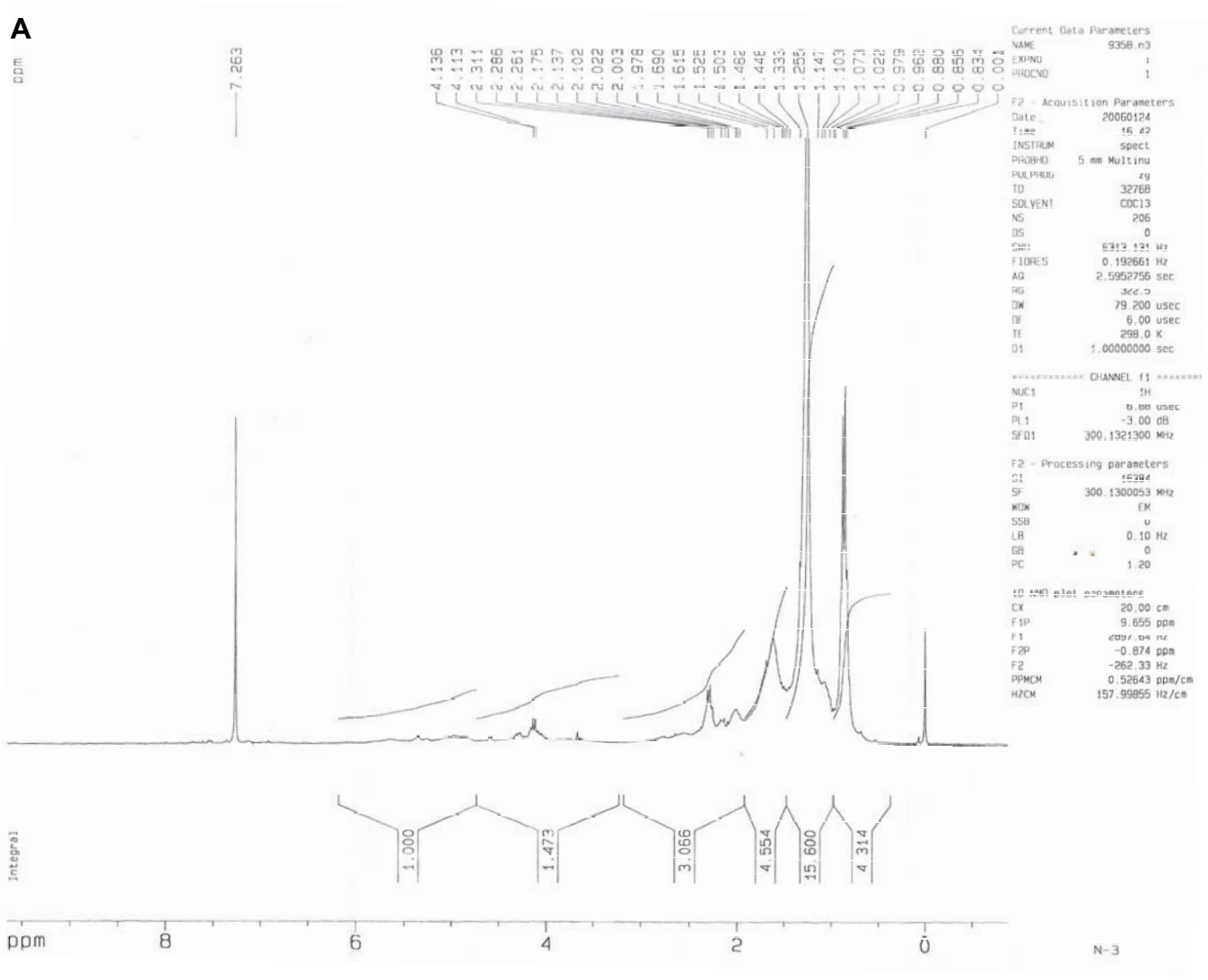

Fig. 4 (**A**) ^{1}H NMR Spectrum of β-sitosterol shows two prominent singlets at δ 1.01 and 0.68, other peaks at 0.5-2.5 ppm corresponding to methyl and methylene protons. (**B**) MASS Spectrum of β-sitosterol showed a molecular ion peak at 400 *m/z* corresponding to its molecular weight and fragmentation peak at 383 due to the loss of an –OH group.

Table 2 Antibacterial activity of the crude extract and their pure compounds of *Naravelia zeylanica* against clinical strains of *Pseudomonas aeruginosa*.

Clinical Strains	Diameter of zone of inhibition (mm)			
	Pet. crude	Taraxerol	β-sitosterol	Ciproflaxin
Ps-1	9.40 ± 0.10 f	19.23 ± 0.14 d	12.83 ± 0.20 bc	23.30 ± 0.15 b
Ps-2	10.00 ± 0.12 e	18.33 ± 0.24 e	10.16 ± 0.16 f	20.50 ± 0.28 e
Ps-3	13.23 ± 0.15 b	20.23 ± 0.14 b	11.73 ± 0.33 e	22.23 ± 0.14 c
Ps-4	11.23 ± 0.15 d	19.43 ± 0.23 cd	13.50 ± 0.17 a	20.20 ± 0.26 e
Ps-5	12.30 ± 0.10 c	18.36 ± 0.18 e	13.23 ± 0.14 ab	23.30 ± 0.15 b
Ps-6	8.40 ± 0.10 g	21.50 ± 0.28 a	-	24.33 ± 0.20 a
Ps-7	12.60 ± 0.12 c	18.23 ± 0.14 e	13.43 ± 0.23 a	21.30 ± 0.15 d
Ps-8	12.53 ± 0.29 c	20.00 ± 0.28 bc	12.13 ± 0.13 de	23.16 ± 0.16 b
Ps-9	14.37 ± 0.19 a	19.00 ± 0.00 d	12.33 ± 0.16 cd	20.50 ± 0.28 e
F-value	157.27	27.51	495.37	52.16

Clinical strains of *Pseudomonas aeruginosa* from different clinical sources.
The values are the mean of three experiments ± S.E.
Means followed by the same letter was not significantly different by Tukey's test at the 0.05% probability level.
-: No activity

Table 3 Antibacterial activity of the crude extract and their pure compounds of *Naravelia zeylanica* against clinical strains of *Klebsiella pneumoniae*.

Clinical Strains	Diameter of zone of inhibition (mm)			
	Pet. crude	Taraxerol	β-sitosterol	Ciproflaxin
Kp-1	12.23 ± 0.14 d	18.23 ± 0.14 d	14.23 ± 0.15 a	25.00 ± 0.12 a
Kp-2	10.56 ± 0.12 f	17.40 ± 0.10 e	13.33 ± 0.17 b	20.23 ± 0.15 f
Kp-3	9.23 ± 0.14 g	19.23 ± 0.14 c	12.30 ± 0.15 c	21.37 ± 0.09 e
Kp-4	11.23 ± 0.14 e	20.50 ± 0.28 b	12.77 ± 0.09 c	20.20 ± 0.26 f
Kp-5	9.40 ± 0.10 g	16.73 ± 0.12 f	14.27 ± 0.18 a	23.37 ± 0.09 c
Kp-6	13.23 ± 0.14 c	21.30 ± 0.15 a	13.43 ± 0.23 b	22.53 ± 0.18 d
Kp-7	15.30 ± 0.10 a	20.26 ± 0.14 b	12.33 ± 0.17 c	24.37 ± 0.19 b
Kp-8	13.16 ± 0.16 c	19.17 ± 0.17 c	14.33 ± 0.17 a	23.43 ± 0.12 c
Kp-9	14.06 ± 0.06 b	20.43 ± 0.03 b	13.30 ± 0.15 b	24.43 ± 0.12 b
F-Value	261.56	96.1	23.33	137.2

Clinical strains of *Klebsiella pneumoniae* from different clinical sources.
The values are the mean of three experiments ± S.E.
Means followed by the same letter was not significantly different by the DMRT test at 0.05 % probability level.
-: No activity

Table 4 Antibacterial activity of the crude extracts and their pure compounds of *Naravelia zeylanica* against clinical strains of *Staphylococcus aureus*.

Clinical Strains	Diameter of zone of inhibition (mm)			
	Pet. crude	Taraxerol	β-sitosterol	Ciproflaxin
Sa-1	15.40 ± 0.23 d	18.37 ± 0.19 f	13.33 ± 0.17 d	28.33 ± 0.17 a
Sa-2	19.43 ± 0.23 a	22.60 ± 0.10 b	14.23 ± 0.15 c	26.90 ± 0.21 b
Sa-3	14.27 ± 0.18 e	19.70 ± 0.10 e	15.87 ± 0.37 ab	21.50 ± 0.29 f
Sa-4	12.40 ± 0.21 g	23.63 ± 0.09 a	12.17 ± 0.17 e	24.50 ± 0.29 d
Sa-5	13.47 ± 0.24 f	18.27 ± 0.12 f	16.33 ± 0.17 a	20.43 ± 0.23 g
Sa-6	18.30 ± 0.15 b	20.33 ± 0.22 d	15.17 ± 0.17 b	27.10 ± 0.21 b
Sa-7	17.60 ± 0.15 c	21.30 ± 0.25 c	13.17 ± 0.17 d	25.50 ± 0.29 c
Sa-8	12.57 ± 0.07 g	22.57 ± 0.12 b	16.13 ± 0.12 a	23.50 ± 0.29 e
Sa-9	11.43 ± 0.23 h	23.60 ± 0.10 a	13.83 ± 0.44 cd	23.83 ± 0.44 de
F-Value	214.1	186.23	38.96	88.91

Clinical strains of *Staphylococcus aureus* from different clinical sources.
The values are the mean of three experiments ± S.E.
Means followed by the same letter was not significantly different by DMRT at the 0.05% probability level.
-: No activity

Table 5 Antifungal activity of the crude extract and their pure compounds of *Naravelia zeylanica* against clinically isolated fungal pathogens.

Clinical Strains	Diameter of zone of inhibition (mm)			
	Pet. crude	Taraxerol	β-sitosterol	Fluconozole
Trichophyton rubrum	10.63 ± 0.09 b	17.37 ± 0.19 a	13.30 ± 0.15 c	15.43 ± 0.23 c
Microsporum gypseum	9.37 ± 0.09 c	12.37 ± 0.09 c	15.37 ± 0.09 b	21.37 ± 0.19 a
Tricophyton tonsurans	11.60 ± 0.10 a	13.37 ± 0.09 b	16.23 ± 0.15 a	16.57 ± 0.12 b
Microsporum audouini	-	-	2.77 ± 0.15 e	11.40 ± 0.10 e
Candida albicans	-	-	4.37 ± 0.19 d	12.23 ± 0.15 d
F-value	147.3	420	1876.0	585.7

Clinically isolates fungal pathogens from different clinical sources.
The values are the mean of three experiments ± S.E.
Means followed by the same letter was not significantly different by DMRT at the 0.05% probability level.
-: No activity

solely responsible for antibacterial and antifungal activity. It can be used as a broad-spectrum antimicrobial agent. The anti microbial activity of β-sitosterol (0.076% v/w) was very meager than the petroleum ether extract. However, β-sitosterol is a very common chemical constituent of medicinal plants, and possesses valuable biological activity, such as antihypercholesterolaemic and estrogenic effect (Buckingham 1998). Recently, it was reported to show an important gastroprotective activity in several experimental ulcer models in rats (Navarrete *et al*. 2002).

Medicinal plants constitute an effective source of both traditional and modern medicines, and herbal medicine has been shown to have genuine utility with about 80% of the Indian rural population depending on it as primary health

care (Indian Herbal Pharmacoepia 2006). For a long period of time, plants have been a valuable source of products to treat wide range of medical problems, including ailments caused by microbial infection. Numerous studies have been carried out in different parts of the globe to extract plant products for screening antimicrobial activity (Essawi and Srour 2000; Ravikumar *et al*. 2005). Plants produce highly bioactive molecules that allow them to interact with other organisms in their environment many of these substances are important in the defense against herbivores and contribute to the resistance to disease.

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