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Identification and Characterization of Two Novel Antimicrobial Compounds from *Jasminum grandiflorum* L.

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Abstract: *Jasminum grandiflorum* L. is described in Ayurveda to treat several diseases such as ulcer, stomatilis, gingivitis and skin disease. Leaves are used to apply locally as in single or compound formulation in Leprosy (Kushta roga- Skin disease). In the present study, extracts of *Jasminum grandiflorum* L. leaves in various solvents were screened for antimicrobial activity using micro broth dilution assay against microbial strains. Chloroform extract was found to display activity in the range of 1.56 to 25.0 mg/ml. In bio-assay four compounds of different R_f values exhibited specific antimicrobial activities. Compound having R_f value 0.21 exhibited activity against *Klebisiella pneumoniae* and *Staphylococcus aureus*. Compounds at R_f value 0.50, 0.63, 0.79 and 0.87 exhibited specific activities against *Escherichia coli* and *Salmonella typhi* respectively. Bioassay- guided fractionation of chloroform extract leads to identification of two new antimicrobial compounds namely 3,5-Dihydroxy-2,4-dimethyl-hexanoic acid 4-hydroxy-phenyl ester (JHF-1) and 2-Hydroxymethyl-3-methyl-butric acid phenyl ester (JHF-2) at R_f values 0.214 and 0.50, respectively. Study revealed the importance of use of plants extract over individual isolated compounds. Furthermore, the study of mechanism of action of these compounds may lead toward the identification of specific bacterial targets.

Key words: Jasminum grandiflorum L. · Antimicrobial · 3,5-Dihydroxy-2,4-dimethyl-hexanoic acid 4-hydroxy-phenyl ester · 2-Hydroxymethyl-3-methyl-butric acid phenyl ester

INTRODUCTION

Medicinal plants play a vital role in alleviating human sufferings. About 80 % of individuals from developed countries use traditional medicines, which are derived from medicinal plants. A wide variety of medicinal plants has been described an ancient science of India, in Ayurveda and a large number is still unexplained. Therefore, such plants should be investigated for better understanding of their properties, safety and efficacy and for a search of new potent antimicrobial compounds [1].

*Jasminum i*s a genus having about 200 species and some of these species are known for medicinal values in Indian system of medicine. *Jasminum grandiflorum* L. from Oleaceae commonly known as Spanish jasmine, Royal jasmine, Catalonian jasmine, among others (*chameli* in Hindi) is a species of jasmine native of South Asia. The plant is mentioned as the name of Jati and some places as Swarnjati. In classical texts of Ayurveda leaf of Jati is described in many places in different disease such as ulcer healing and in stomatilis, gingivitis. Leaves are applied locally as in single or compound formulation in Leprosy (kushta roga- Skin disease) including dermatitis, leucoderma (kilas), ring worm (dadru), scabies (pama) [2-4]. *Jasminum grandiflorum* L. is a least explored shrub described in literature of Ayurveda for its healing properties. The Ethanolic leaf extract of *Jasminum grandiflorum* L. has been reported to have antiulcer and antioxidant activities. The antiulcer activity, may be attributed through its antioxidant mechanism of action [5]. Literature suggests the use of this plant as a diuretic and spasmolytic agent, which is given during childbirth [6, 7]. However, detailed information on chemical constitution of such an important plant is not available. The leaves are reported to possess ascorbic acid, anthranilic acid and its glucosides, indole oxygenase, alkaloid jasminine and salicylic acid [8]. The main odorous components present in Indian oil samples have been reported to be; benzyl acetate, benzyl benzoate, phytol, jasmone and methyl jasmonate. Whereas, other species of the genus are reported to have flavanone glucosides, secoiridiod glucosides and alkaloids [9].

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In the present study, various extracts of *Jasminum grandiflorum* L. were screened for their antimicrobial activity against twelve microbial strains viz. *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus nigar*, *Candida albicans* and *Saccharomyces creveaceae*. Bioassay resulted in the identification of two novel antimicrobial compounds, 3,5-Dihydroxy-2,4-dimethyl-hexanoic acid 4-hydroxy-phenyl ester (JHF-1) and 2-Hydroxymethyl-3-methyl-butric acid phenyl ester (JHF-2) at R_f values 0.214 and 0.50 respectively.

MATERIALS AND METHODS

Plant Collection: Leaves of *Jasminum grandiflorum* L. were collected from NRIBAS, Nehru Garden, Kothrud, Pune. Plants were identified by Dr. T. K. Mandal at National Research Institute of Basic Ayurvedic Sciences, Nehru Garden, Kothrud, Pune, where voucher sample (Voucher No. 666) was deposited.

Plant Extract: The powdered material of plant leaves was subjected to successive soxhlet extraction with n-hexane, chloroform, acetone, ethanol and water to obtain respective extracts [10]. Solvents were evaporated in incubator at 37 °C and dry fractions were stored at 4 °C in air-tight bottles.

Bacterial Cultures: Nine quality control strains of *Enterobacter aerogenes* ATCC13048, *Escherichia coli* MTCCB 1662, *Klebsiella pneumoniae* MTCCB 109, *Proteus vulgaris* ATCC 33420, *Pseudomonas aeruginosa* MTCCB 741, *Salmonella typhi* MTCCB 733, *Staphylococcus aureus* MTCCB 737, were purchased from Institute of Microbial Technology, Chandigarh, India and used in each test as recommended by the National Committee for Clinical Laboratories Standards (NCCLS). Five fungal strains, *Aspergillus flavus* NCIM 549, *Aspergillus fumigatus* NCIM 902, *Aspergillus nigar* NCIM 620, *Candida albicans* NCIM 3471 *and Sacromyces creveaceae* NCIM 3284 were used to screen extracts. The bacteria were maintained on nutrient broth (NB) at 37°C and fungus was maintained on Potato dextrose agar at 28°C.

Preparation of Inoculum: The gram positive and gram negative bacteria were pre-cultured in nutrient broth overnight in a rotary shaker at 37°C, centrifuged at 10,000 rpm for 5 min, pellet was suspended in double distilled water and the cell density was standardized spectrophotometrically (A_{610} nm) to a concentration of 10^6 cell/ml.. The fungal inoculum was prepared from 10 days old culture grown on potato dextrose agar medium. The Petri dishes were flooded with 8 to 10 ml of distilled water and the conidia were scraped using sterile spatula. The spore density of each fungus was adjusted with spectrophotometer (A_{595} nm) to obtain a final concentration of approximately 10^5 spores/ml.

Anti-bacterial Activity: The *in vitro* antibacterial activity of plant extract was determined by broth macro dilution method [11]. Stock solutions of plant extracts were prepared in suitable solvents i. e. DMSO, Acetone and Water. Nutrient broth media was used for serial dilutions. Nine serial dilutions were prepared, ranging from concentration of 25 mg/ml to 0.10 mg/ml of plant extract. The tubes were inoculated with 100 µl of bacterial strain inoculums with a concentration of 10^6 cell/ml. Ampicillin was used as a standard drug for comparison as a positive control. Nutrient broth was used as negative control. The tubes were incubated aerobically at 37°C for 18-24 h; the MIC of each sample was determined by using tetrazolium salts to indicate bacterial growth [12].

Antifungal Activity: Microbroth dilution assays [13] were performed to investigate the activity of various extracts against pathogenic strains of fungi. Stock solutions of plant extracts were prepared in suitable solvents i.e. DMSO, Acetone and Water. Seven serial dilutions in Sabourated Dextrose broth were prepared; ranging from concentration 25 mg/ml to 0.39 mg/ml. The tubes were inoculated with 100 µl inoculum with a concentration of approximately 10^5 spores/ml. Tubes were incubated at 35°C. MICs were determined for each extract against the fungal isolates at 48 and 72 h.

Qualitative Antibacterial Activity Assay by Bioautography: Bio-autography was done with agar overlay method. TLC plates were prepared and developed in different solvent systems, dried for overnight under a stream of air to remove residual solvents. Inoculum was prepared by suspending the microorganism in nutrient broth media with an approximate concentration of 10^6 cell/ml just before applying the overlay. The TLC plates were placed in a sterile Petri dish and covered with 4.5 ml of inoculum. It was k ept at low temperature for some time, once the media has solidified; the plates incubated for 15 h at 36 °C. Plates were sprayed with 2.0 mg/ml an aqueous solution of phenyl tetrazolium

chloride. After incubating for about 1 h at 36°C; clear zones on chromatograms indicating inhibition of growth were noted [14].

Liquid-Liquid Fractionation: This was carried out to simplify chloroform extract of *Jasminium grandiflorum* L. with promising activity by fractionating chemical compounds into broad groups based on their solubility. The residue was dissolved in 1:1 mixture of chloroform and water and two phases was separated by seperatory funnel. Water fraction was mixed with equal volume of n-butanol in a seperatory funnel to yield water (W) and butanol (BT) fraction. The chloroform fraction was taken to dryness and after complete drying was extracted with equal volume of hexane and 10% water in methanol. This yielded Hexane (H) fraction and the 10% water/methanol fraction was further diluted to 20% water/methanol by addition of water. This was mixed with equal volume of carbon tetrachloride in a seperatory funnel; yielded carbon tetrachloride fraction (CT) and the 20% water/methanol fraction was further diluted to 35% water/methanol with water by addition of water and was mixed with equal volume of chloroform in seperatory funnel and yielded chloroform (CH) and 35% water/methanol fraction (MW) [15]. TLC and bio-autography assay was carried out for the entire six fractions obtained and R_f values were also determined from chromatogram. Zone of inhibition and were compared with that of standard antibiotic Kanamycin.

Isolation of the Bioactive Compounds: Analytical and preparative TLC were carried out on Merck precoated silica gel plates (F245 thickness-0.25 mm and 2.0 mm, respectively) using the Chloroform : Ethyl Acetate (7:3) solvent system.

Identification of the Bioactive Compounds: The purified compounds were characterized by spectroscopic methods (IR, ^{1}H-NMR and MS). IR was obtained on a JASCO V5300 FT-IR Poona College of Pharmacy, Pune. ^{1}H-NMR spectra were recorded in $CDCl_3$ on a Varian Mercury VXR 300 spectrometer (300 MHz). The chemical shifts (ppm) were related to that of the solvent. Mass spectra were recorded on GC/MSQP 100 Ex Shimadzu Mass spectrometer at 70 e.v. Spectroscopic analysis (^{1}H-NMR and MS) were performed at University of Pune, Pune.

RESULTS

Percent yield of petroleum ether, chloroform, acetone, methanol and water extracts was found to be 1.50, 10.59, 7.71, 9.50, 19.0 percent, respectively. All the extracts displayed antibacterial activity in a range of 1.6 to 12.5 mg/ml. Whereas, chloroform extract exhibited broad range activity in a range of 1.6 to 6.3 mg/ml. Antifungal activity in water extract was observed at higher range 25 mg/ml (Table 1). TLC profile of chloroform extract developed in chloroform: ethyl acetate (7:3) revealed the presence of several compounds. When chloroform extract was subjected to bio-assay it exhibited five zones of inhibition at different R_f values, indicating the presence of five active components in the fraction. Compound at R_f value 0.21 exhibited activity against *Klebisiella pneumoniae* and *Staphylococcus aureus*. Compounds at R_f value 0.50, 0.63, 0.79 and 0.87 exhibited specific activities against *Escherichia coli* and *Salmonella typhi* respectively.

Direct bio-autography helps to localize antibacterial activity on chromatogram [16]. The agar overlay technique is a hybrid of the two other methods and works successfully with a range of microorganisms; including *P. aeruginosa, S. aureus* and *S. typhi* [17]. The CH and W/M fractions were tested for antimicrobial activity using bioassay for *S. aureus* and *K. pneumoniae*. CH fraction showed presence of five active compounds JHF1, JHF2, JHF3, JHF4 and JHF5 at R_f values 0.21, 0.50, 0.63, 0.79 and 0.87 respectively. Compounds JHF1, JHF2 and JHF5 were active against *S. aureus* and JHF2, JHF3, JHF4 and JHF5 for *K. pneumoniae*. In W/M fraction zone of inhibition was at spot of application for *S. aureus*. Diameter of zone of inhibition for CH fraction were compared with that of Kanamycin. Diameter of zone of inhibition (±14.5 mm at a concentration of 300 μg of CH fraction) at R_f 0.50 and 0.21 was almost equivalent to diameter of zone of inhibition developed by kanamycin (± 16.5 mm at a concentration of 300 μg). Therefore, compound of R_f value 0.21 (JHF-1) and 0.50 (JHF-2) were subjected to purificated by preparative TLC and characterized by UV, IR, NMR and Mass Spectroscopy.

Compound JHF-1: UV (MeOH) λ_{max} ($\log_\varepsilon$): 225 (14.14), 278 (3.61) nm, IR (Nujol) V_{max}: 3306 (broad OH str.), 1719 (CO str.), 1624, 1426 (Ar CH str.) and 1082 cm^{-1} (C-O-C str.), ^{1}H-NMR ($CDCl_3$, 300 MHz): 0.85-1.28 (m, 10 H, all methyl carbon bearing protons), 1.28 (m, J = 11.1 Hz, 8.1 Hz, 1 H, Hd), 2.3 (m, J =16 Hz,1 H, Hc), 3.5 (m, J =11.1 Hz, 16 Hz, 1 H, Ha), 3.6 (m, J = 8.1 Hz, 1 H, Hb), 7.21 (d, J = 8 Hz, 2 H, aromatic protons, both ortho to the -OH group), 7.26 (d, J = 8 Hz, 2 H, aromatic protons, both meta to the -OH group).

Mass Spectrum: FABMS m/z 268: (C_{14} H_{20} O_5) : Observed m/z 267 [M^+-1].

Table 1: ^{1}H-NMR spectra of 3,5-Dihydroxy-2,4-dimethyl-hexanoic acid 4-hydroxy-phenyl ester (Compound JHF-1) in $CDCl_3$.

Atom Number	^{1}H-NMR
Ha	3.5
Hb	3.6
Hc	2.3
Hd	1.28

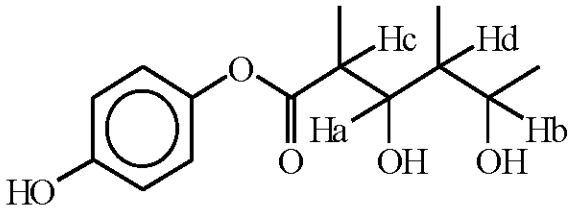

Table 2: ^{1}H-NMR spectra of 2-Hydroxymethyl-3-methyl-butric acid phenyl ester (Compound JHF-1) in $CDCl_3$.

Atom Number	^{1}H-NMR
Ha	3.4
Hb	3.3
Hc	2.3
Hd	2.3

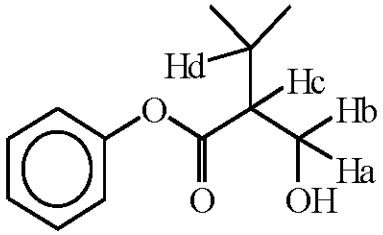

Table 3: Minimum Inhibitory concentration of *Jasminum grandiflorum* L. Extracts.

	MIC in mg/ml											
Extracts	A	B	C	D	E	F	G	H	I	J	K	L
Hexane	-	-	-	3.0	-	-	12.5	-	-	-	-	-
Chloroform	1.6	3.0	3.0	3.0	3.0	6.3	6.3	-	-	-	-	-
Acetone	-	6.3	6.3	6.3	6.3	-	-	-	-	-	-	-
Ethanol	12.5	-	-	12.5	-	12.5	-	-	-	-	-	-
Water	12.5	12.5	-	12.5	12.5	12.5	-	-	25.0	-	-	25.0
Ampicillin	0.2	0.2	0.05	0.1	0.2	0.1	0.1	ND	ND	ND	ND	ND
Kanamycin	ND	ND	ND	ND	ND	ND	ND	0.02	0.03	0.02	0.03	0.05

Note: A= *E. coli;* B= *E. aerogenes;* C= *K. pneumoniae;* D= *P. aeruginosa;* E= *P. vulgaris;* F= *S. aureus;* G= *S. typhi;* H= *A. flavus;* I= *A. fumigatus;* J= *A. niger;* K= *C. albicans and* L= *S. creveaceae.*

Compound JHF-2: UV (MeOH) λ_{max} ($\log_\epsilon$): 225 (14.14), 278 (3.61) nm, IR (Nujol) V_{max}: 3264 (broad OH str.), 1718 (CO str.), 1626, 1459 (Ar CH str.) and 1083 cm^{-1}(C-O-C str.), ^{1}H-NMR ($CDCl_3$, 300 MHz): 0.85-1.25 (m, 6H, all methyl carbons bearing protons), 2.3 (m, J=16Hz, 11Hz, 1H, Hc), 2.3 (m, 1H, Hd), 3.3 (d, J=11Hz, 1H, Hb), 3.4 (d, J=16Hz, 1H, Ha), 7.2-7.24 (m, 5H, all aromatic protons).

Mass Spectrum: FABMS m/z 208: ($C_{12}H_{16}O_3$): Observed m/z 207 [M^+-1].

Interpretation and correlation of spectral data showed presence of two new compounds, JHF-1 and JHF-2 in the chloroform extract of the plant.

DISCUSSION

A number of medicinal plants described in Ayurveda still need to testify according to the modern parameters to ensure their activity and efficacy. Drugs used in Ayurveda are mostly prepared by extraction with water; as in ancient time people do not usually have the access to more lipophillic solvents. This is of concern, as mostly healers do not extract all the active compound (s) that are present in the plant and consequently the prepared drug might not contain all the pharmacologically active compounds. In the present investigation, comparative study of MIC of extracts in different solvents indicated,

broad spectrum activity for aqueous extracts, however the required concentration is comparatively higher than other solvents (12.5-25mg/ml).

Chloroform exhibited lowest MIC indicating better activity against bacterial species however found to be inactive against fungal species at the concentration under consideration and ethanol extract were found to be active against bacterial species with average MIC 6.3 and 12.5 mg/ml respectively.

Interpretation and correlation of spectral data of two out of five antimicrobial compounds showed presence of two new compounds, 3,5-Dihydroxy-2,4-dimethyl-hexanoic acid 4-hydroxy-phenyl ester (JHF-1) and 2-Hydroxymethyl-3-methyl-butric acid phenyl ester (JHF-2) in chloroform extract of the plant.

Study indicates that chloroform extract have broad range activity due to presence of several compounds of specific antimicrobial activities. Further studies of these compound may explore specific antibacterial markers.

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