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Original Article

PHYTOTHERAPEUTIC CONTROL OF FOODBORNE PATHOGENS BY *JASMINUM SAMBAC* L. FLOWERS

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ABSTRACT

Objective: This study is aimed to determine the antibacterial effect of *Jasminum sambac* against foodborne pathogens.

Methods: Antibacterial activity of methanol and chloroform extract of *J. sambac* flowers against foodborne pathogens (*Bacillus cereus, Listeria monocytogenes, Shigella flexeneri*, *Salmonella serovar* enterica Typhi, *Staphylococcus aureus* and *Escherichia coli*) were performed using disc diffusion method and their minimal inhibitory concentration (MIC) was also determined. The preliminary phytochemical screening and gas chromatography-mass spectroscopic (GC-MS) analysis of methanol and chloroform extract of *J. sambac* was analyzed using GC Clarus 500 Perkin Elmer System and gas chromatograph interfaced with a mass spectrometer.

Results: Phytochemical and GC-MS studies revealed the presence of bioactive compounds and found to possess antibacterial activity against foodborne pathogens.

Conclusion: The present study supports the possible use of these phytotherapeutic agents in the clinical management of foodborne diseases.

Keywords: GC-MS analysis, Foodborne pathogens, *Jasminum sambac* L., Antibacterial activity

INTRODUCTION

Foodborne illness is caused by food or drinking beverages contaminated with pathogenic microorganisms. Foodborne illness can cause symptoms that range from a stomach upset to more serious symptoms, including diarrhea, fever, vomiting, abdominal cramps, and dehydration. The most common foodborne illness causing bacteria are *Escherichia coli* and *Salmonella serovar* enterica Typhi, and other species of *Salmonella* also have been implicated in a significant number of cases. The continuing research has been focused on new and novel antimicrobials and anti-pathogenic agents. The plants surviving in an environment with high bacterial density have been identified to possess protective means against infections [1]. The rapid spread of multi-drug resistance and the development of new antimicrobial or anti pathogenic agents that act upon new microbial targets has become a very pressing priority [2].

Jasminum sambac belonging to the family *Oleac* are an important group of flowers, and they are widely cultivated for their attractive, fragrant flowers. Traditionally, different parts of the plant such as the leaf, stem, bark, and roots are very useful medicine in India for a number of skin diseases [3]. The extracts of their flowers also contain Seco iridoids, [4-6] triterpenoid saponins and some other glycosides [7]. They have antiulcer and antioxidant properties [8] and contain enzyme inhibitors to convert angiotensin [9]. Their antimicrobial assay revealed that the extracts showed comparatively better activity and can be used as antibiotics [10]. Plants provide abundant resources of antimicrobial compounds and have been used for centuries to inhibit microbial growth. The present study focuses on antimicrobial compounds from *J. sambac* and its antibacterial efficacy on foodborne illness causing bacterial strains.

MATERIALS AND METHODS

Collection of plant materials

The fresh flowers of *J. sambac* were collected from Hosur, Krishnagiri District, Tamil Nadu, India. The collected plant was identified and authenticated by Dr. G. Prabakaran, Assistant Professor, Research Department of Botany, Government Arts College, Dharmapuri, Tamil Nadu, India. Fresh flowers were washed, shade-dried and then powdered using the blender and stored in airtight bottles.

Preparation of flower extracts

The flowers powder was loaded into the clean, dry Soxlet apparatus tightly using the soft metal rod. Then the apparatus was run to get flower extract with methanol and chloroform. The time was noted to get clear solvent in the side tube. Then, the methanol and chloroform extracts of this plant were evaporated using a rotary vacuum evaporator to remove the solvents. The appearance and amount of the extract of this plant were observed and measured using electronic balance. A loop full of this plant extract was streaked on sterile nutrient agar plates to check the sterility of the extract.

Preliminary phytochemical screening

The extracts were tested for the presence of active phytochemicals constituent viz. alkaloids, proteins, amino acids, anthraquinone, glycosides, flavonoids, tannin and phenolic compounds, carbohydrates, saponins and steroids [11].

Phytochemicals analysis by gas chromatography–mass spectrometry (GC-MS)

GC-MS analysis of methanol and chloroform extracts of *J. sambac* was performed using GC Clarus 500 Perkin Elmer system and gas chromatograph interfaced with a mass spectrometer equipped with Elite–5 MS (5% diphenyl/95% Dimethylpolysiloxane), 30 x 0.25 mm x 0.25 µm df. For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1 ml/min and an injection volume of 2 µl was employed (Spilt ratio 10:1). The injector temperature was programmed at 250 °C; the ion source temperature was maintained at 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, Mass spectra were taken at eV; a scan interval of 0.5 sec and fragment from 45-450 Da. Total GC running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to total areas. Software adapted to handle mass spectra and chromatograms were Turbo Mass Ver 5.2.

Bacterial strains

Bacterial strains (*Listeria monocytogenes* MTCC-1143, *Shigella flexeneri* MTCC-1457, *Staphylococcus aureus* MTCC-3381, *Escherichia coli* MTCC-443, *Salmonella serovar* enterica Typhi MTCC-1251, *Bacillus cereus* MTCC-1305) were obtained from the Microbial Type Culture Collection (MTCC), Chandigargh, India.

Preparation of discs using flower extracts

The observing capacity of 5 mm, sterile discs (Hi-media, India) ranges from 10 µl to 40 µl was selected. For the preparation of the stock solution, 10 mg of each different crude extract was dissolved in 1 ml of DMSO (Dimethyl sulfoxide). From these stocks, 10 µl, 20 µl, 30 µl and 40 µl was added on the sterile discs to get 100 µg, 200 µg, 300 µg and 400 µg respectively of plant extracts. Then, these prepared discs were used for testing antibacterial activity against the bacterial strains.

Antibacterial activity of plant extracts

Antibacterial activity of plant extracts was determined by the agar disc diffusion method using Muller-Hinton agar (Hi-media, India) medium. The bacterial strains were inoculated separately into the culture plates and allowed to dry for 5 min. Then, the prepared discs with compounds were placed on the upper layer of the inoculated plates using sterile forceps. All the plates were incubated at 37 °C for 24 h. Then, the presence of a zone of inhibition was observed and measured on the plates [12]. All the experiments were done in triplicate.

Minimal inhibitory concentration (MIC)

The MIC of the methanol and chloroform extracts was determined using serial dilution technique according to the method of John *et al.* [13]. All the experiments were done in triplicate.

Statistical analysis

The significance of the antimicrobial activity was determined statistically using one-way analysis of the variance (Minitab version 15) via T-test. Significant differences were determined using the Duncan multiple range test at P≤ 0.05. The graphs were analyzed using Microcal Origin 6.0 (Microcal software, Inc.).

RESULTS AND DISCUSSION

Preliminary phytochemicals analysis

Preliminary phytochemical analysis of *J. sambac* methanol and chloroform extracts revealed the presence of alkaloids, flavonoids, saponins, tannins, proteins and amino acids, phytosterols, anthraquinone glycosides, phenolic compounds and absence of carbohydrates (table 1). Interpretation and correlation of spectral data of this plant *J. sambac* showed the presence of more compounds in methanol and chloroform extracts of the plant. Saponins have been reported to possess a wide range of activities. The toxicity of saponinsto insects, parasite worms, mollusks and fish, and their antifungal, antiviral, antibacterial activities have been well documented by Lacaille-Duois and Wagnor [14]. Flavonoids are known to synthesize by plants in response to microbial infection, and they have been found to be containing effective antimicrobial substances against a wide array of microorganisms [15].

Table 1: Preliminary phytochemical screening of methanol and chloroform extracts of *J. sambac*

Constituents/tests	*J. sambac* (Methanol)	*J. sambac* (Chloroform)
Alkaloids		
Mayer's test	++	++
Dragendorff's test	++	++
Hangers test	++	++
Wagers test	++	++
Proteins and amino acids		
Millon's test	+	+
Ninhydrin test	+	+
Biuret test	+	+
Anthraquinone glycosides		
Borntragerstest	+	+
Flavonoids		
Shinoda's test	++	++
Ferric chloride test	++	++
Tannins and Phenols		
Ferric chloride test	++	++
Lead acetate test	++	++
Gelatin contains NaCl test	++	++
Carbohydrates		
Molisch's test	-	-
Barfoed's test	-	-
Fehling test	-	-
Saponins		
Frothing test	++	++
Steroids		
Liebermann-Burchard test	++	++

(++) = moderately present; (+) = slightly present; (-) = absent

Determination of bioactive phytochemicals compounds by GC-MS

The GC-MS study of methanol and chloroform extracts of *J. sambac* was performed and their active compounds were identified with their retention time, molecular formula, molecular structure, molecular weight and concentration (%). The prevalent compounds in chloroform extract of *J. sambac* are 2-methyl-3-oxobutyro nitrile, propanimidamide, 2-chloro-N-(1,2-dichloro-1-propenyl), propanal, 2-methyl-, oxime N-chloro-2-methyl aziridine, 1,2-trans, 1,2, 4, 5-tetrazine, 3,6-diethyl, 2-propen-1-amine, 2-methyl-N-(2-propynyl)-N-ethylamine, cycloheptylamine, cyclo-octanamine, 2–propen–1-amine, N–2-propenyl-[diallyl-

amine], E-2-octadecadecen-1-ol and 3-[3-[1-aziridinyl] propoxy]-2, 5-dimethylpyrazine. The major compounds in methanol extract of *J. sambac* are methoxy carbonyl isothiocyanate [carbon isothiocyanic acid, methyl ester], dibutyl phthalate [phthalic acid, dibutyl ester], 1,2-benzene dicarboxylic acid, diisooctyl ester [di isooctyl phthalate], cyclo butane carbonitrile, 3,3-dimethyl-[3,3-dimethylcyclobutanecarbonitrile], acetonitrile, hydroxy-[glycolonitrile], 1, 2–benzene dicarboxylic acid, butyl octyl ester [phthalic acid, butyl octyl ester], didodecyl phthalate, phthalic acid, 2-hexyl ester, 2-benzene dicarboxylic acid, monobutyl ester [phthalic acid, monobutyl ester], ethane peroxoic acid, 1–cyano–1-[2-(2-phenyl-1, 3–dioxolan–2-yl) ethyl] pentyl ester, 1, 2-benzene dicarboxylic acid and butyl 2-ethylhexyl ester [phthalic acid, butyl 2-ethylhexyl ester] (table 2 and 3).

Table 2: Bioactive components identified in the *J. sambac* methanol extract by GC-MS analysis

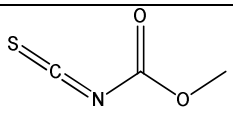
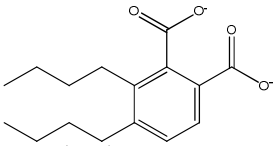
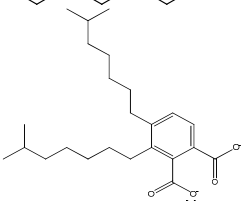
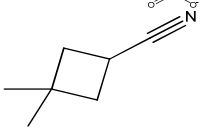
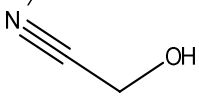
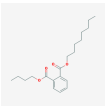
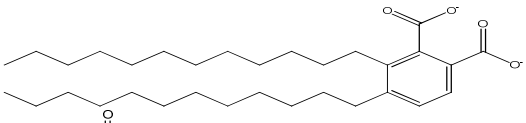
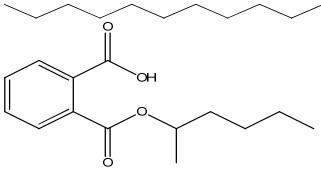
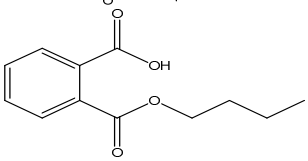
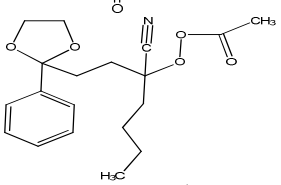
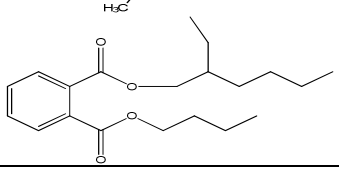
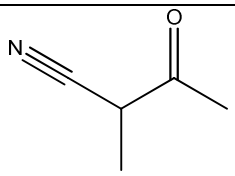
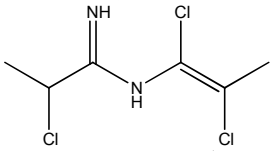
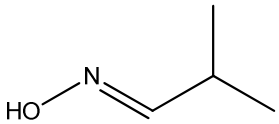
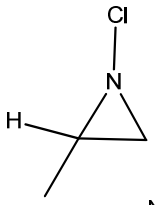
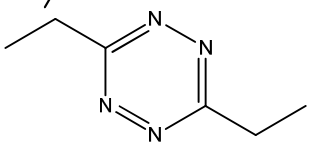
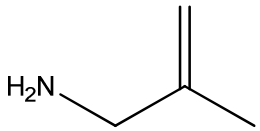
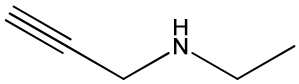
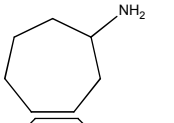
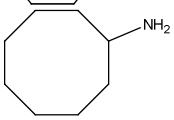
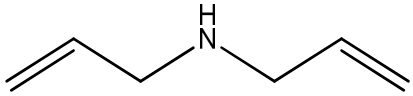
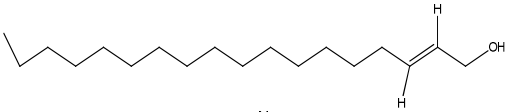
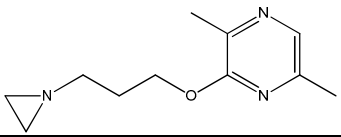
RT	Compounds	Molecular formula	MW	Peak Area %	Molecular structure
10.82	Methoxycarbonyl isothiocyanate [Carbonisothiocyanic acid, methyl ester]	C3H3NO2S	117	11.92	
13.06	Dibutyl phthalate [Phthalicacid, dibutyl ester]	C16H22O4	278	0.33	
20.94	1,2-Benzene dicarboxylicacid, di isooctyl ester [Di isooctyl phthalate]	C24H38O4	390	74.83	
23.70	Cyclobutane carbonitrile, 3,3-dimethyl-[3,3-dimethyl cyclo butane carbonitrile]	C7H11N	109	0.99	
24.17	Acetonitrile hydroxy- [Glycolonitrile]	C2H3NO	57	2.65	
24.51	1,2-Benzenedicarboxylicacid,butyloctyl ester [Phthalicacid, butyloctylester]	C20H30O4	334	1.99	
24.89	Di-dodecyl phthalate	C32H54O4	502	0.33	
25.26	Phthalic acid, 2-hexyl ester	C14H18O4	250	2.65	
25.37	1,2-Benzene dicarboxylic acid, mono butyl ester [Phthalicacid, monobutyl ester]	C12H14O4	222	2.32	
25.65	Ethane peroxoic acid, 1-cyano-1-[2-(2-phenyl-1, 3-dioxolan-2-l)ethyl] pentyl ester	C19H25NO5	347	1.66	
26.04	1,2-Benzene dicarboxylic acid, butyl 2-ethyl hexylester [Phthalic acid, butyl 2-ethyl hexyl ester]	C20H30O4	334	0.33	

Table 3: Bioactive components identified in the *J. sambac* chloroform extract by GC-MS analysis

RT	Name of the compound	Molecular formula	MW	Peak area %	Molecular structure
11.62	2-Methyl-3-oxo butyronitrile	C5H7NO	97	0.09	
14.95	Propanimidamide, 2-chloro-N-(1,2-dichloro-1-propenyl)	C6H9Cl3N2	214	0.09	
21.73	Propanal, 2-methyl-, oxime	C4H9NO	87	0.23	
23.11	1-Chloro-2-methyl aziridine	C3H6ClN	91	0.32	
23.13	1,2,4,5-Tetrazine, 3,6-diethyl	C6H10N4	138	0.05	
24.47	2-Propen-1-amine, 2-methyl	C4H9N	71	0.37	
24.64	N-(2-Propynyl)-N-ethyl amine	C5H9N	83	0.78	
25.80	Cyclo heptyl amine	C7H15N	113	0.69	
27.12	Cyclo octanamine	C8H17N	127	0.73	
28.52	2-Propen-1-amine, N-2-propenyl [Diallyl amine]	C6H11N	97	3.62	
28.93	E-2-Octa decadecen-1-ol	C18H36O	268	81.08	
30.44	3-[3-[1-Aziridinyl] propoxy]-2,5-dimethyl pyrazine	C11H17N3O	207	11.91	

Antibacterial activity of flower extracts

The methanol and chloroform extracts of *J. sambac* showed maximum antibacterial activity against *L. monocytogenes, S. flexeneri, S. aureus, E. coli, S. typhi* and *B. cereus*. Both the extracts were able to inhibit the growth of all tested organisms (Fig.1 and 2). The significant number of studies has been used to obtain purified plant chemicals, and very few screening programs have been initiated on crude plant materials [16]. Heinrich and Simon [17] suggested that the extract of *J. sambac* was effective against the

tested pathogens. The ethanol extracts of *J. sambac* was tested and proved their activity against an array of Gram-positive *(S. aureus, B. subtilis* and *B. cereus)* and Gram-negative bacteria *(E. coli, K. pneumoniae, P. aeruginosa* and *S. typhimurium*) [10, 18].

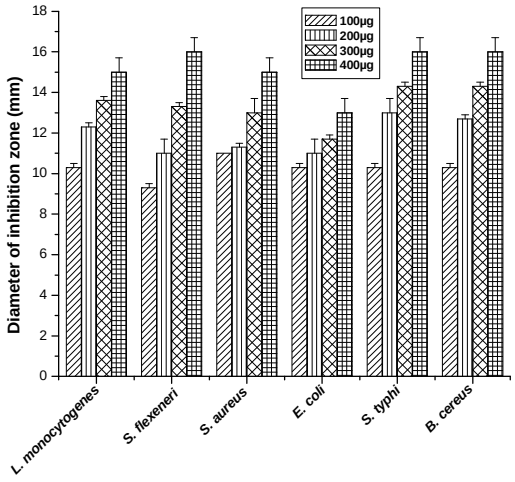


Fig. 1: Antibacterial activity of methanol extract of *J. sambac* against foodborne pathogens

Minimal Inhibitory concentration (MIC) of flower extracts

The MIC values of *J. sambac* flower extracts were determined by the microdilution method and spread plate method. The dilution range was 1000 µg/ml to 7.25 µg/ml. The MIC value of methanol and chloroform extracts of *J. sambac* against *L. monocytogenes, S. typhi* and *B. cereus* were found to be 62.5µg/ml. The MIC values of both extracts against *S. aureus* and *E. coli* were 125µg/ml and for *S. flexeneri* was 250 µg/ml, respectively (table 4). This study is also consistent with the earlier reports on the minimal inhibitory concentration of *Phyllanthusamarus* [19] and *Aervalanata* [20] against all the tested bacterial strains.

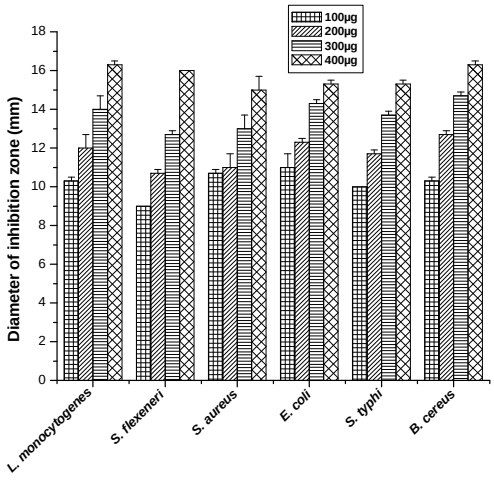


Fig. 2: Antibacterial activity of chloroform extract of *J. sambac* against foodborne pathogens

Table 4: Minimal inhibitory concentration (MIC) of *J. sambac* against foodborne pathogens

Flower extracts	Concentration (µg/ml)							
	7.25	13.65	31.25	62.5	125	250	500	1000
L. monocytogenes								
Methanol extract	+	+	+	β	-	-	-	-
Chloroform extract	+	+	+	β	-	-	-	-
S. flexeneri								
Methanol extract	+	+	+	+	+	B	-	-
Chloroform extract	+	+	+	+	+	B	-	-
S. aureus								
Methanol extract	+	+	+	+	β	-	-	-
Chloroform extract	+	+	+	+	β	-	-	-
E. coli								
Methanol extract	+	+	+	+	β	-	-	-
Chloroform extract	+	+	+	+	β	-	-	-
S. typhi								
Methanol extract	+	+	+	β	-	-	-	-
Chloroform extract	+	+	+	β	-	-	-	-
B. cereus								
Methanol extract	+	+	+	β	-	-	-	-
Chloroform extract	+	+	+	β	-	-	-	-

Note: (-)–No growth; (+)–Growth; β–MIC value

CONCLUSION

The present study concludes that *J. sambac* extracts were found to have potential antibacterial activity against both Gram positive and Gram negative bacterial pathogens. These antibacterial principles can be used to overcome the antibiotic resistance, which poses a great problem with treating bacterial diseases.

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CONFLICT OF INTERESTS

We declare that we have no conflict of interest

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