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Phytochemical analysis and antagonistic activity of *Ixora macrothyrsa* on multidrug resistant bacteria

Femina Wahab, Kalidass Subramaniam*, Sembian Suriyamoorthy, Sweetin Preethi Subburaj

Department of Biotechnology, School of Biotechnology & Health Sciences, Karunya University, Coimbatore, India

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ABSTRACT

Objective: The present investigation was an attempt to identify the potent drug principles of *Ixora macrothyrsa* flower against Methicillin Resistant *Staphylococcus aureus* and *Acinetobacter baumannii*. **Methods:** Hot extraction soxhlet method was performed for the extraction of *Ixora macrothyrsa* flower powder with different solvents. Antagonistic activity checked for all extracts with MTCC standard pathogenic strains and multiple drug resistant strains. Followed this minimal inhibitory concentration, phenol estimation, High performance thin layer chromatography analysis and Gas chromatography- mass spectrometry analysis were performed for *Ixora macrothyrsa* ethanolic extract. **Results:** The results from the present study indicate that the flowers of the plant has rich source of phytochemicals in ethanol extracts compare to acetone and methanol extracts. The quantitative estimation of ethanolic extracts of *Ixora macrothyrsa* showed phenol content 83.67±.04 μg/g of ascorbic acid equivalent. The ethanol extract exhibited good antibacterial activity against all tested pathogens. In this study we point out that ethanol and methanol extracts showed supreme activity against Methicillin Resistant *Staphylococcus aureus* (MRSA) and *Acinetobacter baumannii*. The MIC of *Ixora macrothyrsa* ethanolic extract was identified as 22 μg/ml for MRSA and 200 μg/ml for *Acinetobacter baumannii*. The HPTLC analysis of the ethanol plant extract inferred that it contain both polyphenol and terpenoids. In GC-MS analysis the major constituent was identified as Ethene (2-chloroethoxy)-, 1-propanol 2-chloro, urethane with 16.39 area %. **Conclusions:** Hence it is inferred from our study that the plant flower would be a promising source of phytomedicine against multidrug resistant strains MRSA and *Acinetobacter baumannii*.

1. Introduction

With widespread and often unwarranted use of antibiotics a new threat began to arise in the form of the emergence and the spread of organisms resistant to commonly using antibiotics[1]. Since ancient times human has used medicinal plants which had ameliorated diseases. Medicinal plants also have a tremendous impact on qualitative human life[2].

According to literature report, *Ixora* is a common flowering shrub native to Asia including Bangladesh, Southern India and Srilanka[3]. The *Ixora* genus consists of about 400 species of which 28 are cultivated variety[4]. The various species of *Ixora* flowers are used in the treatment of dysentery, leucorrohea, desmenorrhoea, hemoptysis, catarrhal bronchitis[5], hepatoprotective activity, anti-asthmatic, anti-inflammtory, wound healing, cytotoxic and antitumor activity [4] and antiviral activity[6].

Even though there are more reports in the plant genus *Ixora* there is a lacuna in the report of macrothyrsa species and its therapeutic activities. So the aim of the present study is to evaluate the phytochemicals analysis, antagonistic activity, HPTLC and GC-MS analysis of *Ixora macrothyrsa*. The plant flower extract is also looked for its phenol content.

2. Material and Methods

2.1. Materials

All chemicals and solvents are of analytical reagent grade were procured from HI MEDIA and SD FINE chemicals, Chennai. The plant materials were collected in Kumily vicinity, Kerala State, India and were taxonomically identified by Dr. GVS. Murthy, Botanical Survey of India, Tamilnadu Agricultural University. The voucher Specimen was deposited with register number BSI/SRC/5/23/2010 - 11/ Tech - 1476.

2.2. Preparation of plant material

*Corresponding author: Dr. S. Kalidass, Associate Professor, Department of Biotechnology School of Biotechnology and Health sciences, Karunya University, Coimbatore.
Phone No: +91 9171780742
email id: *kalidass@karunya.edu, kallidass@gmail.com

The Plant flowers were shade dried, powdered and extracted with different solvents acetone, methanol and ethanol by hot percolation using Soxhlet apparatus. Finally the solvents were evaporated using rotary vaccum evaporator and the concentrated crude extracts were stored in small glass screw cap tubes and were used for further analysis[7].

2.3. Screening for Phytochemicals

The preliminary screening test for the presence of the following secondary metabolites such as Alkaloid, Phenols, Flavonoids, Saponins, Phlobatannins, Proteins, Lipids, Steriods, Glycosides, Tannins, Reducing sugar, Terpenoids and acidic compounds were performed as per the standard procedures[8-10].

2.3.1. Phenol estimation

The phenol estimation was performed for ethanol *Ixora macrothyrsa* extract using the standard protocol[11]. Ascorbic acid was used as standard.

2.4. Selected test microorganisms

The acetone, methanol and ethanol plant flower extracts were tested against pathogenic microbes including the gram positive bacteria, *Staphylococcus aureus* (MTCC code - 3160), *Bacillus subtilis* (MTCC code - 121) and gram negative bacteria, *Pseudomonas aeruginosa* (MTCC code - 4676) *Escherichia coli* (MTCC code - 390), *Salmonella paratyphi* (MTCC code - 735), *Shigella sonnei* (MTCC code - 2957), *Klebsiella pneumoniae* (MTCC code - 3384), obtained from IMTECH, MTCC, Chandigarh, India. Two multidrug resistant strains MRSA, *Acinetobacter baumannii* obtained from Microbiological laboratory, Coimbatore, Tamilnadu, India.

2.5. Commercial antibiotic susceptibility of the test organisms

The test organisms taken for the study were checked for their behavior against commercially available antibiotic discs. Antimicrobial screening was carried out using the standard disc diffusion test[12].

2.6. Antagonistic activity of plant extract

The agar-well diffusion assay was adopted for checking antagonistic activity of plant extract[13]. The respective solvents were used as negative control. All the tests were performed in triplicates.

2.7. Determination of minimum inhibitory concentration

Minimum Inhibitory Concentration (MIC) was determined by serial dilution method[14]. The crude extract was serially diluted in nutrient broth. Varying concentrations of the extracts 600 μg to 2.44 μg/ml were prepared from the stock solution and MIC was identified.

2.8. Statistical analysis

The data was pooled in triplicate and subjected to analysis of one way analysis of variance (ANOVA) using SPSS/16 software. P values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as Mean±SEM for the all experiments.

2.9. GC-MS and HPTLC analysis

The Gas Chromatography and Mass Spectrometry was analyzed by GC-MS electron impact ionization (EI) method on GC-8000 gas chromatograph (FISONS Instruments) coupled to a MD- 800 Mass Spectrometer (FISONS Instruments). Compound identification was done by comparing the NIST (National Institute of Standards and Technology-Chemistry web book by WILEY) library data of the peaks with those reported in literature.

The HPTLC analysis was performed using Hamilton syringe and CAMAG LINOMAT 5 instrument, CAMAG REPROSTAR 3 photo-documentation chamber and CAMAG TLC SCANNER 3. The plant ethanol extract was checked for polyphenol and terpenoids profile in HPTLC analysis.

3. Results

In preliminary phytochemicals screening the acetone extracts exhibited positive result for phenol, flavonoids, saponins, steroids, glycosides, tannins, reducing sugars and terpenoids but it showed negative results for other phytochemicals checked. In methanol extracts all phytochemicals were observed except lipids, flavonoids, glycosides and terpenoids. In ethanol extracts except lipids and pholabtannins all phytochemicals were observed (Table 1). The phenolic content of *Ixora macrothyrsa* ethanol extract was estimated and it was observed as 83.67±.04 μg/g of ascorbic acid equivalent.

Table 1

Qualititative assay of phytochemicals constituent in *Ixora macrothyrsa* plant extract

Phytochemicals	Inference		
	Acetone Extract	Methanol extract	Ethanol extract
Alkaloids	-	+	+
Phenols	+	+	+
Flavanoids	+	-	+
Saponins	+	+	+
Pholabtannins	-	+	-
Proteins	-	+	+
Lipids	-	-	-
Steroids	+	+	+
Glycosides	+	-	+
Tannins	+	+	+
Reducing Sugars	+	+	+
Terpenoids	+	-	+

(+ Present - Absent)

The antagonistic results revealed that the ethanol plant extract controlling all tested pathogens to maximum level. It has maximum activity against MRSA, *Shigella sonnei* and *Staphylococcus aureus*. But acetone extract had very good inhibition against *Shigella sonnei* and *Acinetobacter baumannii*. But it was not controlling *Klebsiella pneumoniae*. Methanol extract had maximum zone of inhibition against Shigella sonnei, *Acinetobacter baumannii* and MRSA. Interestingly we got results that both methanol and ethanol extracts inhibiting *Acinetobacter baumannii* to a maximum

level (Table 2). The minimum inhibitory concentration of ethanol plant extract was identified as 22 μg/ml for MRSA and 200 μg/ml for *Acinetobacter baumannii*.

Among the all antibiotic discs tested Imipenem controlled all the tested pathogens in higher degree (Table 3). Upon comparing Imipenem, the *Ixora* extract has higher degree of inhibition for *Pseudomonas aeruginosa* and *Bacillus subtilis*. Piperacillin exhibited high zone of inhibition for *Acinetobacter baumannii* and Fusidic acid exhibited high zone of inhibition for MRSA (Table 4). Upon comparing the activity of these antibiotics, *Ixora* extract controlled *Acinetobacter baumannii* and MRSA to a good extend.

The drug principles in *Ixora* extract were identified through GC-MS and HPTLC. In GC-MS analysis totally 13 compounds were identified. The Mass Spectrometry indexes of the major peaks are shown in the Figure 1. The GC-MS analysis shown that 5 major peaks, in that maximum area percentage revelaed in 2 major peaks with retention time 15.70, 16.30. The highest peak area % at 16.39 obtained by Ethene (2-chloroethoxy)-, 1-propanol 2-chloro, urethane and peak area % at 13.39 obtained by Urea, Butyl, propylcarbamate (Table 5). All compounds coming under the group of aliphatic and aromatic nature. HPTLC profile of ethanol extract of *Ixora macrothyrsa* was performed. Blue, Brownish blue colored zones at Daylight mode were present in the tracks, it was observed from the chromatogram after derivatization, which confirmed the presence of polyphenol in the polyphenolic standard (Catechin) and in the plant extract. Blue, Blue-violet colored zones observed confirmed the presence of terpenoid in the given standard (Solanesol) and in the plant extract (Figure 2).

Table 3

Antagonistic activity of the selected bacteria to the commercially available antibiotics

Test organism	Zone of inhibition (mm)												
	AC	A	CA	C	E	G	I	NA	NF	NX	T	K	CF
Shigella sonnei	7	0	4	20	19	18	31	10	11	20	16	11	19
Salmonella paratyphi	8	0	13	24	16	15	31	13	13	17	15	11	23
Klebsiella pneumoniae	6	0	11	22	20	17	31	15	12	19	12	12	22
Staphylococcus aureus	27	20	17	22	22	14	43	8	15	17	21	14	18
Pseudomonas aeruginosa	4	0	16	17	11	10	12	15	5	26	3	0	23
Bacillus subtilis	42	30	25	27	39	20	31	4	15	30	24	3	29
Escherichia coli	2	0	0	17	0	12	19	0	7	4	7	10	4

AC - Amoxyclav, A - Ampicillin, CA - Ceftazidime, C - Chloramphenicol, E - Erythromycin, G - Gentamicin, I - Imipenem, NA - Nalidixic acid, NF - Nitrofurantoin, NX - Norfloxacin, T - Tetracycline, K - Kanamycin, CF - Ciprofloxacin

Table 2

Antagonistic activity of *Ixora macrothyrsa* against various pathogens

Test organisms	Zone of inhibition (mm)		
	AE	ME	EE
Salmonella paratyphi	14±0.88	15±2.6	22±0.57
Staphylococcus aureus	16±0.57	17±0.3	24±1.76
Shigella sonnei	23±1.4	26±3.17	24±0.66
Bacillus subtilis	11±0.58	12±0.33	17±0.21
Pseudomonas aeruginosa	12±0.57	15±0	19±1.2
E-coli	11±0.88	13±1.15	20±0.33
Klebsiella pneumoniae	3±0.57	16±1.15	19±1.9
Acinetobacter baumannii	23±0.57	29±0.33	20±0.88
MRSA	23±0.57	29±0.33	33±0.33

(Values are presented as mean±SEM, $n = 9$)

AE- Acetone extract, ME- Methanol extract, EE- ethanol extract

Table 5

Compounds identified in the GC-MS analysis for the ethanol extract of *Ixora macrothyrsa*

RT	Compound name	Molecular weight	Molecular formula	Area %	Compound nature
15.69	Ethene (2-chloroethoxy)-	106.55	C4H7ClO	16.39	Aliphatic hydrocarbon
	1-propanol 2-chloro	94.54	C3H7ClO	16.39	Aliphatic hydrocarbon
	urethane	89.09	C3H7NO2	16.39	Aliphatic hydrocarbon
16.29	Urea, Butyl	116.16	C5H12N2O	13.39	Aliphatic hydrocarbon
	benzene 1,4-dinitro-	168.10	C6H4N2O4	13.39	Aromatic
	propylcarbamate	103.11	C4H9NO2	13.39	Aliphatic hydrocarbon
19.96	1-Alanine N-(1-oxopentyl) Methyl	187.23	C9H17NO	7.29	Aliphatic hydrocarbon
	benzyl alcohol Alpha-(1-aminoet)	151.2	C9H13NO	7.29	Aromatic
	3-propoxyamphetamine	193.28	C12H19NO	7.29	Aromatic
17.06	1-Octadecanamine	269.50	C18H39N	5.74	Aliphatic hydrocarbon
	Tridecylamine	199.37	C13H29N	5.74	Aliphatic hydrocarbon
18.85	Benzenemethanol 2-(2-aminopropoxy)	195.25	C11H17NO2	5.60	Aromatic
	Cyclobutanol	72.10	C4H8O	5.60	Aromatic

RT - Retention time

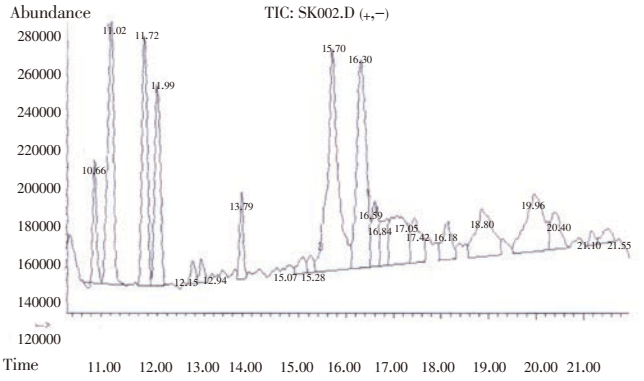

Figure 1. GC-MS chromatogram of ethanol extract of *Ixora macrothyrsa*

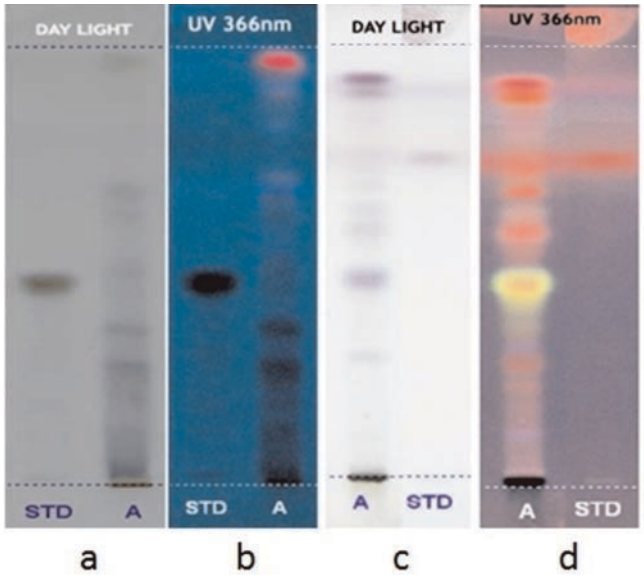

Figure 2. HPTLC pattern for PolyPhenol and Terpenoid profile in ethanol extract of *Ixora macrothyrsa*. a & b Polyphenol chromatogram of standard and plant extract respectively after dervitatization under day light and UV 366nm. c & d, Terpenoids chromatogram of standard and plant extract respectively after dervitatization under day light and UV 366nm.

Table 4

Antagonistic activity of the *Acinetobacter baumannii* and MRSA to the commercially available antibiotics

Acinetobacter baumannii		*MRSA*	
Antibiotic discs	Zone of inhibition (mm)	Antibiotic discs	Zone of inhibition (mm)
PI	22	MU	25
CX	18	FC	27
CF	13	C	16
MRP	18	MI	13
GEN	17	AMP	9
VA	–	OF	–
CEP	–	OX	–
		LE	–

Vancomycin (VA), Cephalothin (CEP), Piperacillin (PI), Cefoxitin (CX), Cefaclor (CF), Gentamicin (GEN), Mupirocin (MU), Fusidic acid (FC), Chloramphenicol (C), Minocycline (MI), Ampicillin (AMP), Oxacillin (OX), Levofloxacin (LE), Ofloxacin (OF), Meropenem (MRP)

4. Discussions

The phytochemicals study showed that the plant would be a promising source as phytomedicine. So qualitatively we inferred that the ethanol extract was best in this plant for checking biological activities because most of the phytochemicals were observed in the ethanol extract. Our study is agreement with the study to say that ethanol is good in phytochemicals extraction[15]. Phenolic compounds widely distributed in plants which have been reported to exert multiple biological effects, including antimicrobial, antioxidant, free radical scavenging abilities, anti-inflammatory and anticarcinogenic activities[16]. The presence of phenolic compounds in the ethanol extract exhibiting that may be the responsible for antagonistic property of this plant.

The antagonistic activity of plant extract results was good in ethanol extract. Hence the results give an inference that the phytochemicals constituent of *Ixora macrothyrsa* flower extracted well by ethanol and it is responsible for controlling the bacterial pathogens. Methicillin resistant *Staphylococcus aureus* causes many severe infections to humans and also it is resistant to many available antibiotics[17]. We have also checked the activity of plant extracts against multiple drug resistant strain *Acinetobacter baumannii*. Because currently there are no antimicrobials working against *Acinetobacter baumannii* in clinical trials[18]. But it's our privilege to point out that the *Ixora macrothyrsa* ethanol plant extract had the ability to control MRSA and both methanol and ethanol extract had ability to control *Acinetobacter baumannii*.

In GC-MS analysis the presence of compounds with maximum area percentage has been already reported for many pharmacological activities. The compound Ethene (2-chloroethoxy) which coming under derivatives of chloroethene already reported for many activities like analgesic, anesthetic[19]. The polyurethane coatings and the polyurethane derivatives possessed antimicrobial properties[20,21,22]. The benzene compounds and its derivatives have many pharmacological activities like antiseptic, antioxidant, antimicrobial, antidermatitic fungicide, insecticide and candidicide[23]. Hence these compounds in synergistic or alone may provide the property of antagonistic activity against tested pathogens especially against MRSA and *Acinetobacter baumannii*. The ethanol plant extract was analysed for polyphenol and terpenoid because both have many pharmacological activities[24]. The presence of both polyphenol and terpenoid was observed in the plant extract by HPTLC analysis.

In conclusion this is the first scientific information reported regarding the plant species *macrothyrsa*. There are many bioactive compounds were identified in *Ixora macrothyrsa* ethanol extract by Gas Chromatography Mass Spectrometry. The presence of these various bioactive compounds may emerge as more effective therapeutic agent to counter the problem of multidrug resistance of Methicillin Resistant *Staphylococcus aureus* and *Acinetobacter baumannii*. Further studies we have to carry out to separate and purify these compounds and check in *in vivo* models.

Conflict of interest statement

We declare that we have no conflict of interest.

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