Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(11):136-143

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Evaluation of antimicrobial activity of different solvent extracts of aromatic plant: *Jasminum sambac* Linn.

T. Gowdhami[1*], A. K. Rajalakshmi[1], N. Sugumar[1] and R. Valliappan[2]

[1]*Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India*
[2]*Department of Chemistry, Annamalai University, Annamalai Nagar, Tamil Nadu, India*

ABSTRACT

Antimicrobial efficiency of Jasminum sambac aromatic plants leaf extracts were examined using petroleum ether, chloroform, ethyl acetate and ethanol as solvents and tested against eight human pathogens like Bacteria: Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Fungi: Aspergillus niger, Aspergillus flavus, Candida albicans using agar disc diffusion method. The ethanol extracts of Jasminum sambac showed highest antibacterial activity against than that moderate the ethyl acetate, petroleum ether and chloroform the bacterial strains tested. The mean zone of inhibition produced by the extracts in disc diffusion assays were ranged from 5 mm to 27 mm. All the plants showed significant activity against all pathogens. The minimum zone of inhibition and comparatively greater inhibitory concentration were determined in petroleum ether and chloroform extract of Jasminum sambac showing less antimicrobial activity against all the experimental strains. The preliminary phytochemical analysis of presence and absence of different solvent extracts of Alkaloid, Flavonoid, tannin, Saponin, glycoside, steroid and terpinoid. The Spectrum of activity observed in the present study may be indicative of the present study ethanolic extracts of these plants could be a possible source to obtained new and effective herbal medicines to treat infections, hence justified the tribal uses of Jasminum sambac against various infectious diseases.

Keywords: Antimicrobial activity, *Jasminum sambac* Linn. Agar Disc diffusion method, Minimum Inhibitory Concentration

---

INTRODUCTION

Many of the plant materials used in traditional medicine are readily available in rural areas. Medicinal plants are valuable source of natural active constituents that are used to maintain human health and also used for the treatment of many human diseases (17). Plants are good source of economically important compounds such as phenolic compounds, nitrogen containing compounds, vitamins and minerals which have anti-oxidant, anti-tumor, anti-mutagenic, anti- carcinogenic and diuretic activities. In Indian traditional medicine, herbs are used as beautification of the body and for preparation of various cosmetics and colours. Nature has been a source of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources. Various medicinal plants have been used for years in daily life to treat various diseases all over the world. They have been used as remedies and for health care preparations (15).

The medicinal plants have always played a key role in the maintenance of world health by providing the best source of remedies for a variety of ailments (1). Infectious diseases are the leading cause of death and disabilities worldwide. Food-borne infections have been one of the major public health concerns and they account for considerably high cases of illness. The numbers of invasive fungal and bacterial infections have dramatically increased in both developed and developing countries (9).Therefore researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infection (6). The

increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (4)

Multiple drug resistance in both human and plant pathogens has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The limited life span of antibiotics has rendered a necessity to search for new antimicrobial substances from various sources such as medicinal plants. Antibiotic resistance has become a global concern (5). There has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years and in many parts of the World. Hence researchers have recently paid attention to safer phytomedicines and biologically active compounds isolated from plant species used in herbal medicines with acceptable therapeutic index for the development of novel drugs (13).

The *Jasminum sambac* flowers and leaves are largely used in folk medicine to prevent and treat breast cancer. Flowers of *Jasminum sambac* are useful to women when brewed as a tonic as it aids in preventing breast cancer and stopping uterine bleeding (12).It is widely used in the Ayurveda, as an antiulcerative, anti cancer, antileprotic, skin diseases and wound healing. And antidiabetic (18), antitumor(14), antimicrobial (11), antioxidant (3) anti-acne (8), suppression of puerperal lactation (16), A.N.S stimulating effect (10).

The present study has been designed to determine the leaf extract (petroleum ether, chloroform, ethyl acetate and ethanol) of *Jasminum sambac* for potential antimicrobial activity against five gram positive and gram negative bacteria viz..*Bacillus subtilis (MTCC 10224), Bacillus cereus (MTCC 10211), Staphylococcus aureus (MTCC 9542) ,Escherichia coli(MTCC 1563), Pseudomonas aeruginosa (MTCC 14676)* and Three fungi *Aspergillus niger, Aspergillus flavus, Candida albicans.* The observed inhibition zones were measured (in nm) and compared against standard antibiotics Ampicillin.

EXPERIMENTAL SECTION

COLLECTION OF PLANT MATERIAL

The fresh leaves of *Jasminum sambac* were collected from Villupuram District, Alagramam Village (Latitude 11.30886 and Longitude 79.816361) TamilNadu, India. Different leaf Extraction (Petroleum ether, Chloroform, Ethyl acetate and Ethanol) were used for further studies.

EXTRACTION OF LEAVES OF *JASMINUM SAMBAC* IN DIFFERENT SOLVENTS

The collected plant Material was washed with water to remove other undesirable material and dried under shade. The air-dried leaves (500 gm) of *Jasminum sambac* were Crushed. The crushed leaves extracted with different solvents of increasing polarity viz. Petroleum ether, Chloroform, Ethyl acetate and Ethanol by hot percolation method using Soxhlet Apparatus. The extract was evaporated till dryness to obtain residue.

PHYTOCHEMICAL ANALYSIS OF DIFFERENT EXTRACTS (2)

The different extracts of leaves of *Jasminum sambac* were tested for various
Components as follows

1. Test for alkaloids

Small portion of solvent free extract was stirred with few drops of dil HCl and filtered .The filtrate was then tested for following colour test

Mayer's test

(a) 1.36 gm of mercuric chloride was dissolved in 60 ml distilled water. **(b)** 5gms of potassium iodide was dissolved in20 ml of distilled water.(a) and (b) was mixed and the volume adjusted to 100ml with distilled water .Appearance of cream colour precipitate with Mayer's reagents showed the presence of alkaloids.

2. Test for flavonoids

Shinoda's test

5 ml of 20% sodium hydroxide was added to equal volume of the sample extract. A yellow solution indicates the presence of flavonoids.

3. Test for tannins and phenolic compounds
10% lead acetate solution, 0.5g of the extract was added and shanken to dissolved. A white precipitate observed indicate the presence of tannins and phenolic compounds.

4. Detection for carbohydrates and glycosides
Molisch's test
10 gm of alpha naphthol was dissolved in 100 ml of 95% alcohol. Extract was treated with this solution and 0.2 ml of conc. sulphuric acid was slowly added through the sides of the test tube, purple or violet colour appeared at the junction.

Fehling's Test
6.932 gm of copper sulphate was dissolved in distilled water and make volume up to 100 ml (solution A). 34.6 gm of potassium sodium tartarate and 10 gm of sodium hydroxide was dissolved in distilled water and make volume up to 100 ml (solution B). Two solution was mixed in equal volume prior to use and few drop of sample was added and boiled, a brick red precipitate of cuprous oxide was formed, if reducing sugars were present.

5. Test for sterols and terpenoids
Salkowski test
Extract was treated with few drops of conc. Sulphuric acid , shake well and allowed to stand for some time, red colour appear at the lower layer indicated the presence of steroids and formation of yellow collared lower layer indicated the presence of terpenoids.

6. Test for proteins and amino acids
Ninhydrin test
1gm of ninhydrin (indane1, 2, 3 trione hydrate) was dissolved in n-butanol and make the volume to 100ml. Extract treated with this solution gave violet colour on boiling.

7. Test for Saponin
Foam test
1ml of extract was diluted with distilled water to 20ml and shake in a graduated cylinder for 15 minutes. A one centimetre layer of foam indicated the presence of Saponin.

8. Test for gums and mucillages
About 10ml of various extracts were treated with absolute alcohol and filtered. Occurrence of precipitate indicates the presence of gum and mucilage's.

9. Test for fats and fixed oil
Spot test
Small quantity of the extract is placed between two filter papers. Oil stains produced with any extract shows the presence of fixed oils and fats in the extract.

COLLECTION OF BACTERIAL STRAINS
The antibacterial activity was tested using leaf extracts from each individual against two strains of gram positive bacteria viz, *Bacillus subtilis* (MTCC 10224), *Bacillus cereus (*MTCC 10211*), Staphylococcus aureus* (MTCC 9542), gram negative bacteria viz., *Escherichia coli* (MTCC 1563), *Pseudomonas aeruginosa* (MTCC 14676), were procured from Microbial Type Culture Collection (MTCC), Chandigarh. The Clinical isolates of fugal strains *viz Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* were obtained from the Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai Nagar, Tamil Nadu, India. These strains were maintained on nutrient agar slant at 4 °C. *In vitro* antibacterial activity was determined by using Muller Hinton Agar (MHA) and Muller Hinton Broth (MHB) was obtained from Hi media, Mumbai

ANTIBIOTIC SENSITIVITY TEST
Antibiotic sensitivity of the bacterial strains were determined by standard CLSI disc diffusion method (M100-S22, 2012). Antibacterial agents from different classes of antibiotics viz., Methicilin (ME 5 µg/disc), Oxacillin (OX µg/disc), Linezolid (LIN 30 µg/disc), Vancomycin (VAN 30 µg/disc), Amikacin (AK 30 µg/disc), Ampicillin (AMP 10 µg/disc), Cefixime (CFM 5 µg/disc), Ceftazidime (CAZ 30 µg/disc), Ciprofloxacin (CIP 5 µg/disc), Chloramphenicol (C 30 µg/disc), Erythromycin (E 15 µg/disc), Gentamycin (GEN 10 µg/disc), Norfloxacin (NX 10 µg/disc), Nalidixic acid (NA 30 µg/disc), Ofloxacin (OF 5 µg/disc), Streptomycin (S 10 µg/disc) and Tetracycline (TE 30 µg/disc), were obtained from Himedia, Mumbai

PREPARATION OF TEST SOLUTION AND DISC

The test solution was prepared with known weight of crude extracts, dissolved in 5 percent of Dimethyl sulphoide (DMSO).Whatmann NO.1sterile filter paper discs (6mm) were impregnated with 20µl of the extract allowed to dry at room temperature.

ANTIBACTERIAL AND ANTIFUNGAL ASSAY

DISC DIFFUSION METHOD

The agar diffusion method (7) was followed for antibacterial susceptibility test. Petri plates were prepared by pouring 20ml Mueller Hinton Agar and Saboruarud Dextrose Broth allowed to solidity for the use in susceptibility test against bacteria and fungi. Plates were dried and 0.1ml of standardized inoculums suspension was poured and uniformly spread. The excess inoculums were drained and the plates were allowed to dry for 5 minutes. After drying the discs with extract were placed on the surface of the plates with sterile forceps and gently pressed to ensure the content with the inoculated agar surface. Ciprofloxacin (5µg/disc) for bacteria and Amphotericin-B (100units/disc) was used as positive control. 5 percent DMSO was used as blind control in these assays. Finally the inoculated plates were incubated at 37^0c for 24h (bacterial) and 28^0c for 24-72 hours (fungi). The zone of inhibition was observed and measured in millimetres. Each assay in this experiment was repeated three times

MEASUREMENT OF BIO ASSAY

For the present in vitro experiment 3 replicates were maintained. After 24 to 72 hours incubation the diameter of inhibition zone was measured for the edge of the disc to the inner margin of the surrounding pathogens.

STATISTICAL ANALYSIS

The results were expressed as the mean ± SD. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Student's t-test was performed to determine any significant difference between different extracts for *in vitro* antibacterial assays. Comparison of means for *in vitro* antibacterial assessment was carried out using one-way analysis of variance (ANOVA) and Duncan test. *P* value < 0.05 was considered statistically significant

RESULTS

PHYTOCHEMICAL SCREENING

The colour, nature and the total yield of each extract obtained from different solvents are presented in **(Table:1)**. Phytochemical evaluation of the various extracts from of the leaf of *Jasminum sambac* were done for the presence Alkaloids, Flavonoids, Saponin, Tannin, Phenol, Treterpinoid, Glycoside, Steroids the results are presented in**(Table:2)**.

Table-1: Percentage yield of different extracts *Jasminum sambac* leaves

S.No	Solvent system	Colour of the extract	% age yield
1	Petroleum ether	Dark green	2.88
2	Chloroform	Black	1.34
3	Ethyl acetate	Greenish yellow	3.4
4	Ethanol	Dark green	6.12

Table-2: Qualitative Phytochemical Chemical Analysis of Extract of *Jasminum sambac* Leaves

S.No	Test for constituents	Petroleum ether	Chloroform	Ethyl acetate	Ethanol
1	Alkaloids	+	-	+	-
2	Carbohydrates	-	-	-	+
3	Flavanoids	-	-	+	+
4	Tannin	-	-	+	+
5	Phenol	-	-	-	+
6	Protein	-	-	-	-
7	Mucilage	-	-	-	-
8	Steroids	-	+	+	+
9	Terpenoids	-	-	-	+
10	Glycosides	-	+	-	+
11	Saponins	+	-	+	+
12	Fats and fixed oils	+	-	-	-

(-) Absence, (+) Presence

ANTIMICROBIAL ACTIVITY

The antimicrobial activity was examined by agar well diffusion method. The petroleum ether, chloroform, ethyl acetate and ethanol extract from *Jasminum sambac* leaf exhibited potent antimicrobial activity towards all the microbes. The zone of inhibition values are presented in **(Table: 3; Figure: 1 and Plate: 1)**.

Bacillus cereus was found to be more highest activity towards the ethanol extract from the leaf with maximum inhibitory zone (27mm) followed by Ethyl acetate (23mm), Chloroform(18 mm) and Petroleum ether (20mm). *Bacillus subtilis* was found to be more higher activity towards the ethanol extract from the leaf with maximum inhibitory zone (22mm) followed by Ethyl acetate (20mm), Petroleum ether (18.4mm) and Chloroform (16mm). *Staphylococcus aureus* was found to be more higher activity towards the ethanol extract from the leaf with maximum inhibitory zone (20mm) followed by ethyl acetate (19mm), petroleum ether (17mm) and chloroform (16mm). *Escherichia coli* was found to be more higher activity towards the ethanol extract from the leaf with maximum inhibitory zone (15mm) followed by ethyl acetate (13mm), petroleum ether (12mm) and chloroform (10mm). *Pseudomonas aeruginosa* was found to be more higher activity towards the ethanol extract from the leaf with maximum inhibitory zone (13mm) followed by ethyl acetate (12mm), petroleum ether (10.5mm) and chloroform (9.5mm). *Candida albicans* was found to be more higher activity towards the ethanol extract from the leaf with maximum inhibitory zone (10.5mm) followed by ethyl acetate (9mm), petroleum ether (9mm) and chloroform (8.5mm). *Aspergillus niger* was found to be more higher activity towards the ethanol extract from the leaf with maximum inhibitory zone(10mm) followed by ethyl acetate (9mm), petroleum ether (8.6mm) and chloroform (7.5mm). *Aspergillus flavus* was found to be more higher activity towards the ethanol extract from the leaf with maximum inhibitory zone (9mm) followed by ethyl acetate (8.5mm), petroleum ether (8mm) and chloroform (7.5mm). The result obtained shown that all the extracts showed very significant antimicrobial activity against the tested organisms.

Table:3 Antimicrobial activity (zone of inhibition, mm) of various leaf extracts *Jasminum sambac* against clinical pathogens

S.No	Organisms	Mean zone of inhibition (mm) concentration (µg/ml)												Ampicillin (10 µg/disc
		Petroleum ether			chloroform			Ethyl acetate			ethanol			
		50	100	150	50	100	150	50	100	150	50	100	150	
1	*B.subtilis*	8	9.2	18.4	7.2	8.1	16	8.5	11	20	10.9	12	22	23
2	*B.cerus*	7.5	8	20	6	7	18	8	9	23	10	11	27	28
3	*P.aeruginosa*	6.2	9.6	10.5	5	8.5	9.5	6.5	11.2	12	7	12.1	13	15
4	*S.aures*	7	8	17	6.2	5	16	7.6	10	19	8	13	20	21
5	*E.coli*	7.2	8.5	12	6	6.9	10	7	9	13	8.9	10	15	16
6	*A.niger*	6.4	7.4	8.6	5.5	6.2	7.5	7.5	8.5	9	7.3	8.5	10	12
7	*A.flavus*	6	7	8	5.1	6	7.5	7	7	8.5	6	8	9	10
8	*C.albicans*	7.8	8.6	9	6.4	7.0	8.5	8	8.5	9	8.5	9	10.5	11

Figure :1 Activation index against various microorganisms

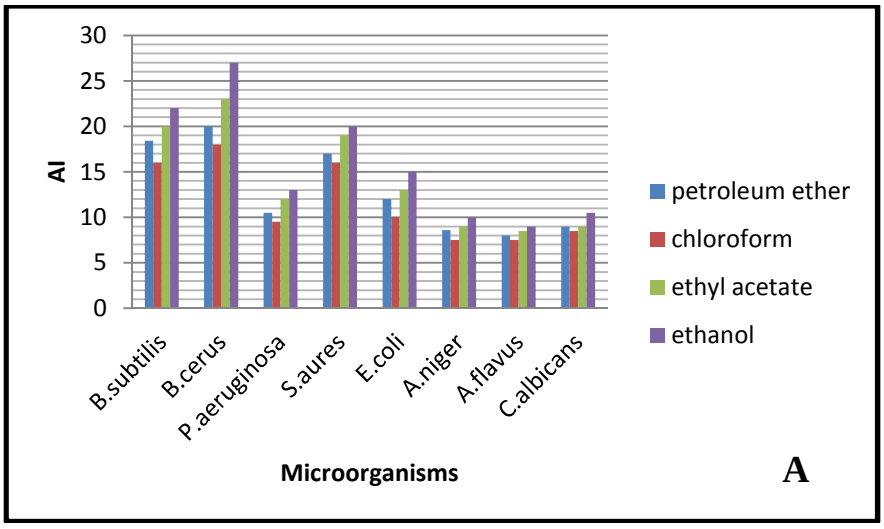
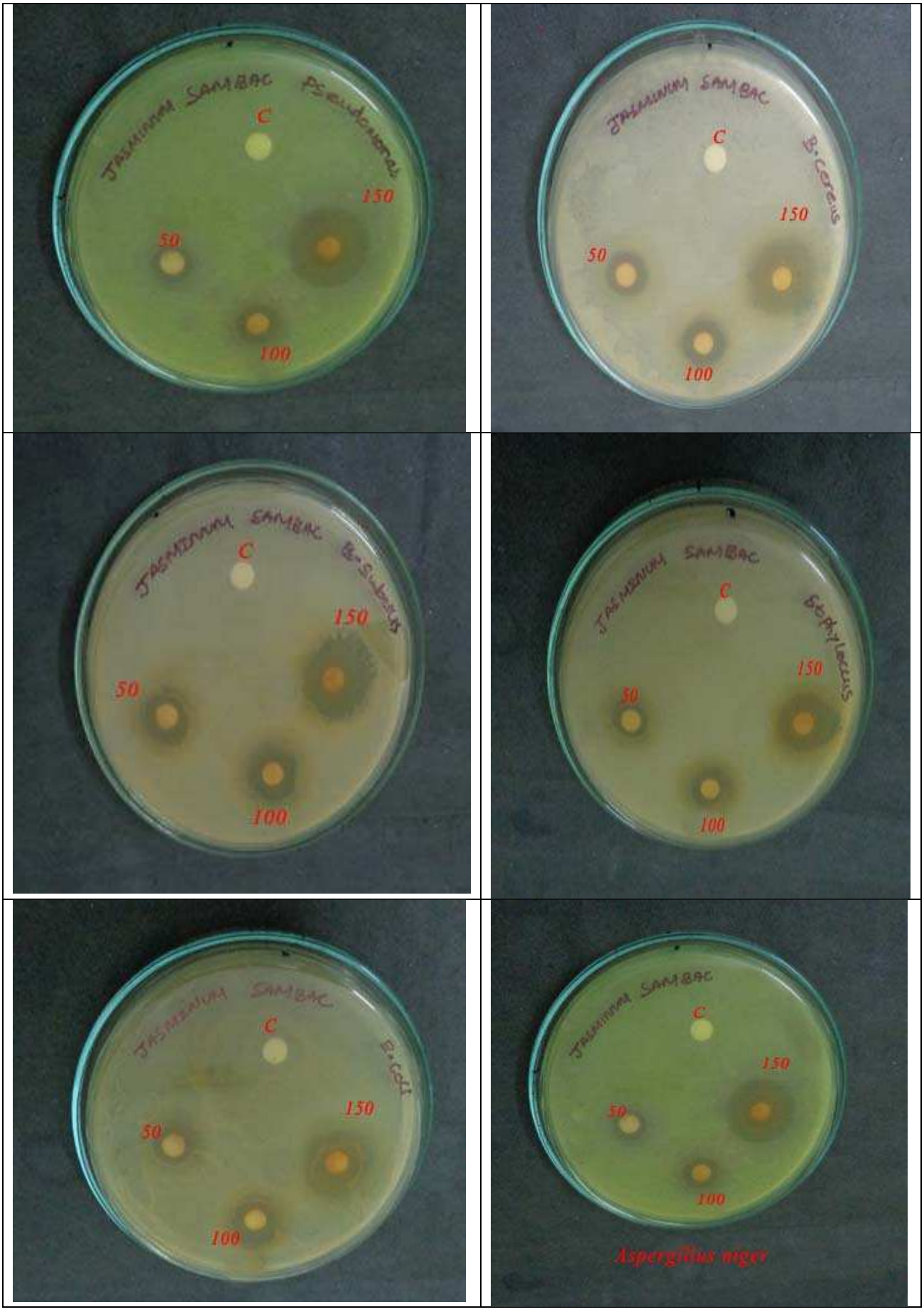
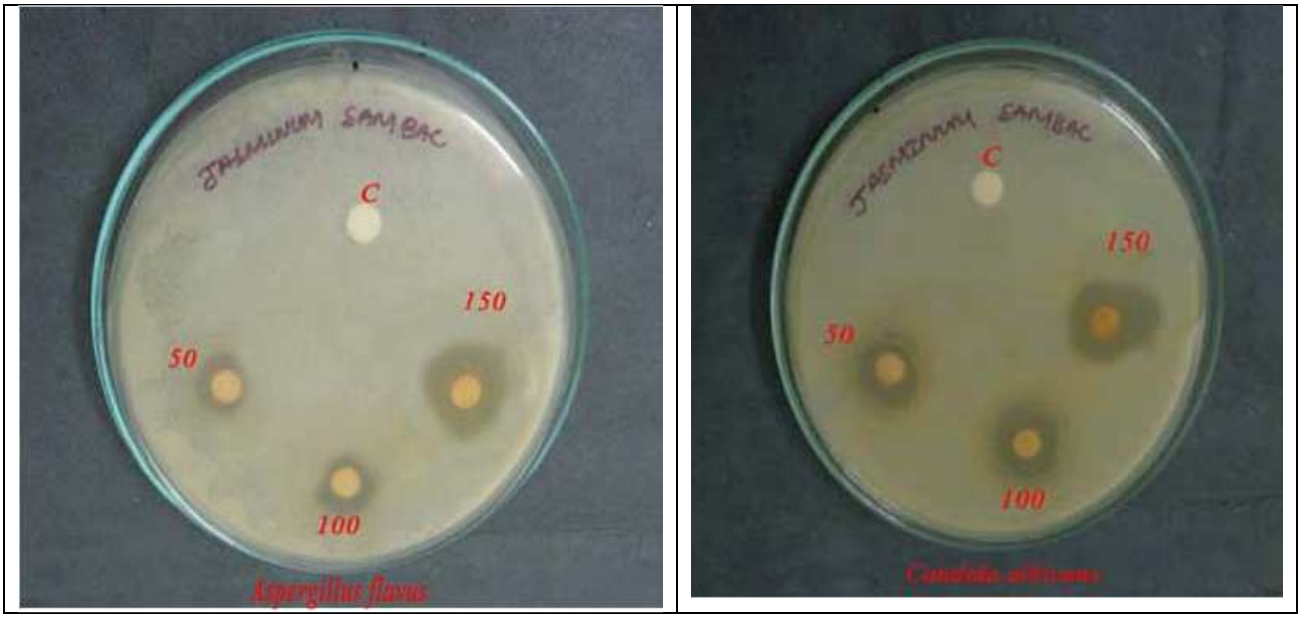


Plate :1 Antimicrobial activity of ethanolic extracts of *Jasminum sambac* L


JASMINUM SAMBAC
C
150
50
100
Pseudomonas
JASMINUM SAMBAC
C
B. cereus
150
50
100
JASMINUM SAMBAC
C
B. Subtilis
150
50
100
JASMINUM SAMBAC
C
Staphylococcus
150
50
100
JASMINUM SAMBAC
C
E. coli
150
50
100
JASMINUM SAMBAC
C
150
50
100
Aspergillus niger



DISSCUSSION

The extract of leaves of *Jasminum sambac* undergoes various qualitative chemical tests. It was found out that methanol extract was the richest extract for phytoconstituents. It contained all tested phytoconstituents viz. Alkaloids, flavanoids, carbohydrate, phenolic compounds, saponin, glycosides, terpenoids and tannins. Chloroform extract showed the presence of steroids and glycosides compounds while Petroleum ether contained alkaloids and saponins compounds. All extracts showed antibacterial activity. Ethanol extract showed maximum anti-bacterial activity in comparison to other extracts. All extracts showed anti-fungal activity against bacterial culture at a concentration of 150 mg/ml. Ethanol extract showed the maximum anti-fungal activity in comparison to other extracts.

CONCLUSION

From the above study it is concluded that the ethanol extract showed the maximum antimicrobial activity in comparison to other extracts. The antimicrobial activity of *Jasminum sambac* was found active against *Escherichia coli, Bacillus subtilis, Bacillus Cerus, Pseudomonas aeruginosa* in ethyl acetate and petroleum ether extracts whereas extracts of chloroform was found inactive against *Escherichia coli,* and Pseudomonas. The extract of ethanol was found highly active against *Candida albicans* and *Aspergillus niger*. The extract of ethyl acetate ether also showed activity against these fungi. It may be concluded that further research need to be done to apply antimicrobial property of *Jasminum sambac* for drugs formulation

Acknowledgement
The authors are thankful to Professor and Head, Department of Botany, Annamalai University for providing laboratory facilities.

REFERENCES

[1] AA Dehpour; MA Ebrahimzadeh; A Roudgar; S F Nabavi; SM Nabavi. *World Acad Sci Eng Technol*. **2011**; 73:162-6.
[2] C K Kokate .Pharmacognosy .Nirali publication (39). **2007**; pp: 97-132.
[3] FA Latif ; P Edou ; F Eba ; N Mohamed ; A Ali ; S Djama ; LC Obame ; I Bassole and M Dicko . *African Journal of Plant Science.***2010**;4 (3) : 038-043.
[4] ML Colombo ; E Bosisio. *Pharmacol Res.* **1996**; 33: 127-134,
[5] H Westh ; CS Zinn ; VT Rosdahl . Sarisa Study Group. *Microbial Drug Resistance*.**2004**; 10: 169-176.
[6] N Benkeblia. Lebensm-Wiss u-Technol. **2004**; 37:263-268.
[7] AW Bauer; WMM Kirby ; JC Scherris; M Turck. *American Journal of Clinical Pathology.**1966;*** 45 :493-496
[8] RS Harisaranraj ; S Babu ; K Suresh. *Ethnobotanical Leaflets.***2010**; 14: 84- 94.
[9] DP Kisangau ; KM Hosea ; VM Herbert ; CC Lyaruu ; ZH Joseph ; PJ Mbwambo. *Pharm Biol* .**2009**; 4:708-16.
[10] T Hongratanaworakit. *Natural Product Communications*.**2010**; 5(1):157-162.

[11] RA Hussaini and AM Mahasneh. *Molecules* **2009**;14: 3425-3435.
[12] SG Joshi . Oleaceae: Joshi SG. (Ed.). Medicinal Plants. Oxford & IBH Publishing Co. Pvt Ltd, New Delhi. **2000**: 298-300.
[13] PS Pavithra ; VS Janani ; KH Charumathi ; R Indumathy ; S Potala and RS Verma. *Int. J. Green Pharma.* **2010**; 10: 22-28.
[14] S Radu and CY Kqueen . *Malaysian Journal of Medical Sciences*.**2002**;9(2): 23-33.
[15] S Shanmugam ; K Manikandan and K Rajendran. *Ethnobotan. Leaf lets*. **2009**; 13: 189-94.
[16] P Shrivastav ; K George ; N Balasubramaniam ; M Padmini Jasper ; A Thomas ; AS Kanagasabhapathy . *Australian and New Zeland Journal of Obstetrics and Gynaecology*.**1988**; 28(1): 68–71.
[17] F Stary and S Hans .The National guides to medical herbs and plants. Tiger Books. Int. Plc. **1998**. UK.
[18] AB Upaganlawar ; A Bhagat, CR Tenpe and PG Yeole . *Pharmacologyonline*. **2003**;1: 1-6