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PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF SALICYLIC ACID (SA) AND JASMONIC ACID (JA) TREATED PLANTS OF *ACALYPHA INDICA*.LINN.

S. Shireesha and A.A Haleem Khan*
Deparment of Botany, Telangana University, Dichpally, Nizamabad
Email- bunnishireesha@gmail.com & aahaleemkhan@gmail.com*

ABSTRACT:
Acalypha indica Linn. belongs to *Euphorbiaceace* family, distributed in the world wide. It is major source of bio active compounds. In the present work, we try to assess the phytochemical and antimicrobial activity of Salicylic acid (SA) and Jasmonic acid (JA) treated *Acalypha* plants. Plants were treated with alone and different concentration combinations of SA and JA. After 55 days treated plant leaves were used for extractions. Methanol and Chloroform extractions were prepared by Soxhlet method and antimicrobial activity carried out by using agar well diffusion method. The extractions were evaluated against on gram positive and negative bacteria and fungi like *Bacillus subtilis, Escherichia coli, Proteus vulgarius, Salmonella typhi, Pseudomonas aeruginosa, Aspergillus niger, Candida albicans and Fusarium oxysporum.* In this work, we report SA and JA treated plant methanol extracts were showed promising secondary metabolites, antibacterial and antifungal activity when compared to control plants. Especially, alone 400µM JA treated plant were showed more activity against *Bacillus subtilis* (21mm) and combination of SA & JA treated plants are showed high activity against *Bacillus subtilis* (18mm), *Pseudomonas aeruginosa* (12mm), *Salmonella typhi* (19mm), *Escherichia coli* (14mm), *Proteus vulgarius* (23mm) *and Candida albicans* (21mm) respectively. The result indicated that, combination of SA and JA treated plant methanol extracts were a major source of bio active compounds, which are leads to high antimicrobial activity against bacterial and fungal cultures, when compared to alone and combination of SA and JA treated chloroform extractions.

Keywords: *Acalypha indica*.L, Agar well diffusion method, Antimicrobial activity.

INTRODUCTION:

Acalypha indica Linn. (Euphorbiaceae) is an annual erect herb and it is cosmopolitan distributed common weed plant(Ramachandran, 2008). It is used in treating pneumonia, asthma, rheumatism and also an emetic emmenagogue and anthelmintic (Chopra and Nayar, 1956; Hiremath *et al.*, 1999). The juice of this plant used to treat a number of skin disorders and also been reported to possess contraceptive activity (Bourdy and Walker, 1992). In India, *Acalypha indica* has been extensively used in Ayurvedic system of medicine for various ailments like hepato protective, anti-inflammatory, antitissive, antifungal, wound healing and also antibacterial agent (Gupta, 2010). The ethanol and water extract of leaves from *Acalypha indica* were effective against Gram negative bacteria, Gram-positive bacteria and fungi. Sensitivity towards

dose dependent aqueous leaves and root extracts. *Aspergillus niger*and, *Candida albicans* were resistant to both ethanol and water extract of all *Acalypha indica*. (Jagatheeswari *et al.*, 2013).

The production of secondary metabolites could be enhanced by using elicitors, the pathogen-derived (exogenous) or plant-derived (endogenous) signal molecules, which are inducers of the plant defense response. These defense responses are activated through a signal transduction pathway via recognition of the elicitor by the receptors located in the plasma membrane and formation of secondary messengers, such as jasmonates, ethylene, and salicylic acid (SA) (Odjakova and Hadjiivanova,2001; Talarczyk and Hennig, 2001) Salicylic acid(SA) and Jasmonic acid (JA) is one of the endogenous signal molecules, which are enhance the production of secondary metabolites and essential oils, the most commonlyused elicitors like Jasmonic acid(JA) and methyl Jasmonate (Me- JA)(Zhao *et al.*, 2005). The broad possibilities of using the plant are linked to its production of essential oils, which are a mixture of chemical compounds, mainly mono-and sesquiterpens (Kennedy and Wightman, 2011; Sharopov *et. al.*, 2015). They showed strong antimicrobial (Moon *et al.*, 2006), antifungal activities (D'Auria et *al.*, 2005). SA are two key signal molecules widely used as elicitor compounds inducing secondary metabolites in many plants, plant cell and callus cultures (Sirvent and Gibson, 2002)

The present effort has been made objective of this current study was to evaluate the influence of Jasmonic acid (JA) and SalIcylic acid (SA) on secondary metabolite production and antimicrobial activity of *Acalypha indica* L.

MATERIALS AND METHODS:

Process of plant treatment with SA and JA:

Acalypha indica L plants were transplanted in to the field. Plants were treated with alone and combination of Salicylic acid (SA) and Jasmonic acid (JA) with different concentrations for every 10 days intervals up to 55 days. The treatment concentrations like T1 (Control), T2 (1mM SA), T3 (3mM SA) T4 (200 µM JA) T5 (400 µM JA) T6 (1mM SA + 200µM JA) T7 (1mM SA+400µM JA) T8 (3mM SA+200µM JA) and T9 (3mM SA + 400µM JA).

Sample preparation:

After 55 days of grown plants leaves were used as extraction material. The obtained leaves were washed with tap water to remove dust and then were shade dried at room temperature. Then crushed to fine powder using mortar and pestle and stored at refrigerator (4^0C) for further use.

Preparatio of extracts:

Preparation of extracts by using Soxhlet extracting method. 100g of dried leaf material was taken in a soxhlet and 500ml of 80% methanol was added. The temperature is set to 70^0C and the extraction was carried out upto 5hours. The extract obtained is filtered and concentrated at 70^0C. Dried extracts were kept in refrigerator and used for

further study (Kuluvar *et al.*, 2009).

Preliminary Qualitative Phytochemical Analysis:

Preliminary phytochemical screening was carried out to identify the secondary metabolites present in the leaf extracts of *Acalypha indica* L (Harborne, 1973).

Alkaloids: To 2 ml of extract, 2 ml of Wagner's reagent wasadded. The appearance of a brownish precipitate indicates the presence of alkaloids.

Flavonoids: To 2 ml of extract, 2 ml of 10% lead acetate was added. Yellowish- green colour indicates the presence of flavonoids.

Saponins: To 2 ml of extract, 2 ml of Benedicts reagent was added. Bluish-black precipitate indicates the presence of saponins.

Tanins: To 2 ml of extract, 0.1% ferric chloride was added. Brownish-green colour indicates the presence of tannins.

Terpenoids: To 2 ml of extract, 2 ml of chloroform and conc. H_2SO_4 was carefully added to form a layer. A reddish-brown colour indicates the presence of terpenoids.

Reducing sugars: The extract was shaken with distilled water and filtered. The filtered was boiled with Fehlings solution A and B for few minutes. An orange-red precipitate indicates the presence of reducing sugars.

Glycosides: The extract was hydrolysed with HCL solution and neutralized with NaOH solution. A few drops of Fehlings solution A and B were added. Red precipitate indicates the presence of glycosides.

Proteins: The extract was treated with $CuSO_4$ solution. Formation of a violetcolor complex indicated the presence of proteins.

Amino acids: To 0.5 ml of extract two drops of freshly prepared 0.2% Ninhydrin reagent was added and heates. The appearance of pink or purple colour indicates the presence of peptides or amino acids.

Steroids: To 2 ml of solvent extract, 2 ml of chloroform and 2 ml of conc. H_2SO_4 was added slowly and shaken well. The chloroform layer turned red and sulphuric acid layer turned greenish yellow, which indicated the presence of sterols.

Detection of Oils and Resins: Test solution was applied on filter paper. Transparent appearance on the filter paper indicates the presence of oils and resins.

Carbohydrates: A few drops of Molisch reagent were added to the extract followed by conc. H_2SO_4. The formation of a violet ring at the junction of two liquids indicate the presence of carbohydrates.

Phenols: To 1 ml of extract, 2 ml of distilled water and a few drops of10% aqueous ferric chloride solution were added. Formation of blue or green color indicates the presence of phenols.

Quinones: 1 ml of extract was treated with alcoholic KOH solution. quinonesgive coloration ranging from red to blue.

Antimicrobial activity against test organisms:

The antimicrobial activity of the crude extract of *Acalypha Indica* was determined by agar well

diffusion method (Naragani *etal.*, 2014). Nutrient agar (NA) and Czapek-Dox (CD) agar media were used for culturing the test bacteria and fungi respectively. Nutrient agar (NA) medium (100 ml) was sterilized at 15 lbs pressure (121^0C) for 15min, cooled and inoculated with 0.5 ml of test bacterial suspension. After solidification of agar medium, wells of about 5mm diameter were punched with sterilized cork borer, In case of antifungal assay, spore suspension of test fungi was mixed with the cooled, Czapek-Dox (CD) agar medium and poured into petri dishes. The crude extract dissolved in ethanol at a concentration of 50,100,150 µg/ml (100,200 and 300µg/L for fungi) were added to the wells made in the medium. Adding only ethanol to the wells served as control. The plates were incubated at 30^0C for 24h for bacteria and 24-72h for fungi and thediameter of the inhibition zones was measured.

The antimicrobial activity of the leaf methanol and chloroform extracts produced by the *Acalypha indica* were tested against bacteria viz. *Bacillus subtilis* (ATCC6644), *Salmonella typhi (*ATCC6538*) Escherichia coli* (ATCC15597). *Candida albicans* (ATCC 10231), *Proteus vulgaris* (ATCC29905), *Pseudomonas aeruginosa* (ATCC27853) *Fusarium oxysporum* (ATCC52429) and *Aspergillus niger* (ATCC10864) byagar-diffusion assay.

RESULTS AND DISCUSSION:

Plants are major source of bio active compounds, which are used in treatment of human diseases. Based on the temperature and soil conditions might be to change the concentration of phyto chemicals. Some of the plant hormones (Salicylic acid & Jasmonic acid) are also influence the phytochemical concentration in plant species. The leaf of *Acalyphaindica* has presence of tannins, cardiac glycosides, alkaloids, flavonoids, terpenoids, Antharaquinones and steroids are absent (Darshini, 2015).

SA and JA have been proved to be able to produce several compounds (alkaloids, terpenoid and phenolic, phytoalexins, coumarins and taxanes) in many plant species (Fits and Memelink., 2000). In the qualitative analysis, maximum bioactive compounds were present in leaf methanol extract except oils and steroids compounds (Table.1) whereas, chloroform leaf extraction contains low levels of phytochemicals (Table.2).

Due to JA and SA treatments, the plants were containing high concentrations of secondary metabolite scompared to the control plants. The maximum phytochemials are present in leaf methanol extract compared to chloroform extract.

Foliar application of SA at the conentration of 50 mg/L, *Simarouba glauca* plants produce the secondary metabolites at water stress conditions (Awate and Gaikwas, 2014). Addition of jasmonic acid in plant micropropagation media, JA involved in the ativation of genes, that may help in biosynthesis of secondaray metabolites (Pirbalouti *et al.*, 2014). Similarly, methanol extract of SA and JA treated plants were containing high

content of alkaloids, flavonoids, terpenoids and steroids were absent. Due to Alone and combination of SA and JA treated plants are contain high phytochemical constituents (Table 1,2) when compared to control plants. Whereas, some of the phyochemicals such as, steroids, phenols, quinones and oils were not present in the both extractions. *Acalypha indica.*L methanol extracts were exhibiting strong antimicrobial activity when compared to chloroform solvent extract (Darshini., 2015). Methanol extract showed maximum inhibition zone against *Salmonella typhi* (20.1±1.3) and no effect on *Proteus Vulgaris* and *Pseudomonas* (Govindarajan *et al.*, 2008; Vijayarekha *et al.*, 2015). Similarly, chloroform extract of *Acalypha indica.L* plants has been showed maximum inhibition zone against *Bacillus subtilis* (5mm), *Salmonella typhi* (4mm), *Escherichia coli* (5mm) and *Cadida albucans* (8mm) no effect on *Proteus vulgarius* and *pseudomonas aeruginosa* (Table.4).

At the concentration of 50%, all the essential oils isolated from the plants grown on the media with the addition of JA, regardless of its concentration, exhibited stronger activity towards *C. albicans* (9.7-12.3mm) in comparison with the control. The antimicrobial activity of Lavandula essential oils has been commonly tested. It has been determined that essential oils inhibit the propagation of *Proteus vulgaris* (Prabuseenivasan *et al.*, 2006), *Escherichia coli* (Abroom and Azar *et al.*, 2011; Criste *et al.*, 2014), *Bacillus subtilis* (Prabuseenivasan *et al.*, 2006; Abroom and Azar *et al.*, 2011). Similarly, methanol extract of (1MmSA+200µMJA) treated plants are showed more activity against *Proteus vulgaris* (22mm) (Table.3).

We observed at 150µg/mg concentration of methanol extract of 3mm SA treated plants were showed maximum inhibition against *Bacillus subtilis* (21mm) (Fig.3) and combination of (1mMSA+200uMJA) & (3mMSA+400uMJA) methanol extract showed more activity against gram-positive and negative bacteria and fungi like *Bacillus subtilis* (21mm), *Proteus vulgarius* (22mm) (Fig. 2), *Samonella typhi* (15mm), *Pseudomonas* (12mm) and *Cadida albicans* (19mm) respectively, when compared to control plants. Rest of the alone and combinations of SA and JA treated plant methanol and chloroform extracts were showed minimum inhibition zone observed against above mentioned bacterial and fungal cultures (Table 3&4).

Because of salicyclic acid and jasmonic acid treatment may influencethe secondary metabolite production like alkaloids and essential oils, due to the high production of secondary metabolite might be a reason for performed maximum inhibition activity on bacteria and fungi.

CONCLUSION:

In the present work, the data indicates alone and different combinations of SA and JA treated plants exhibit the more phytochemical constituents and promising activity against gram-positive and gram-negative bacteria and fungi when

compared to control plants.

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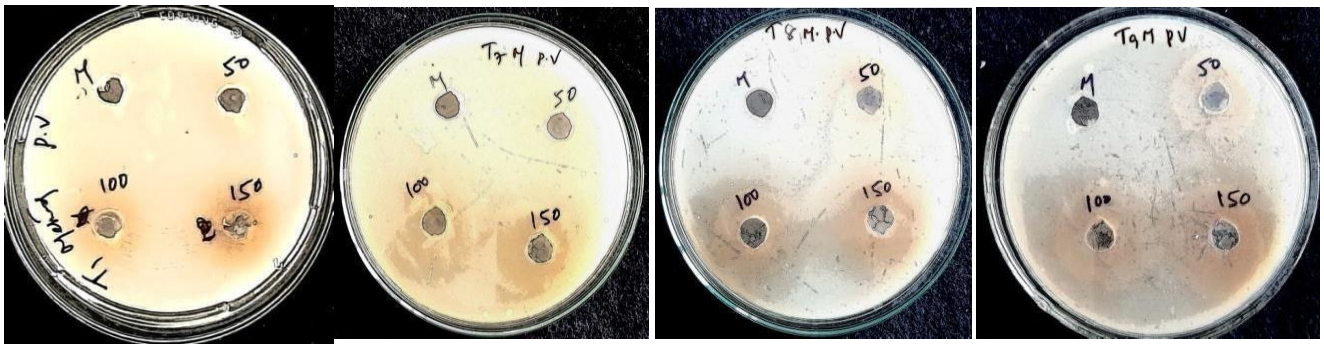
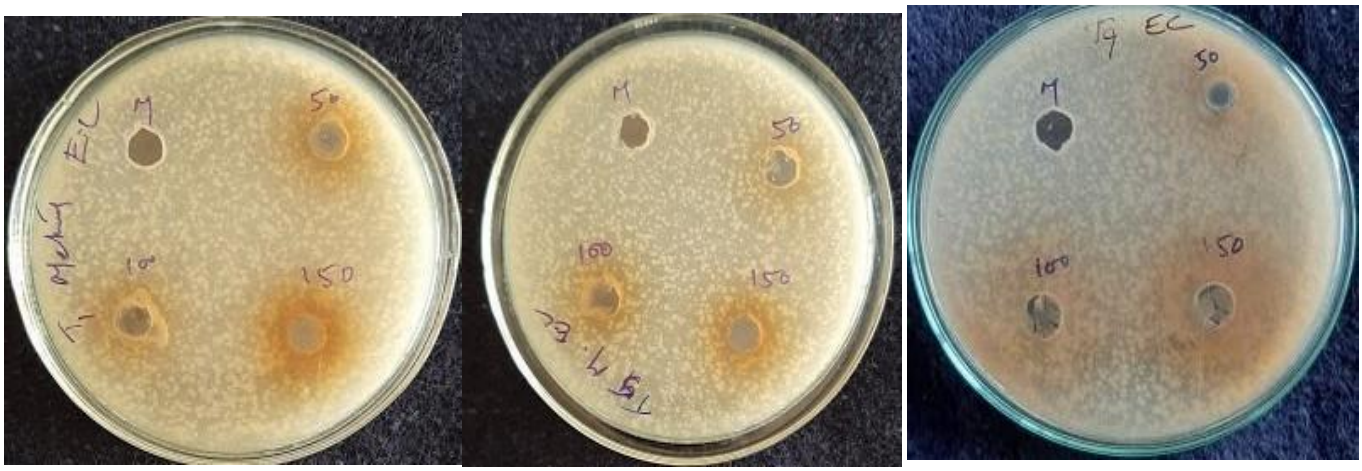


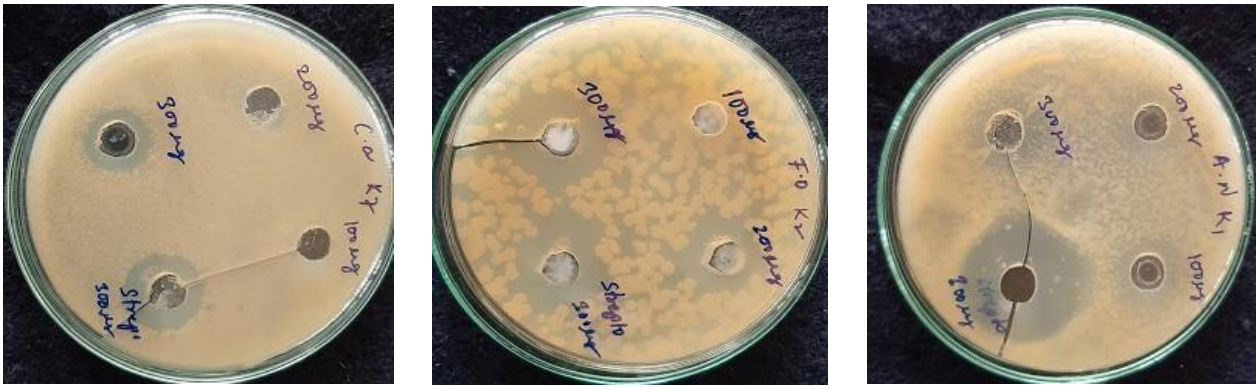
T1 (5mm) T7 (15mm) T8 (22mm) T9 (23mm)

Fig.1 T8 & T9 Methanol extract showed maximum inhibition zone against *Proteious vulgarius*



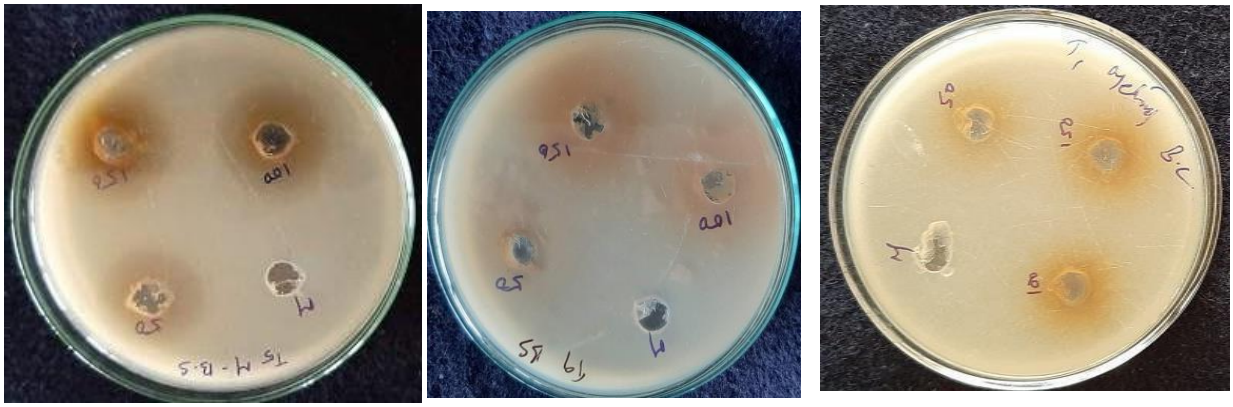
T1 (3mm) T5 (9mm) T9 (14mm)

Fig.2 T9 Methanol extract showed maximum inhibition zone against Escherichia *coli*



T8 C.a (21mm) T7 F.O (10mm) T8 (10mm)

Fig.3 Methanol extract showed maximum inhibition zone against *fungi*



T9 (18mm) T5 (21mm) T1 (5mm)

Fig.4 Methanol extract showed maximum inhibition zone against *Bacillus*

Table1. Phytochemical analysis from methanol extract of SA&JA treated *Acalyphaindica.*L plants.

Sr. No.	Secondary metabolite	Test	Methanol extract								
			T1	T2	T3	T4	T5	T6	T7	T8	T9
1	**Alkaloids**	Wagner's test	+	++	++	+	+	+	++	+++	+
2	**Glycosides**	Keller-Kilianii test	---	--	--	---	---	---	+	+	--
3	**Flavonoids**	Shinoda test	+	+	+	+	++	+	++	++	++
4	**Saponins**	Forthing test	--	---	--	--	--	+	+	+	+
5	**Tannins**	Braemer's test	---	+	+	-	--	--	--	+	---
6	**Terpenoids**	Salkowski test	+	+	+	+	+	+	++	++	++
7	**Reducing sugars**	Fehling test	---	--	+	+	+	+	+	+	+
8	**Proteins**	Fraction + CuSo4	++	+	+	+	+	+	++	++	++
9	**Amino acids**	Ninhydrin test	++	+	+	+	+	+	++	+	+
10	**Steroids**	Fraction+chloroform+Con H2So4	---	--	--	--	---	---	---	---	--
11	**Carbohydrates**	Fehling test	+	+	+	++	++	++	+++	++	++
12	**Oils & Resins**	Filter paper test	--	--	--	--	--	--	---	--	--
13	**Phenols**	Forthing test	---	--	--	+	+	+	+	+	+
14	**Quinones**	Forthing test	---	---	--	--	--	--	--	--	--

-- (Absent), + (low) and +++ (High).

Table.2 Phytocemical analysis from choroform extracts of SA&JA treated *Acalypha indica L* plants.

Sr. No.	Secondary metabolite	Test	Chloroform extract								
			T1	T2	T3	T4	T5	T6	T7	T8	T9
1	**Alkaloids**	Wagner's test	--	+	+	--	+	+	++	+++	+
2	**glycosides**	Keller-Kilianii test	---	--	--	---	---	---	--	---	--
3	**Flavonoids**	Shinoda test	--	--	+	+	+	+	+	+	+
4	**Saponins**	Forthing test	--	---	--	--	--	--	--	--	--
5	**Tannins**	Braemer's test	---	+	+	-	--	--	--	+	---
6	**Terpenoids**	Salkowski test	-	--	--	+	+	+	+	+	+
7	**Reducing sugars**	Fehling test	---	--	+	--	--	+	+	+	+
8	**Proteins**	Fraction + CuSo4	++	+	+	+	+	+	+	+	+
9	**Amino acids**	Ninhydrin test	+	+	+	+	+	+	++	++	+
10	**Steroids**	Fraction+chloroform +Con H2So4	---	--	--	--	---	---	---	---	--
11	**Carbohydrates**	Fehling test	+	+	+	+	++	++	+++	++	++
12	**Oils & Resins**	Filter paper test	--	--	--	--	--	--	---	--	--
13	**Phenols**	Forthing test	---	--	--	-	---	--	--	--	--
14	**Quinones**	Fraction + KOH	---	---	--	--	--	--	--	--	--

-- (Absent), + (low) and +++ (High).

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Table.3 Antimicrobial activity of leaf methanol extract of SA&JA treated and controlplants of *Acalypha indica* L

Sr. No.	Micro organism	Zone of inhibition (mm)																										
		T1 (ug/ml)			T2 (ug/ml)			T3 (ug/ml)			T4 (ug/ml)			T5 (ug/ml)			T6 (ug/ml)			T7 (ug/ml)			T8 (ug/ml)			T9 (ug/ml)		
		50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150
1	Bacillus subtilis	00±00	00± 00	02± 0.45	00± 00	03± 0.44	05± 0.88	00± 00	02± 0.19	08± 0.33	00± 00	04± 0.12	06±0 .47	05 ±0 .76	10±0 .42	**21**±0 .04	00± 00	05± 86	10±0 .43	06± 0.05	12±0 .59	**17**± 0.07	05± 0.92	12±0 /55	**16**± 0.64	02 ±0 .77	05± 0.04	18± 0.04
2	pseudomonas	00±00	00±0 0	00±0 0	00± 00	00± 00	04± 0.34	00± 00	04± 0.55	06± 0.22	00± 00	02± 0.05	07±0 .06	00±0 0	05±0 .06	08±0 .46	02± 0.09	04± 0.14	09±0 .66	05± 0.35	10±0 .42	**10**± 0.19	05± 0.87	07±0 .03	**12**± 0.16	02 ±0 .65	04± 0.66	07± 0.66
3	Salmonell typhi	00±00	00±0 0	02± 0.18	00± 00	02± 0.09	05± 0.22	00± 00	03± 0.27	07± 0.06	00± 00	05± 0.08	10±0 .14	00±0 0	07±0 .65	11±0 .53	07± 0.42	09± 0.06	15±0 .52	08± 0.25	12±0 .04	**18**± 0.34	10± 0.25	14±0 .92	**19**± 0.29	05 ±0 .66	09± 0.43	10± 0.35
4	Escherichia coli	00±00	00±0 0	03± 0.40	00± 00	00± 00	04± 0.12	00± 00	05± 0.09	08± 0.03	00± 00	08± 0.33	10±0 .38	00±0 0	07±0 .23	09±0 .78	02± 0.15	06± 0.50	10±0 .59	04± 0.57	08±0 .19	**12**± 0.90	04± 0.03	06±0 .23	**14**± 0.05	03 ±0 .56	07± 0.25	09± 0.76
5	Proteious vulgaris	00±00	00±0 0	05± 0.09	00± 00	03± 0.04	06± 0.48	02 ±0. 37	18± 0.45	20±. 24	00± 00	05± 0.29	08±. 77	00±0 0	10±0 .5	12±0 .34	02± 0.48	17± 0.19	17±0 .07	04± 0.10	08±0 .23	**15**± 0.63	06± 0.23	20±0 .75	**22**± 0.61	06 ±0 .10	15± 0.05	23± 0.63
6	Candida albicans	00±00	00±0 0	05± 0.32	00± 00	00±0 0	05± 0.33	00± 00	03± 0.22	03± 0.65	04 ±0. 51	10± 0.25	12±0 .27	04 ±	09±0 .74	12±0 .84	05± 0.09	10± 0.58	19±0 .16	07± 0.04	14±0 .05	**20**± 0.72	09± 0.05	15±0 .36	**21**± 0.33	06 ±0 .30	10± 0.89	15± 0.22
7	Aspergillus niger	00±00	00±0 0	03± 0.49	00± 00	00±0 0	05± 0.42	00± 00	04± 0.03	04± 0.30	00± 00	06± 0.65	10±0 .79	02 ±0 .66	05±0 .17	09±0 .25	00± 00	06± 0.04	08±0 .74	04± 0.12	06±0 .48	**08**± 0.26	03± 0.58	05±0 .09	**10**± 0.52	02 ±0 .24	05± 0.23	07± 0.03
8	Fusariu m oxyporo us	00±00	00±0 0	02± 0.28	00± 00	00±0 0	00± 00	00± 00	00± 00	03± 0.11	00± 00	00± 00	09±0 .44	00 ±0 0	06±0 .68	08±0 .33	02± 0.27	04± 0.78	08±0 .13	05± 0.49	08±0 .04	**10**± 0.82	04± 0.63	07±0 .26	**09**± 0.76	03 ±0 .56	06± 0.25	08± 0.51

Table.4 Antimicrobial activity of leaf chloroform extract of SA&JA treated and control plants of *Acalypha indica*.L

Sr. No.	Micro organism	Zone of inhibition(mm)																									
		T1 (ug/ml)			T2 (ug/ml)			T3 (ug/ml)			T4 (ug/ml)			T5 (ug/ml)			T6 (ug/ml)			T7 (ug/ml)			T8 (ug/ml)			T9 (ug/ml)		
		50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150	50	100	150
1	Bacillus subtilis	00±00	00±00	02±0.05	00±00	00±00	03±0.08	00±00	00±00	04±0.04	00±00	02±0.07	04±0.26	1±0.6	02±0.41	05±0.04	1±00	02±0.51	10±0.47	02±0.61	02±0.11	05±0.05	02±0.03	06±0.10	10±0.50	02±0.18	04±0.03	04±0.55
2	pseudomonas	00±00	00±00	00±00	00±00	00±00	02±0.19	00±00±00	02±0.42	04±0.19	00±00	00±00	04±0.19	0±00	00±00	00±00	2±00	00±00	09±0.62	00±00	03±0.35	04±0.69	03±0.28	08±0.44	11±0.07	00±00	05±0.82	07±0.07
3	Samonell typhi	00±00	00±00	00±00	00±00	02±0.04	03±0.03	00±00	02±0.17	05±0.21	00±00	02±0.32	05±0.09	0±00	05±0.08	08±0.36	1.1±00	05±0.28	05±0.07	00±00	0.0±0.10	06±0.04	00±00	04±0.29	09±0.08	05±0.44	09±0.31	10±0.62
4	Escherichia coli	00±00	00±00	03±0.02	±000	009±00	02±0.18	00±00	00±00	05±0.06	00±00	02±0.03	06±0.01	0±00	02±0.03	04±0.09	1.3±00	05±0.01	08±0.01	04±0.48	0.4±0.68	03±0.29	04±0.07	04±0.51	06±0.22	00±00	06±0.39	09±0.59
5	Proteious vulgaris	00±00	00±00	00±00	00±00	00±00	00±00	00±00	02±0.05	05±0.15	00±00	00±00	04±0.03	±000	03±0.14	04±0.33	1.34±00	03±0.05	00±0	04±0.03	2±0.27	05±0.06	00±00	06±0.02	09±0.83	00±00	05±0.06	05±0.08
6	Candida albicans	00±00	00±00	05±0.13	00±00	00±00	04±0.21	00±00	00±00	04±0.03	04±0.17	03±0.08	06±0.52	00±00	02±0.07	06±0.67	1.33±00	04±0.29	08±0.04	07±0.22	3±0.54	14±0.81	00±00	03±0.06	08±0.28	00±00	07±0.01	07±0.02
7	Aspergillus niger	00+00	00±00	00±00	00±00	00±00	00±00	00±00	02±0.24	03±0.06	00±00	04±0.01	04±0.14	0±00	02±0.58	03±0.05	2±00	02±0.73	02±0.41	04±0.35	1±0.02	06±0.49	02±0.31	02±0.39	05±0.11	02±0.09	05±0.07	06±0.57
8	Fusarium oxyporous	00±00	00±00	00±00	00±00	00±00	00±00	00±00	00±00	02±0.12	00±00	00±00	0202±0.02	00±00	00±00	02±0.023	3±00	02±0.07	09±0.55	05±0.02	1.5±0.51	08±0.35	03±0.59	06±0.04	06±0.64	03±0.80	05±0.18	05±0.03