WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 5, 1263-1271. Research Article ISSN 2277 – 7105

ANTIBACTERIAL SCREENING ON LEAVES OF *ARGYREIA CYMOSA* ROXB., AGAINST PATHOGENIC BACTERIA

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Article Received on
25 May 2014,

Revised on 20 June 2014,
Accepted on 15 July 2014

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ABSTRACT

The present study deals with the aqueous leaf extract of *Argyreia cymosa* (Roxb) were evaluated for antibacterial activity.The aqueous leaf extract of *Argyreia cymosa* is active against *E.coli*, *P.aeruginosa*, *S.epidermidis*, *Proteus* sps. The compound was separated using column chromatography. This compound was also evaluated for antibacterial study. The plant is well reputed in traditional system of medicine; present studies will help in further validation and standardization of the plant.

KEY WORDS: *Argyreia cymosa* leaves, Convolvulaceae, Antibacterial activity, Aqueous extract.

INTRODUCTION

Argyreia cymosa (Roxb) is stem woody, terete, pubescent,leaves deltoid to cordiform,6-8×4-6 cm Chartceous, thins pubescent on both sides, entire acute or obtuse, base truncule or cordate, flowers pinkins in axillary, carymbone cymes, fruit globose, 1.7× 1.4 cm, glabrous, seeds 2or 3 ovate to elliptic black.The paste of leaves applied on wounds and cracks[(1)(5)] . *Argyreia cymosa* bark have antioxidant activity[(2)]and it has various pharmacognostic activity[(3)].

TAXONOMIC CLASSIFICATION

Kingdom:	Plantae
Phylum:	Tracheophyta
Class:	Magnoliopsida
Order:	Solanales

Family:	Convolvulaceae
Genus:	*Argyreia*
Species:	*Argyreia cymosa*

MATERIALS AND METHOD

Collection Of Plant Materials

The plant *Argyreia cymosa* was collected from region of Tiruchirappalli district and identified by local flora. The leaves were separated from the collected plant and dried under shade. After drying, it was powdered and used for our studies.

Antibacterial Screening

Microbial Strains Used

Different microbial strains were used to evaluate the antimicrobial effect of which one were gram positive bacterial strain (i.e) *S.epidermidis* and three were gram negative bacterial strains (i.e) Escherichia coli, Pseudomonas *aeruginosa*, *Proteus*. The strains were obtained from Jamal Mohamed College, Trichy, Tamil Nadu, India and maintained on agar slants.

Chromotography

Column chromatography is used to purify liquids by separating an organic solvents from a mixture of solvent.

Preparation of Leaf Extract

The leaf extract was prepared by grinding the mixture in mortar pestle containing 22 ml of acetone, 3 ml of petroleum ether, and calcium carbonate. The pigments was filtered and mixed with 20 ml of petroleum ether and 20 ml of 10% aqueous sodium chloride solution. The separating funnel was shaken carefully and the lower was allowed to drain in to the beaker.

Preparation of Column

A plug of cotton is placed to the bottom of the column so that silica and soil won't fall out. Slurry of silica was prepared and poured into the column carefully. It is allowed to settle and sand is added.

Loading Of Sample

The sample was added using pasture's pipette carefully above the sand. The eluent is added on top of the sand. The mobile phase slowly flows down through the silica gel column by gravity leaving behind zone of colour and a component was eluted from the column.

Disc Diffusion Method

Disc diffusion method was carried out for antibacterial susceptibility testing according to the standard method to assess the presence of antibacterial activities of the plant extract. Muller Hinton agar (MHA) plates were prepared. Overnight nutrient broth culture of test organisms were seeded over the MHA plates using sterile cotton swab so as to make lawn culture. The discs which had been impregnated with aqueous extracts of leaf and compound separated from column were on the MHA plates with the control disc and subjected to antibacterial screening. The plates were then incubated at 37^0 c for 18 to 24 hours depending on the species of bacteria used in this test. After the incubation , the plates were examined for inhibition zone.

Chi-Square Test

In this study chi-square test was applied. The purpose of chi-square test was to decide whether the set of observed data agrees with the standard antimicrobial disc susceptibility test(NCCLS, 2002).

IR Spectrum Analysis

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. This absorption corresponds specifically to the bonds present in the molecule. The frequency ranges are measured as wave numbers typically over the range 4000-600 cm^2-1.

Procedure

FTIR spectrum of the compound obtain from column chromatography was done using Shimadzu IR Affinity 1 instrument.

RESULTS

The present study showed that aqueous extract of leaves and compound separated from the leaves showed antibacterial activity against the organism *S.epidermidis*, Escherichia coli, Pseudomonas aeruginosa, *Proteus*.

Table 1. Zone of inhibiton formed by aqueous extract of leaf *Argyreia cymosa* against bacterial strains

S.NO	Sample	Bacterial strains	µg	Zone of inhibition in Diameter (mm)		$X^2=\sum[(O-E)^2]/E$
				Standard value	Observed value (Aqueous)	Observed crude
1	*Argyreia cymosa* leaf powder	*E.coli*	128µg	20	19	0.05
2		*P.aeruginosa*	128µg	20	19	0.05
3		*Proteus*	128µg	20	17	0.45
4		*S. epidermidis*	128µg	20	13	2.45

Table value x^2 (0.05) = 3.841 Chi-square value significance at 5% level

Table 2. Zone of inhibition formed by compound separated from column

S.no	Bacterial strains	Zone of inhibition(mm)
1	*E.coli*	11
2	*P.aeruginosa*	14
3	*Proteus*	12
4	*S. epidermidis*	-

Table 3. Infrared spectrum analysis by *Argyreia cymosa* leaf powder(crude)

S.no	Peak value	Stretching	Interpretation
1	470.63	-	Benzene
2	534.28	C-Br Stretching	Bromine
3	671.23	C-Br Stretching	Bromine
4	779.24	C=C Stretching	Hydro carbon
5	1022.27	C-O Stretching	Ethers
6	1047.35	C-O Stretching	Ethers
7	1105.21	C-F Stretching	Halogen
8	1149.57	C-O Stretching	Ethers
9	1244.09	C-O Stretching	Ethers
10	1323.17	N=O Stretching	Nitrogroup
11	1381.03	C-O Stretching	Phenols
12	1404.18	C-O Stretching	Phenols
13	1440.83	C-C Stretching	Aromatics
14	1525.69	N=O Stretching	Nitrogroup
15	1631.78	C=C Stretching	Alkenes
16	1635.00	C=O Stretching	Alkenes
17	1730.15	C=O Stretching	Esters
18	1807.30	C=O Stretching	Anhydrides
19	1853.59	C=O Stretching	Anhydrides
20	1876.74	C=O Stretching	Esters

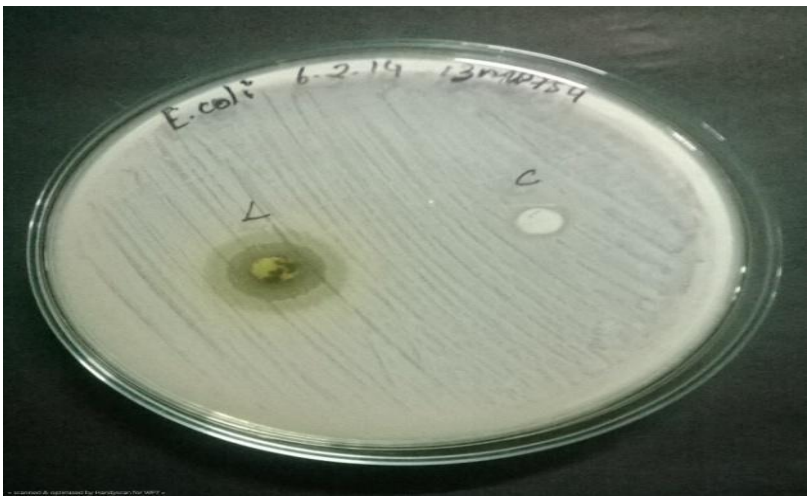
21	1901.81	C=O Stretching	Carboxylic acid
22	1930.74	C=O Stretching	Carboxylic acid
23	2370.51	C=C Stretching	Alkenes
24	2852.72	O-H Stretching	Carboxylic acid
25	2922.16	O-H Stretching	Carboxylic acid
26	3408.22	N-H Stretching	Amides
27	3697.54	O-H Stretching	Free OH group
28	3782.41	N-H Stretching	Amines

Table 4. Infrared spectrum analysis by leaf *Argyreia cymosa* powder(compound)

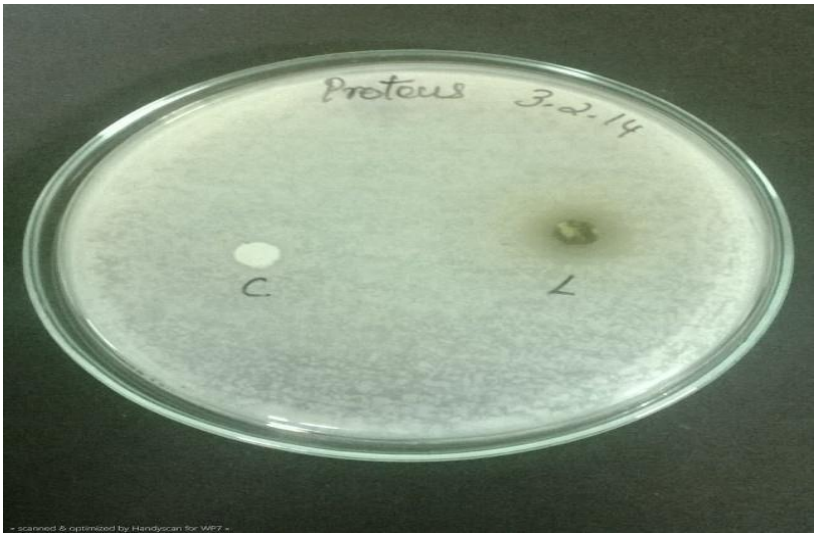
S.no	Peak value	Stretching	Interpretation
1	441.70	-	Benzene
2	601.79	C-H Bending	Alkynes
3	665.44	C- H Bending	Alkynes
4	900.76	S-S Stretching	Esters
5	1016.49	C-N Stretching	Amines
6	1074.35	S=O Stretching	Sulfone
7	1379.10	S=O Stretching	Sulfate
8	1591.27	NH_2 Scissoring	Amines
9	1656.85	C=C Stretching	Alkenes
10	1757.15	C=O Stretching	Anhydrides
11	2270.22	-N=C Stretching	Nitrites
12	2374.37	P-H Stretching	Phosphorous
13	2854.65	CH_3 Stretching	Alkanes
14	2922.16	O-H Stretching	Carboxylic acid
15	3693.68	O-H Stretching	Free OH group
16	3778.55	N-H Stretching	Amines

Table 5. Infrared spectrum analysis by compared with two tables

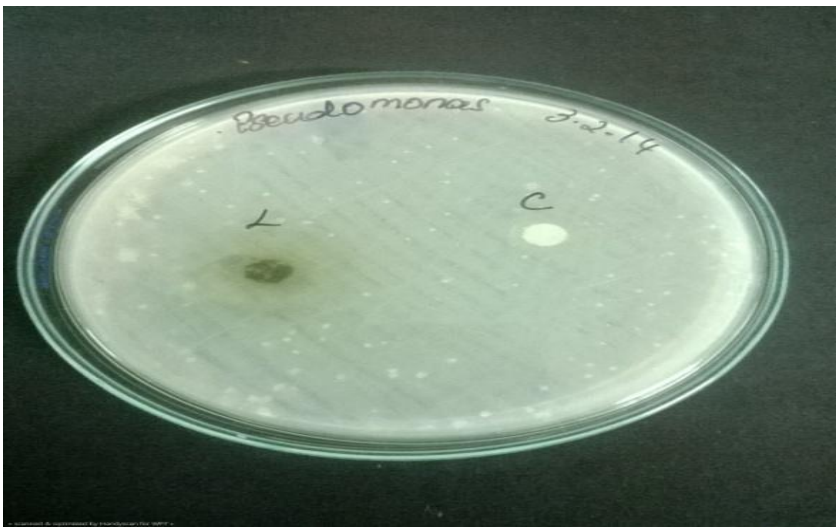
S.no	Peak value	Stretching	Interpretation
1	2922.16	O-h stretching	Carboxylic acid



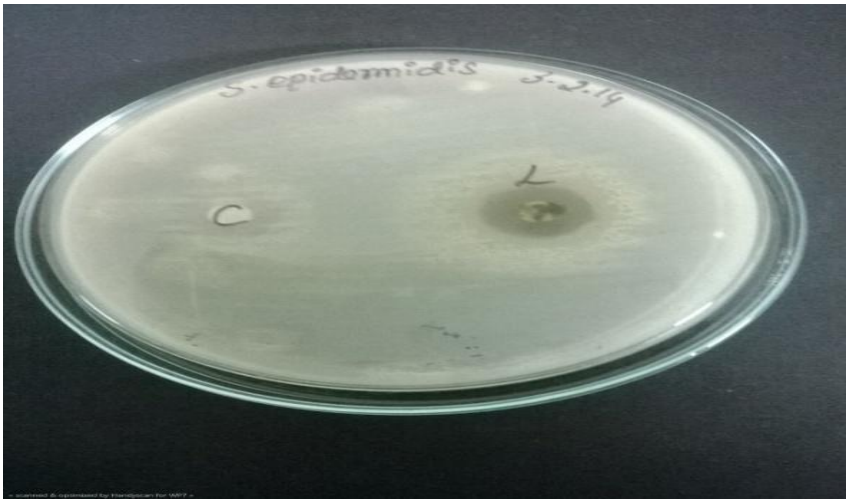
E.coli



***Proteus* sps**

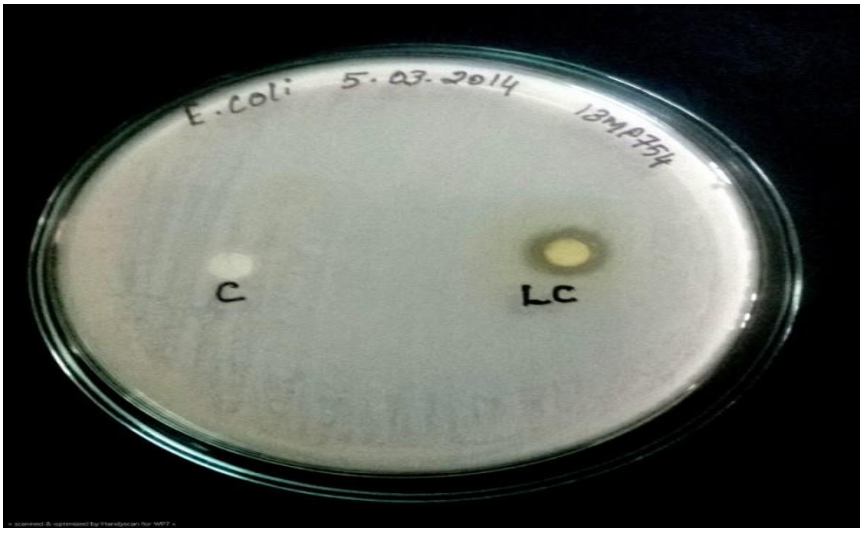


S.epidermidis

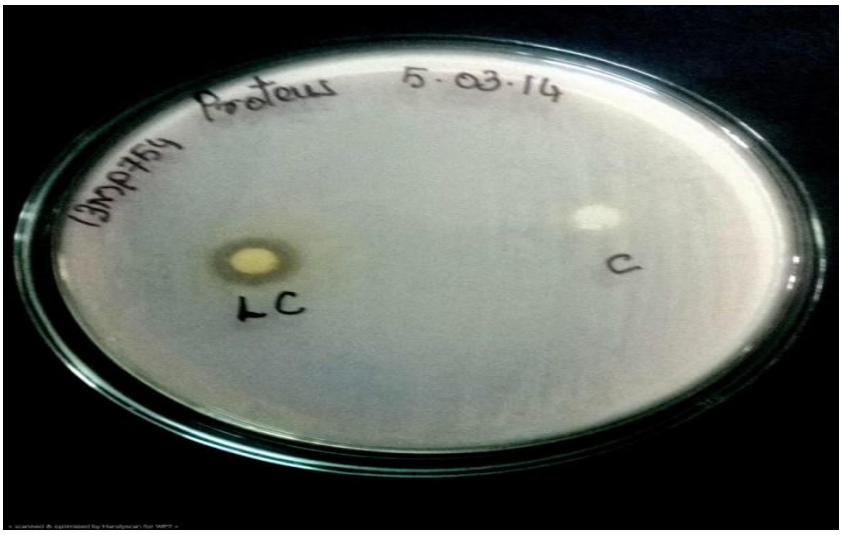


P.aeruginosa

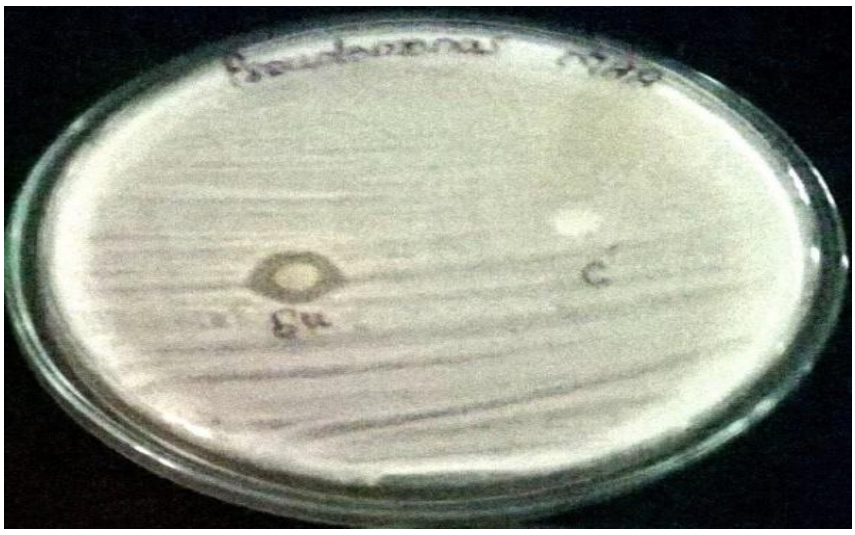
Figure 1.Antibacterial activity of crude extract of *Argyreia cymosa* leaves



E.coli



***Proteus* sps**



Psedomonas aeruginosa

Figure 2. Antibacterial activity of compound of *Argyreia cymosa* leaves

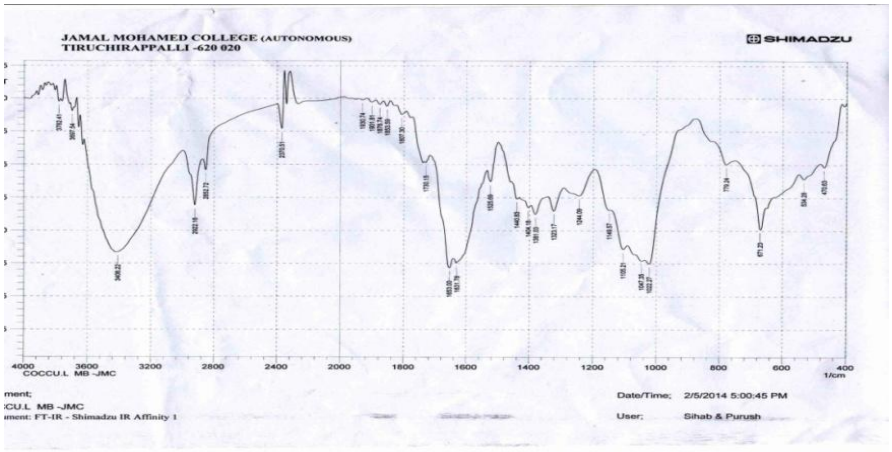


Figure 3. Showing ir results for leaves of *Argyreia cymosa*

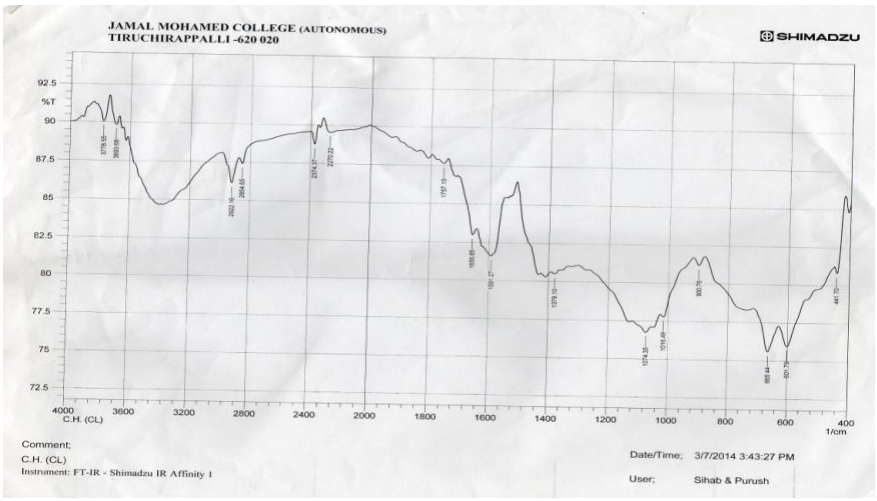


Figure 4. Showing ir results for compound of *Argyreia cymosa*

DISCUSSION

In earlier study an ethnobotanical survey was carried out to collect information on the use of medicinal plants by paliyan tribes in sirumalai hills of eastern ghats[(1)] In earlier study, *Argyreia cymosa* bark extracts were subjected to invivo antioxidant activity with different methods. The petroleum ether extract has shown antioxidant activity in ABTS,nitric oxide, hydroxyl radical (by P-NDA) and lipid peroxidation methods. The ethyl acetate extract has shown antioxidant activity [(2)]. The previous study evaluated that the Cercospora aecidiicola on the aecia of Trochodium sampathens on the leaves of A. cymosa and C.riveae on the aecia of T.ajrekari on the leaves of Rivea ornate, and also C. cladosporiodes on Aecidium oleae on the leaves of Olea diocia.[(3)] In previous study reaveled the leaves of *Argyreia cymosa* and Argyreia capitata with respect to the trichomes , stomata, epidermal characteristics and anatomical features were studied[(4)].In our study, the aqueous extract *Argyreia cymosa* showed

maximum activity against Escherichia coli (19mm), Pseudomonas aeruginosa (19mm), *Proteus* (17mm), *S.epidermidis*(13mm) and the compound of l *Argyreia cymosa* eaves showed activity against Pseudomonas aeruginosa (14), Escherichia coli (11mm), *Proteus* (12mm).

CONCLUSION

The present study of the plant *Argyreia cymosa* showed maximum activity against pathogenic organisms, Hence leaf extract of *Argyreia cymosa* is highly recommended for herbal preparations to the traditional medicinal practioners and for the pharmaceutical industries for the mass scale extraction of therapeutic agents.

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