Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.6968

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **6 (5):** 290-296 (2018)

Research Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Eco- Friendly Management of *Xanthomonas axonopodis* pv. *punicae* Causing Bacterial blight on Pomegranate

A. Chowdappa[1*], A. Kamalakannan[1], S. Kousalya[2], C. Gopalakrishnan[1] and K. Venkatesan[3]

[1]Department of Plant Pathology, Center for Plant Protection Studies,

[2]Vanavarayar Institute of Agriculture, Pollachi,

[3] Department of Horticulture, Tamil Nadu Agricultural University, Coimbatore,

*Corresponding Author E-mail: chowdaagri008@gmail.com

Received: 3.06.2018 | Revised: 30.07.2018 | Accepted: 10.07.2018

ABSTRACT

Bacterial blight of pomegranate (Punica granatum L.) caused by Xanthomonas axonopodis pv. *punicae was considered as serious threat for pomegranate cultivation in recent years throughout India. The use of herbal plant extracts and is gradually becoming a method of choice in the management of plant diseases as these are more ecofriendly and safe. There are several plant extracts such as Neem, Nochi, Turmeric, Ginger, Garlic, Nithyakalyani, Aloe vera etc., used for control Xanthomonas axonopodis* pv. *punicae which helps the organic cultivation of pomegranate. Among several plant extracts screened, aqueous, methanol and ethanol leaf extracts of Coleus and Periwinkle effectively retarded the growth of pomegranate bacterial blight pathogen under in vitro condition.*

Key words: *Bacterial blight of pomegranate, Plant extracts, Aqueous, Methanol, Ethanol leaf extracts*

INTRODUCTION

Pomegranate is one of the important commercial fruit crops belongs to the family Lythraceae. It was grown since ancient times for its fruit, ornamental and medicinal purposes. It is native to Iran and crop has been cultivated extensively in all Mediterranean countries like Spain, Morocco, Egypt, Iran, Afghanistan, Arabia, Baluchistan, and other Mediterranean countries since ancient times. It is widely cultivated in tropical regions of India, Southeast Asia, Malaya, Myanmar, China, Japan, USA (California)[8]. Pomegranate is severely infected with *Xanthomonas axonopodis* pv. *punicae* which causes bacterial blight. This is one of the most destructive diseases of pomegranate. Due to bacterial blight of pomegranate, the yield loss was recorded up to 90 per cent. Infected fruit and twigs are potential sources of primary inoculum. The secondary spread of bacterium is mainly through rain and spray splashes, irrigation water, pruning tools, humans, and insect vectors. Entry is through wounds and natural openings.

Cite this article: Chowdappa, A., Kamalakannan, A., Kousalya, S., Gopalakrishnan, C. and Venkatesan, K., Eco- Friendly Management of *Xanthomonas axonopodis* pv. *punicae* Causing Bacterial blight on Pomegranate, *Int. J. Pure App. Biosci.* **6(5):** 290-296 (2018). doi: http://dx.doi.org/10.18782/2320-7051.6968

To control bacterial blight of pomegranate, there are several management practices has been developed, the present objective was concentrated on eco-friendly management to promote the organic cultivation of pomegranate and reduce the pesticide usage.

MATERIAL AND METHODS

Preparation of aqueous plant extracts

Different parts of the medicinal plants were used for *in vitro* screening. Fifty grams of leaves, rhizome was collected from botanical garden, TNAU, Coimbatore and was washed thoroughly under tap water to remove soil and dust particles. It was then dried under shade and was ground with 50 ml of sterile distilled water using sterile pestle and mortar. The extracts were passed through double layered cheese cloth, then through Whatman No.1 filter paper. The extracts were then centrifuged at 5000 rpm for 20 minutes and the filtrate was collected separately. Finally the filtrate was passed through syringe filter of 0.2 µm pore size for sterilization. This filtrate served as 100 per cent standard solution. Filtrate was diluted to 5 per cent and 10 per cent concentration using sterile distilled water. The standard solution was stored at 4°C for further use[6].

List of medicinal plants used against *Xanthomonas axonopodis* pv. *punicae*

S.No.	Common name	Scientific name	Parts used
1.	Neem	*Azadirachta indica*	Leaves
2.	Nochi	*Vitex negundo*	Leaves
3.	Turmeric	*Curcuma longa*	Rhizome
4.	Ginger	*Gingiber officinalis*	Rhizome
5.	Garlic	*Allium sativum*	Clove
6.	Nithyakalyani	*Vinca rosea*	Leaves
7.	Aloe vera	*Aloe vera*	Stem
8.	Omavalli	*Coleus aromaticus*	Leaves
9.	Chilli	*Capsicum annum*	Fruit
10.	Pepper	*Piper nigrum*	Seed
11.	Pungam	*Pongamia sp*.	Leaves
12.	Palmarosa	*Cymbopogan martinii*	Leaves
13.	Lemongrass	*Cymbopogan citrates*	Leaves
14.	Guava	*Psidium gujava*	Leaves
15.	Eucalyptus	*Eucalyptus globus*	Leaves
16.	Prosophis	*Prosopis juliflora*	Leaves
17.	Adathoda	*Adothoda vesica*	Leaves
18.	Vilvam	*Aegle marmelos*	Leaves
19.	Tulsi	*Oscimum sanctum*	Leaves
20.	Siriyanangai	*Andrographis paniculata*	Leaves

In vitro screening of plant extracts against *Xanthomonas axonopodis* pv. *punicae*

Forty-eight hours old bacterial culture of *X. axonopodis* pv. *punicae* was seeded into the nutrient agar medium at lukewarm temperature (40°C), mixed well and poured into sterile Petri plates. Filter paper discs of 5 mm diameter was soaked in plant extracts and placed on four sides of the Petri plates. Three replicates were maintained for each extract at 5 per cent and 10 per cent separately. Filter paper discs dipped in sterile water served as control and incubated at room temperature (28± 2°C) for 2 days the zone of inhibition was measured in cm after 48 hours of incubation. The experiment was conducted in completely randomized block design with three replications.

Preparation of solvent extract from medicinal plants

Leaves of the plants were thoroughly washed and dried under shade at the room temperature (20 ± 2°C). The dried leaves were then ground to a fine powder in an electric grinder. Stock solutions of the extract were prepared by adding ground leaf powder to 200 ml of each solvent (w/v, 1 g/ 10 ml). Methanol and ethanol solvents were used for extraction. Prepared extracts were then shaken for 6 hours for homogenous mixing of ground leaf powder in the solvent. After that each extract was passed through Whatmann filter paper no.1. Final filtrate was then concentrated to 10 per cent crude extract on a mini rotary evaporator under vacuum at 20°C and was utilized for the experiments[2].

Efficacy of solvent extracts of plant products against *Xanthomas axonopodis* pv. *punicae*

Forty eight hours old bacterial culture was seeded into the nutrient agar medium at lukewarm temperature (40°C), mixed well and poured into sterile Petri plates. Filter paper discs of 5 mm diameter was soaked in solvent extracts and placed on four sides of the Petri plates. The plates were incubated at three replicates were maintained for each extract at 5 per cent and 10 per cent separately. Filter paper discs dipped in sterile water served as control and incubated at room temperature (28± 2°C) for 2 days the zone of inhibition was measured in cm after 48 hours of incubation.

RESULTS

In vitro* screening of plant extracts (aqueous extract) against *Xanthomonas axonopodis* pv. *punicae

The results revealed that most of the plant extracts inhibited the pathogen except *Vitex negundo*. Higher concentration of aqueous plant extract showed high inhibition compared lower concentration in all three replications. Among the plant extracts, Coleus exhibited maximum inhibition zone (3.47 cm) followed by tulasi (2.63 cm). Other isolates were also recoded inhibition zone to a lesser extent at 5 per cent concentration. In case of 10 per cent concentration, periwinkle exhibited maximum inhibition (4.90 cm) followed by the Coleus (3.53 cm). Other isolates also recoded inhibition zone to a lesser extent (Table 1 and Plate 1).

In vitro* screening of methanol plant extracts against *Xanthomonas axonopodis* pv. *punicae

The results revealed that most of the plant extracts inhibited the pathogen except *Vitex negundo* at 5 per cent concentration. The Higher concentration of methanol plant extracts showed higher inhibition compared lower concentration. Among the plant extracts, Coleus exhibited maximum inhibition zone (6.13 cm) followed by tulasi (5.12 cm). Other isolates also recoded inhibition zone to a lesser extent at 5 per cent concentration. In case of 10 per cent concentration, Coleus exhibited maximum inhibition (7.15 cm) followed by the periwinkle (6.60 cm). Other isolates were also recoded inhibition zone to a lesser extent (Table 2, Fig. 1 and Plate 2).

In vitro* screening of plant extracts (Ethanol extract) against *Xanthomonas axonopodis* pv. *punicae

Ethanol extract of all plant species inhibited the growth of *X. axonopodis* pv. *punicae* under *in vitro* at 5 and 10 per cent concentrations. The 5 per cent ethanol extracts showed lesser inhibition than 10 per cent. Among the eight plant extracts tested, Coleus recorded 5.13 cm and 6.20 cm inhibition zones at 5 and 10 per cent concentrations, respectively. This was followed by tulasi with the inhibition zone 4.22 cm and 5.47 cm at 5 and 10 per cent concentrations, respectively. All other ethanol extracts showed lesser inhibitory effect on the growth of *X. axonopodis* pv. *punicae* (Table 3 and Plate 3).

DISCUSSION

Effect of plant extracts on the growth of *Xanthomonas axonopodis* pv. *punicae* The use of herbal plant extracts and is gradually becoming a method of choice in the management of plant diseases as these are more ecofriendly and safe. The antimicrobial activity of herbal plant extracts were studied by several authors includes Mahesh and Satish[7] and Kagale *et al.*[5]. Gargade and Kadam[3], studied the antibacterial activity of bark extracts of *Acasia nilotica*, *Datura metal*,

by using different solvents against *X. axonopodis* pv. *punicae.* They observed aqueous extracts more effectively inhibited the *X. axonopodis* pv. *punicae* growth than the other solvents, among the different solvents more inhibitory effect was observed on the methanol. Alane and swamy[1]. studied the inhibitory effect of aqueous ethanol extract, acetone extracts of different plants against the growth of *X. axonopodis* pv. *punicae* and found that solvent extracts provide good inhibition than the aqueous extracts. Jayachitra and Chitra[4]. studied antibacterial activity of Coleus leaf extracts using different solvents against *Bacillus subtilis, Pseudomonas fluoreoscens, Escherichia coli* and *Streptococcus nimonic.* Suman kumar *et al.*[9], reported that various phytochemical like alkaloids, safonins, tannins, flavonoids, cumonins, phenols present in the Coleus leaf extracts are responsible for its antimicrobial activity. Valarmathy *et al.*[10], studied that antimicrobial activity of ethanol extracts of various plants against *Bacillus subtilis, Escherichia coli* and found that ethanol extracts of Neem and *Cynodon dactylon* inhibited the growth of bacteria. In the present study inhibition effect of *X. axonopodis* pv. *punicae* might be due to various phytochemicals present in the Coleus and periwinkle.

Table 1. *In vitro* screening of plant extract (Aqueous extract) against *Xanthomonas axonopodis* pv. *punicae*

S. No.	Common Name	Botanical Name	Inhibition Zone (cm)*	
			5 %	10 %
1	Pepper	*Piper nigrum*	2.03 (1.59)^{c}	2.93 (1.85)^{b}
2	Periwinkle	*Catharanthus roseus*	3.10 (1.90)^{a}	4.90 (2.32)^{a}
3	Seemai karuvel	*Prosopis juliflora*	1.93 (1.56)^{c}	2.17 (1.63)^{c}
4	Notchi	*Vitex negundo*	0.00 (0.71)^{d}	2.70 (1.79)^{b}
5	Coleus	*Coleus forskohlii*	3.47 (2.01)^{a}	3.53 (1.99)^{a}
6	Pongamia	*Pongamia pinnata*	2.17 (1.73)^{c}	2.5) (1.63)^{d}
7	Palmarosa	*Cymbopogon martinii*	1.80 (1.54)^{c}	1.87 (1.52)^{d}
8	Tulasi	*Ocimum sanctum*	2.63 (1.77)^{b}	2.97 (1.86)^{b}
9	Control	*Sterile Distilled water*	0.00 (0.71)^{d}	0.00 (0.71)^{e}
SEd			**0.14**	**0.15**
CD (0.05)			**0.31**	**0.31**
CV%			**12.13**	**10.92**

*- Means of three replications

Values in the parentheses are square root transformed values

Table 2. *In vitro* screening of methanol extracts of plants against *Xanthomonas axonopodis* pv. *punicae*

S. No.	Common Name	Botanical Name	Inhibition Zone (cm)*	
			5 %	10 %
1	Pepper	*Piper nigrum*	3.12 (1.90)^{c}	4.23 (2.18)^{d}
2	Periwinkle	*Catharanthus roseus*	4.47 (2.23)^{b}	6.60 (2.66)^{a}
3	Seemai karuvel	*Prosopis juliflora*	4.45 (2.22)^{b}	4.49 (2.23)^{d}
4	Notchi	*Vitex negundo*	0.00 (0.71)^{d}	5.52 (2.45)^{b}
5	Coleus	*Coleus forskohlii*	6.13 (2.57)^{a}	7.15 (2.77)^{a}
6	Pongamia	*Pongamia pinnata*	4.52 (2.24)^{b}	4.91 (2.33)^{e}
7	Palmarosa	*Cymbopogon martinii*	4.03 (2.13)^{c}	4.02 (2.13)^{e}
8	Tulasi	*Ocimum sanctum*	5.12 (2.37)^{b}	5.16 (2.38)^{d}
9	Control	*Sterile Distilled water*	0.00 (0.71)^{d}	0.00 (0.71)^{f}
SEd			**0.13**	**0.11**
CD (0.05)			**0.28**	**0.24**
CV%			**8.70**	**6.55**

*- Means of three replication

Values in the parentheses are square root transformed values

Table 3. *In vitro* screening of ethanol extracts of plants against *Xanthomonas axonopodis* pv. *punicae*

S. No.	Common Name	Botanical Name	Inhibition Zone (cm)*	
			5 %	10 %
1	Pepper	*Piper nigrum*	2.63 (1.90)^{c}	3.33 (2.18)^{c}
2	Periwinkle	*Catharanthus roseus*	4.13 (2.23)^{a}	6.10 (2.66)^{a}
3	Seemai karuvel	*Prosopis juliflora*	3.67 (2.22)^{c}	3.73 (2.23)^{b}
4	Notchi	*Vitex negundo*	0.00 (0.71)^{d}	4.00 (2.45)^{b}
5	Coleus	*Coleus forskohlii*	5.13 (2.57)^{a}	6.20 (2.77)^{a}
6	Pongamia	*Pongamia pinnata*	3.50 (2.24)^{b}	3.90 (2.33)^{c}
7	Palmarosa	*Cymbopogon martinii*	2.57 (2.13)^{c}	2.77 (2.13)^{c}
8	Tulasi	*Ocimum sanctum*	4.22 (2.37)^{a}	5.47 (2.38)^{a}
9	Control	*Sterile Distilled water*	0.00 (0.71)^{d}	0.00 (0.71)^{d}
SEd			**0.16**	**0.11**
CD (0.05)			**0.35**	**0.23**
CV%			**11.86**	**6.68**

*- Means of three replication

Values in the parentheses are square root transformed values

Fig. 1: *In vitro* screening of methanol extracts of plants against *Xanthomonas axonopodis* pv. *punicae*

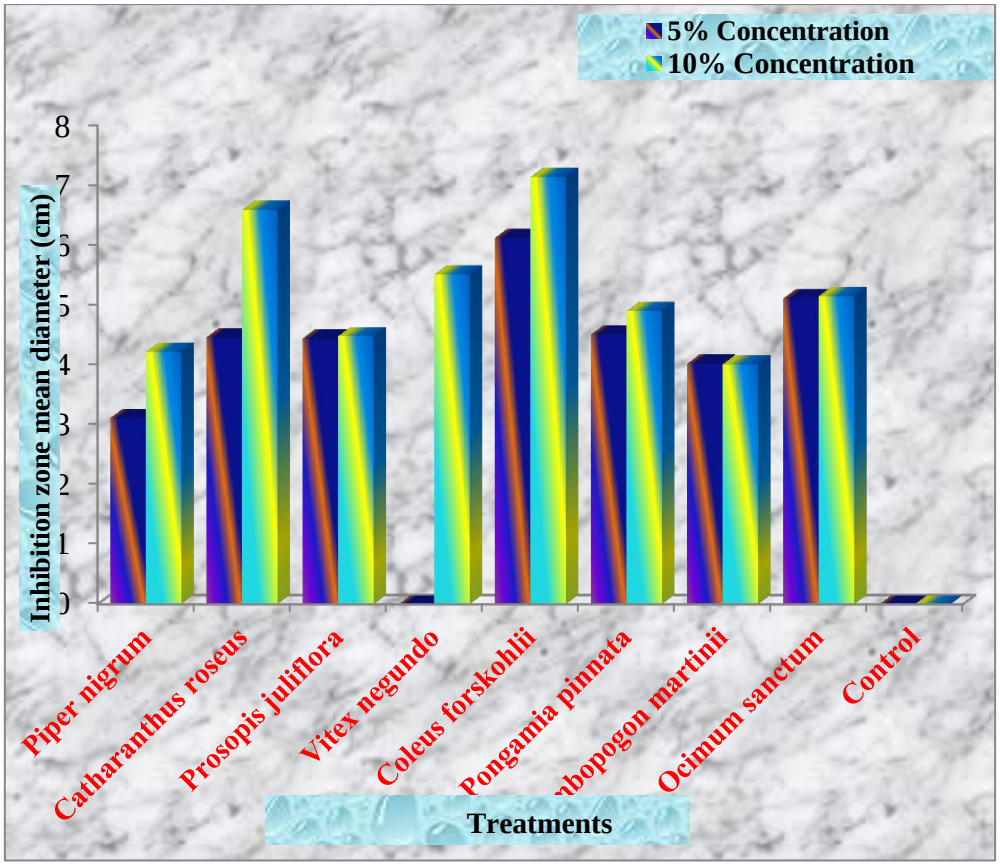


Plate 1. *In vitro* screening of plant extracts (aqueous extract) against *Xanthomonas axonopodis* pv. *punicae*

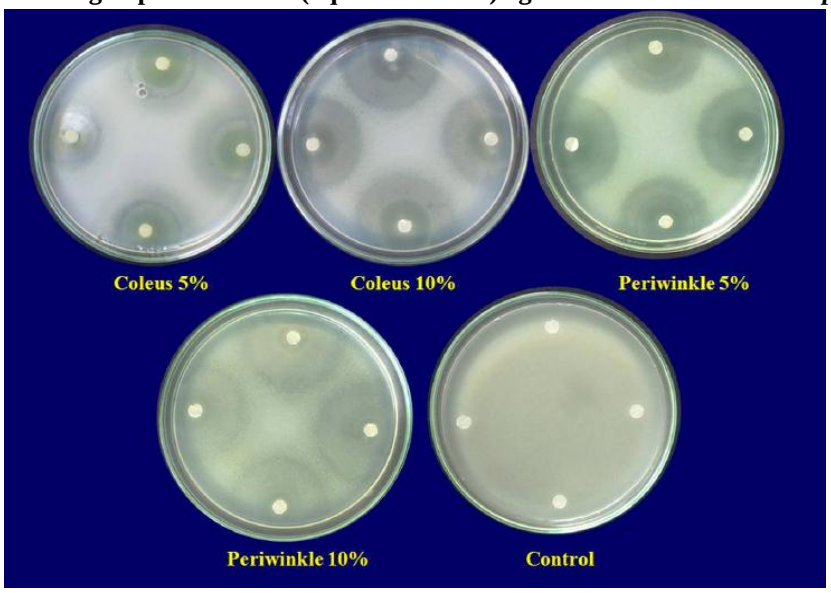


Plate 2. *In vitro* screening of methanol plant extracts against *Xanthomonas axonopodis* pv. *punicae*

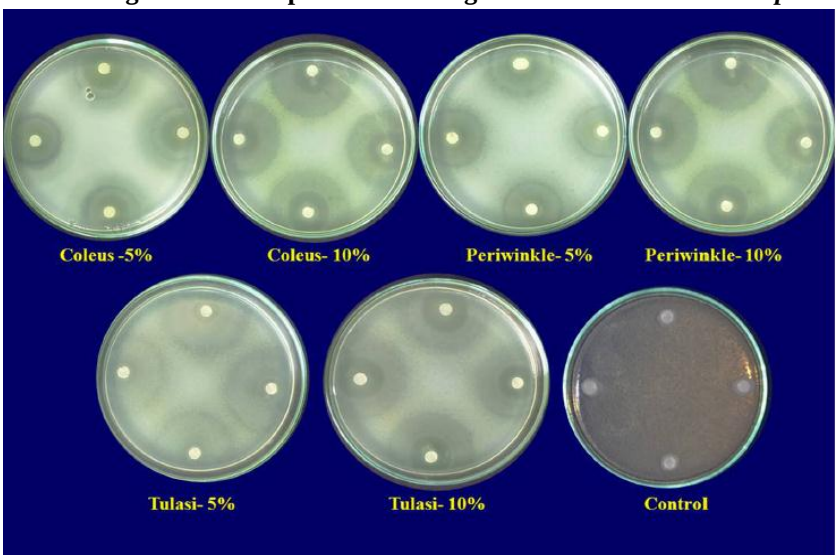
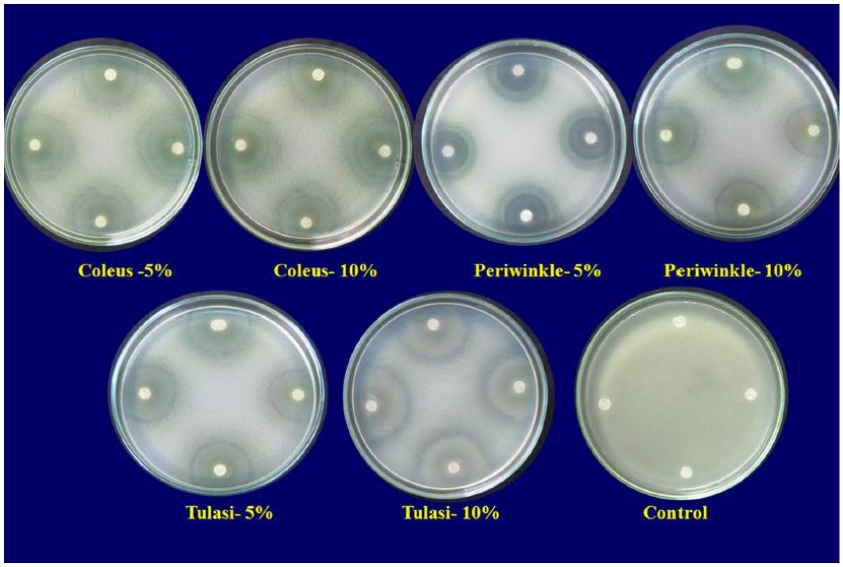


Plate 3. *In vitro* screening of plant extracts (Ethanol extract) against *Xanthomonas axonopodis* pv. *punicae*



REFERENCES

1. Alane, S. K. and Swami, C. S., Antibacterial activity of plant extracts against *Xanthomonas Axonopodis* pv. *punicae* causing bacterial blight of Pomegranate. (*Punica Granatum* L.) **6(2):** 720-721 (2016).
2. Digvijay, Bhardwaj, S. and Kumar,P., Antibacterial activity of *Juniperus communis* and *vetex negundo* against *Xanthomonas axonopodis* pv. *punicae in vitro*.*World Journal of Pharmaceutical Research* **3(10):** 1489-1500 (2014).
3. Gargade, V. A. and Kadam, D. G., Antibacterial activity of some herbal extracts and traditional biocontrol agents against *Xanthomonas axonopodis punicae*. *Journal of International Academic Research for Multidisciplinary* **2(3):** 16-25 (2014).
4. Jayachitra, J. and Chitra, M., Antibacterial activity of *Coleus aromaticus* L. *World Journal of Pharmacy and Pharmaceutical Sciences* **4(5):** 1026-1030 (2015).
5. Kagale, S., Marimuthu, T., Thayumanavan, B., Nandakumar, R. and Samiyappan, R., Antimicrobial activity and induction of systemic acquired resistance in rice by leaf extract of Datura metel against *Rhizoctonia solani* and *Xanthomonas oryzae* pv. *oryzae*. *Physiological and Molecular Plant Pathology* **65:** 91 (2004).
6. Kulshrestha, S., Chaturvedi, S., Jangir, R. and Agrawal, K., *In vitro* Evaluation of Antibacterial Activity of Some Plant Leaf Extracts against *Xanthomonas axonopodis* pv. *phaseoli* isolated from Seeds of Lentil (Lens culinaris Medik.). *International Research Journal of Biological Sciences* **4(7):** 59-64 (2015).
7. Mahesh, B. and Satish, S., Antimicrobial activity of some Important Medicinal plants against Plant and Human Pathogens, *World Journal of Agricultural Sciences* **4:** 839-843 (2008).
8. Raghuwanshi, K. S., Hujare, B. A., Chimote, V. P. and Borkar, S. G., Characterization of *Xanthomonas axonopodis* pv. *punicae* isolates from western Maharashtra and their sensitivity to chemical treatments. *The Bioscan* **8(3):** 845-850 (2013).
9. Suman Kumar R, Ramchandra Reddy P, Gangadhar Rao S and Nethaji K., Phytochemical Screening from leaf extracts of the plant *Coleus forskohlii* (brig). *World journal of pharmacy and pharmaceutical sciences* **3(4):** 829-835 (2014).
10. Valarmathy, K., Gokulakrishnan, M., Kausar, M. S. and Paul, K., A study of antimicrobial activity of ethanolic extracts of various plant leaves against selected microbial species, *International Journal of Pharma Sciences and Research* **1(8):** 293-295 (2010).