Comparative analysis of antibacterial activity of methanolic & aqueous extracts of Nyctanthes-arbor-tristis against X. citri and X. campestris

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Abstract **- The aim of present study was to evaluated comparative analysis of antibacterial efficacy of Nyctanthes-arbor-tristis against X. citri and X. campestris on different level of concentrations by using agar well diffusion method. In this research work we have used two parts of plant like leaf and stem, and we have noted some effective results of methanolic as well as aqueous extract of N.-arbor-tristis against X. citri and X. campestris. The highest zone of inhibition i.e. 16.33±0.60 mm was recorded in methanolic leaf extract of N.-arbor-tristis at 100 µl of concentration against X. citri. So, from this study it can be suggested that methanolic leaf extract of N.-arbor-tristis could be used as a substitute for chemical pesticides and antibiotics.**

Index Terms **- N.-arbor-tristis, X. campestris, X. citri, antibacterial activity, methanol, aqueous.**

Abbriviation- **mm (milli metre), µl (micro litre), ZOI (zone of inhibition), hrs (hours), % (percent), gm (gram), ºC (degree Celsius), MHA (Muller-Hinton Agar), DMSO (Dimethyl sulphoxide), min (minute).**

INTRODUCTION

Recently medicinal plants have paying attention for being potent sources of biologically active compounds or substances (Peyvast and Khorsandi, 2007; Miladi and Darnak, 2008; Malik et al., 2012; Ahmad et al., 2014). Now a day the frequency of infectious microorganisms on effecting human life has increased worldwide. Many microorganisms are being resistant to synthetic chemicals or pesticides (Nagumanthri et al., 2012). The use of synthetic pesticides to control pests is generally a low-cost treatment but laid down several harmful effects by affecting the human health and damage to environment (Bruce, 2010). However, there is a need to discover new generation of bio pesticides against resistant pathogens, which are ecofriendly in nature (Kavitha and Satish, 2011). Nyctanthes-arbor-tristis, is a large shrub, known as Parijata or Harshringar. It is native to many parts of India, widely disseminated in outer Himalayan ranges from China to Nepal, Assam, Burma, Bengal and Central India to Godavari. Phytochemical examination of N. arbor-tristis revealed the presence of various active metabolites like mannitol, sitosterol, flavanolglycosides, astragalin, nicotiflorin, oleanolic acid, nyctanthic acid, tannic acid, ascorbic acid, methyl salicylate, an amorphous glycoside, an amorphous resin, benzoic acidnyctanthin, glucose, carotenoids, β-mono gentiobioside ester of α-crocetin, β-mono gentiobioside-β-D mono glucoside ester of α-crocetin, and β-digentiobioside ester of α-crocetin etc. (Singh and Jindal, 1985; Mathuram et al., 1997; Jain and Mittal, 2011). Xanthomonas citri and Xanthomonas campestris are two fundamental plant pathogen, widely effect citrus, mustard, beans, cotton etc business all over the world and distributed in Asia, South America, The United States, and parts of Oceania and a few islands of the African landmass. The symptomatically X. citri makes sores on leaves, twigs and natural product which results defoliation, untimely organic product abscission and flawed foods grown from the ground passing of the plant. The characteristic "black rot" symptoms in vascular tissues of cruciferous, is generally developed by the variant of X. campestrispv. Campestris (Xcc) and developed on the veins and angular necrotic sores at the foliar edge (Alvarez, 2000). Hence, the main aim of the present study was to select commonly available important medicinal plant i.e. N.-arbor-tristis and to evaluate the efficacy of plant extracts to control the growth of X. citri and X. campestris.

MATERIAL AND METHODS

Collection and authentication of medicinal plants

Different parts of plant like leaves and stems were collected from the campus of Jiwaji University, Gwalior (M.P.) India, and were authenticated by the experts of School of Studies in Botany, Jiwaji University, Gwalior, (M.P).

Preparation of plant extracts and Percent yield

Fresh leaf and stem parts from selected plants were collected and washed 2-3 times with running tap water and allowed to shade drying at room temperature. After the process of natural drying, the dried plant materials were powdered using a clean pestle mortar. The powder was filled in separate airtight containers and stored in a dry place at room temperature until analysis. Plant materials (powder) were extracted in the solvent like methanol and double distilled water by using the method of Harborne, (1984) and Roopashree et al., (2008).

Fifteen gm of dried powdered plant materials were extracted in soxhlet apparatus using solvent methanol and double distilled water. The extraction was done for 48 hrs and after extraction the crude extract was evaporated at 40℃ on hot water bath. After evaporation process obtained extracts were weighed. Yield of extract was calculated by using the formula. The extracts were collected and stored at 4℃ in sterile airtight containers for further analysis.

$$Yield\ (\%) = \frac{W_1 \times 100}{W_2}$$

'W_1' denotes the weight of the extract after lyophilization of solvent and

'W_2'indicates the weight of the powdered material.

Antibacterial assay

Standard pure cultures i.e. *Xanthomonas citri* (# ITCC No. BN 0001) and *Xanthomonas campesris* (# ITCC No. BH 0001), were procured from Indian Agriculture Research Institute, Pusa, New Dehli, India, were maintained by sub-culturing method on Nutrient agar media (Hi Media) and Nutrient broth (Hi Media). The antibacterial activity of plant extracts was determined by agar well diffusion method (Magaldi and Mata-Essayag, 2004). Commercially available dehydrated MHA media (Hi media) was used in antibacterial study and prepared according to the manufacturer's directions. The leaf and stem extract of *M. arvensis* were dissolved in DMSO (Dimethyl Sulphoxide) in a concentration of 100 mg/ml (stock solution). In this method wells were made in MHA medium using sterile cork borer after the spreading of bacteria. The method is suitable for organisms, which grow rapidly at 35-37℃ in 24 hrs. The previously inoculated bacterial strain was spread on MHA. After few minutes five wells were made in each Petri plate and loaded with different concentration (20, 40, 60, 80 and 100 µl). Plates were incubated at 37℃ for 24 hrs. After the diffusion of extracts, Petri plates were left at room temperature for about 30 min and then incubated at 37℃ for 24 hrs. The diameter of zone of inhibition of bacterial growth around each well was measured and the susceptibility was determined by using Hi-media zone scale. Experiments were carried out in triplicates.

STATICALLY ANALYSIS

In this study the results were expressed as the mean$\pm$ standard deviation and to check the significance of data, One way ANOVA was used at the level of 0.05 ($p < 0.05$).

RESULTS

The present study was carried out to check the comparative antibacterial efficacy of leaves and stem of two selected medicinal plant viz. *Nyctanthes arbor-tristis* and *Tinospora cordifolia* against the bacteria *Xanthomonas citri*. The plants were collected shade dried, powdered and were extracting using soxhlet apparatus for phytochemical and antibacterial analysis. Table 1 showed that 6.04% and 6.74% yield was showed in methanolic extract of *N.arbor- tristis* (leaf and stem). While 7.46% and 6.09% yield was recorded in aqueous extract. The highest ZOI of *N. arbor-tristis* (leaf and stem) at 100 µl of concentration against *X. citri* was 16.33±0.60 mm and 12.32±0.60 mm respectively (Table 2). At 80 µl of concentration the ZOI was 14.22±0.60 mm and 10.5±0.75 mm. 11±0.60 mm and 9.55±0.75 mm was recorded at 60 µl of concentration and 10.66±0.75 mm and 8.22±0.75mm was noted at 40 µl of concentration against *X. citri*. At 20 µl of concentration the ZOI was 9±0.60 mm and 4.55±0.75 mm. 14.63±0.60 mm ZOI was recorded at 100 µl of concentration in methanol stem extract of *N. arbor-tristis* against *X. campestris* and 10±0.90 mm was showed in leaf. At 80 µl of concentration the ZOI was 13±0.60mm and 9±0.90

mm. 12±0.75mm and 8.33±0.60mm ZOI was recorded in stem and leaf extracts of *N. arbor-tristis* at 60 µl of concentration. 4±0.60 mm and10.66±0.75 mm ZOI was recorded at 40 µl of concentration. At 20 µl of concentration the ZOI was 2±0.60 mm and 8±0.60 mm. 60 µl, 80 µl and 100 µl of concentration was found to be significant at the level of 0.05($p < 0.05$) against *X. citri* and *X. campestris*.

The maximum ZOI of *N. arbor-tristis* (stem and leaf) at 100 µl of concentration against *X. citri* were 12.32±0.75 mm and 7.33±0.60 mm respectively (Table 3). in aqueous extract. At 80 µl of concentration the ZOI was 6.22±0.60 mm and 11.5±0.60 mm. 4.57±0.62 mm and 11±0.25 mm was recorded at 60 µl of concentration. At 40 µl of concentration the ZOI was 2.25±0.22 mm and 10.5±0.60mm. The stem of *N. arbor-tristis* showed 8.55±0.30mm ZOI at 20 µl of concentration and leaf did not show any activity. At 100 µl of concentration the leaf and stem of *N. arbor-tristis* showed 12.63±0.60mm and 11±0.90 mm ZOI against *X. campestris* and 10.55±0.90 mm and 11±0.60 mm was recorded at 80 µl of concentration. At 60 µl of concentration 8.33±0.60 mm and 10±0.75 mm ZOI was noted. 6±0.60 mm and 9.66±0.75 mm ZOI was showed at 40 µl of concentration. At 20 µl of concentration the ZOI was 4±0.60 mm and 8±0.60 mm against *X. campestris*. 80 and 100µl of concentration were considered to be significant at the level of 0.05($p < 0.05$) and at 60 µl the values of stem of *N. arbor-tristis* was found to be significant.

Table 1. Percentage Yield of *N. arbor-tristis* (Leaf and Stem) using methanol and aqueous solvent.

Plant part used	Extract	Extraction Yield (%)
Leaf	Methanol	6.04
Stem	Methanol	6.74
Leaf	Aqueous	7.46
Stem	Aqueous	6.09

Table 2. Comparative antibacterial activity of methanolic extract of N. arbor-tristis against X. citri and X. campestris

S. No.	Concentration (µl)	Plant part	Zone of Inhibition (mm)	
			X. citri	*X. campestris*
1.	20	Leaf	9±0.60*	2±0.60*
		Stem	4.55±0.75*	8±0.60*
2.	40	Leaf	10.66±0.75*	4±0.60*
		Stem	8.22±0.75*	10.66±0.75*
3.	60	Leaf	11±0.60	8.33±0.60
		Stem	9.55±0.75	12±0.75
4.	80	Leaf	14.22±0.60	9±0.90
		Stem	10.5±0.75	13±0.60
5.	100	Leaf	16.33±0.60	10±0.90
		Stem	12.32±0.60	14.63±0.60

Each presented values were expressed as mean±SD. Mean of triplicate analysis (n=3). Values with symbol * at different concentrations in the table were not considered to be statistically significant at the level of 0.05($p < 0.05$) and rest of the values were significant at the level of 0.05($p < 0.05$).

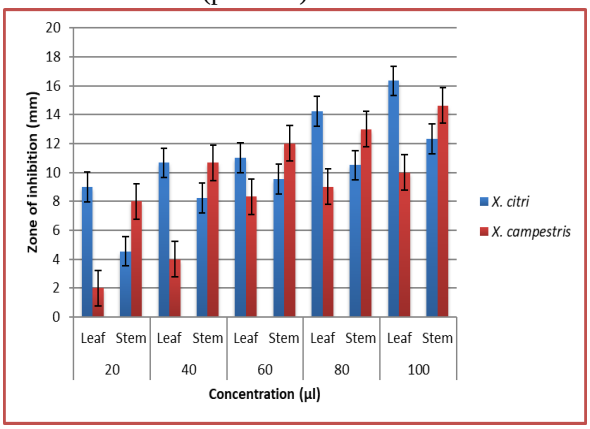

Fig.1 Comparative antibacterial activity of methanolic extract of N. arbor-tristis against X. citri and X. campestris

Table 3. Comparative antibacterial activity of aqueous extract of N. arbor-tristis against X.citri and X.campestris

S. No.	Concentration (µl)	Plant part	Zone of Inhibition (in mm)	
			X. citri	*X. campestris*
1.	20	Leaf	NI	4±0.60*
		Stem	8.55±0.30*	8±0.60*
2.	40	Leaf	2.25±0.22*	6±0.60*
		Stem	10.5±0.60	9.66±0.75
3.	60	Leaf	4.57±0.62*	8.33±0.60*
		Stem	11±0.25	10±0.75
4.	80	Leaf	6.22±0.60	10.55±0.90
		Stem	11.5±0.60	11±0.60
5.	100	Leaf	7.33±0.60	11±0.90
		Stem	12.32±0.75	12.63±0.60

Each presented values were expressed as mean±SD. Mean of triplicate analysis (n=3). Values with symbol * at different concentrations in the table were not considered to be statistically significant at the level of 0.05($p < 0.05$) and rest of the values were significant at the level of 0.05($p < 0.05$).NI = No Inhibition

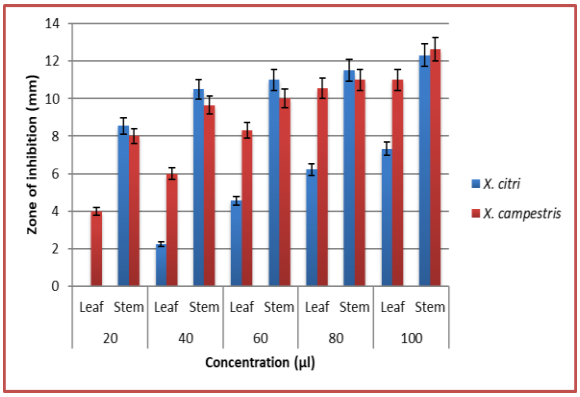

Fig. 2 Comparative antibacterial activity of aqueous extract of N. arbor-tristis against X. citri and X. campestris

DISCUSSION

Since from thousands of year, medicinal plants are being used to treat different diseases of human and plants. Use of synthetic pesticides is cost effective and cause deleterious effect to the environment. Medicinal plants are a good substitute of synthetic pesticide because of the presence of active metabolites. It has been reported by various researchers that medicinal plant extracts showed excellent antibacterial efficacy against plant pathogens. Even at low concentration some plant extracts inhibit the growth of bacteria (Biswas et al., 2013). Chaudhari and Girase, (2017) analysed the extractive value of different solvents like petroleum ether and chloroform and alcohol (1.04%, 1.60%, 10.16%) of Sesbania sesban (L) Merr. And observed significant yield in alcohol. Priya and Ganjewala, (2007) evaluated the ethyl acetate and chloroform extract of Nyctanthesarbor-tristis against Staphylococcus aureu, Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa showed excellent antibacterial activity. Manisha et al., (2009) also studied the antimicrobial potential of Nyctanthes-arbor-tristis. Nyctanthes-arbor-tristis showed good antibacterial activity against both the tested bacteria. Jayalakshmi et al., (2011) reported the presence of flavonoids, terpenoids, tannins steroids, alkaloids and glycosides are responsible for antibacterial activity in plants. Bhagwat and Datar, (2013) also examined the in vitro antibacterial activity of herbal extracts of Garcinia indica, Curcuma aromatica, Glyccyrrhiza glabra, Nyctanthes arbor-tristis and Vernonia anthelmintica against Xanthomonas campestris, Xanthomonas axonopodis pv. Punicae, Erwinia species, Pseudomonas syringae and Xanthomonas citri. In this study the extracts of C. aromatica, G. indica and G. glabra showed minimum bacterial concentration among other tested plant extracts. Ethanolic extract of Nyctanthes-arbor-tristis showed maximum antibacterial activity than aqueous extract against Bacillus subtilis, Staphylococcus aureus, Klebsiella pneumoniae and Proteus mirabilis using agar well diffusion method and used streptomycin as a positive control (Geetha et al., 2014). In Aruveda, Nyctanthes-arbor-tristis is one of the most important plants which possess tremendous medicinal properties (Gulshan et al., 2015). Aggarwal and Goyal, (2013) studied on different solvent extracts of Nyctanthes arbor-tristis and observed that hot water extract showed significant antibacterial activity against tested pathogens. Antibacterial activity of N. arbor-tristis was evaluated by Jain and Singh, (2013) and found maximum ZOI (22.00 mm) by using aqueous extract against Pseudomonas aeruginosa. Antibacterial efficacy of N. arbor-tristis and Nerium oleander was also evaluated by Kumar et al., (2013). Chouhan et al., (2014) also worked on antimicrobial potential of N. arbor-tristis and observed the significant antimicrobial activity in aqueous extract rather than methanolic extract. Observations showed the therapeutic importance of N. arbor-tristis (Gopalkrishnan and Chiranjeev, 2017; Srivastava et al., 2018).

CONCLUSION

Different types of chemical pesticides or antibiotics are usually applied for the treatment of plant diseases, resulting environmental hazards and cause big economic loss for farmers throughout the world. On comparing the antibacterial efficacy of leaf and stem extracts of N. arbor- tristis against both the tested bacteria, we found the significant results in methanolic as well as aqueous extract because of the presence of active metabolites present in the plant parts. But among, the methanolic leaf extract of N. arbor- tristis showed excellent inhibitory activity against X. citri. So, from this study it can be suggested that these plant

parts could be used as a substitute for chemical pesticides and antibiotics.

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AUTHOR'S CONTRIBUTION

AS conceived the study and designed experiments and carried out analysis and interpretation of experimental data including statistical analyses. SP help in wrote the research paper.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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