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COMPARATIVE ANTIBACTERIAL EFFICACY OF METHANOL AND AQUEOUS EXTRACT OF *M. PANICULATA* AGAINST *X. CITRI* AND *X. CAMPESTRIS*

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ABSTRACT

In this study, *Murraya paniculata* was analysed for its antibacterial activity by using different solvents like methanol and aqueous against two plant pathogens i.e. *X. citri* and *X. campestris* and observed the growth inhibition activity ranging from 20 µl - 100 µl. The antibacterial potential of *M. paniculata* (leaf and stem) by using two different solvents was studied by agar well diffusion method and we observed that at 100 µl it's showed significant result against both the tested bacteria. Highest antibacterial activity was observed by the methanolic leaf extract of *M. paniculata* against *X. citri* i.e.13.22±0.05 mm at 100 µl of concentration.

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

INTRODUCTION

The diseases of bacteria are known to cause huge damage to plants all over the world. Most plant species possess resistance against numerous pathogens. Generally, asymptomatically numerous plants can harbour plant pathogens and developed a risk in horticultural practices. Recently, in agricultural practices the bacterial infection on the plant is a severe threat. Because of the phylogeny, the association of bacteria with plants are varied in their habitats and effects badly to the plant (Brittie, 2006). *Xanthomonas citri* and *Xanthomonas campestris* are the two important plant pathogenic species, which cause extreme harm in plants, trees and harvest by causing intense monetary misfortune for farmers around the world. The blister plague is found in Asia, South America, The United States, 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. It is spread to new region through the transportation of tainted citrus foods grown from the ground (Gottwald and Graham, 2000). It is quickly conveyed by water which running over the surfaces of sores and sprinkling into uninfected shoots. Some nations are directing diverse kinds of program for the annihilation of citrus blister around the world. Citrus infections in a few territories of the world have been annihilated and some program going on other region. *Xanthomonas campestris* is a gram-negative bacterium that has placed within the subdivision of Proteobacteria. It was hereditarily separated into more than 141 pathovers (pv.) and each with an explicit host range (Dye *et al*., 1980; Swings and Civerolo, 1993). *X. campestris* taints a wide scope of plants belongs to crucifer family (Brassicaceae) including broccoli cabbage, cauliflower, radish and the model plant *Arabidopsis thaliana*. The characteristic "black rot" symptoms in vascular tissues of cruciferous, is generally developed by the variant of *X. campestris* pv. *campestris* (*Xcc*) and developed on the veins and angular necrotic sores at the foliar edge (Alvarez, 2000). For the treatment of plant diseases, since last several years, various synthetic pesticides or chemicals are used, all over the world resulting, they tend to gather in animal tissues and create a threat to human health. Medicinal plants characterize a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (Balandrin *et al*., 1985; Hostettmann and Wolfender, 1997). In this study we used to check the antibacterial efficacy of medicinal plant *M. paniculata* against *X. citri* and *X. campestris*. *Murraya paniculata*, commonly known as Kamini or Honey bush. It is also known as *Chalcas exotica*, *Chalcas paniculata* and *Camunium exoticum* (Seidemann, 2005). It is a small, tropical, evergreen tree or shrub, distributed throughout the world. There are many phytoconstituents like flavanoid, alkaloids, coumarins, essential oils are obtained from different parts of *M. paniculata* like leaves, fruit, roots, stem and flowers (Chowdhury *et al*., 2008; Ng and Abdulhadi - Noaman, 2012). Therefore, this study is a primary step to evaluate the efficacy of *M. paniculata* against selected plant pathogens.

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 air tight 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).

15 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 air tight 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± standard deviation and to check the significance of data, One way ANOVA was used at the level of 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

In this study we used two plant parts i.e. leaf and stem of *Murraya paniculata* against the bacteria *Xanthomonas citri* and *Xanthomonas campestris*. The plants were collected shade dried, powdered and were extracting using soxhlet apparatus for antibacterial analysis. 7.6% & 7.4% yield was observed in methanolic extract of *M. paniculata* and 7.88% and 7.4% were noted in aqueous extracts (Table 1). At 100 µl of concentration the methanol extract of leaf and stem of *M. paniculata* exhibited the highest ZOI i.e. 13.22±0.05 mm and 12.87±0.07 mm against *X. citri* (Table 2). At 80 µl of concentration the ZOI was 11.23±0.75 mm and 10.54±0.05mm. 10.25±0.03 mm and 9.55±0.12 mm was recorded at 60 µl of concentration and 8.95±0.28 mm and 8.02±0.19 mm at 40 µl of concentration. Minimum antibacterial activity was noted at 20 µl of concentration i.e. 6.02±0.25 mm and 5.22±0.15 mm. The highest 12.25±0.06 mm ZOI was recorded at 100 µl of concentration in methanolic extract of leaf of *M. paniculata* against *X. campestris* and 7.53±0.07 mm was noted in stem (Fig 1). At 80 µl of concentration the ZOI was 10.21±0.03 mm and 5.02±0.18 mm. 8.52±0.18 mm and 4.85±0.85 mm ZOI was recorded in leaf and stem extracts of *M. paniculata* at 60 µl of concentration. Minimum ZOI was recorded against *X. campestris* in 40 µl and 20 µl of concentrations viz.6.52±0.18 mm, 3.65±0.05 mm and 4.85±0.01 mm while stem of *M. paniculata* did not show any activity at 20 µl of concentration. There has been very little literature available against the antibacterial activity of *X. citri* and *X. campestris*. The value of *M. paniculata* (leaf and stem) was found significant at the level of 0.05($p < 0.05$) at 100 µl of concentration against *X. citri* and *X. campestris*. While the leaf of *M. paniculata* also showed significant at the level of 0.05($p < 0.05$) at 80 µl of concentration against *X. citri* and *X. campestris*. At 100 µl of concentration the ZOI of aqueous extract of *M. paniculata* (leaf and stem) against *X. citri* was 8±0.50mm and 12±0.75 mm. 7±0.60 mm and 11±0.80 mm was recorded at 80 µl of concentration (Table 3). At 60 µl of concentration the ZOI was 6±0.03 mm and 10±0.30 mm. Minimum ZOI was noted at 40 µl of concentration i.e. 4.5±0.50 mm and 8±0.20 mm. The ZOI of aqueous extract of *M. paniculata* (leaf and stem) at 20 µl of concentration against *X. citri* was found to be inactive (Fig 2). No significant value was recorded at any level

of concentrations. The aqueous extract of *M. paniculata* (leaf and stem) did not show any activity at any level of concentrations against *X. campestris*.

Table 1. Percentage Yield of *Azadirachta indica* (Leaf and Stem) using methanol and aqueous solvent.

Plant part used	Extract	Extraction Yield (%)
Leaf	Methanol	7.6
Stem	Methanol	7.4
Leaf	Aqueous	7.8
Stem	Aqueous	7.4

Table 2. Comparative antibacterial activity of methanolic extract of M. *paniculata* against *X. citri and X. campestris.*

S.No.	Concentration (µl)	Plant part	Zone of Inhibition (mm)	
			X. citri	*X. campestris*
1.	20	Leaf	6.02±0.25*	4.85±0.01*
		Stem	5.22±0.15*	NI
2.	40	Leaf	8.95±0.28*	6.52±0.18*
		Stem	8.02±0.19*	3.65±0.05*
3.	60	Leaf	10.25±0.03*	8.52±0.18*
		Stem	9.55±0.12*	4.85±0.85*
4.	80	Leaf	11.23±0.75	10.21±0.03
		Stem	10.54±0.05*	5.02±0.18*
5.	100	Leaf	13.22±0.05	12.25±0.06
		Stem	12.87±0.07	7.53±0.07

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

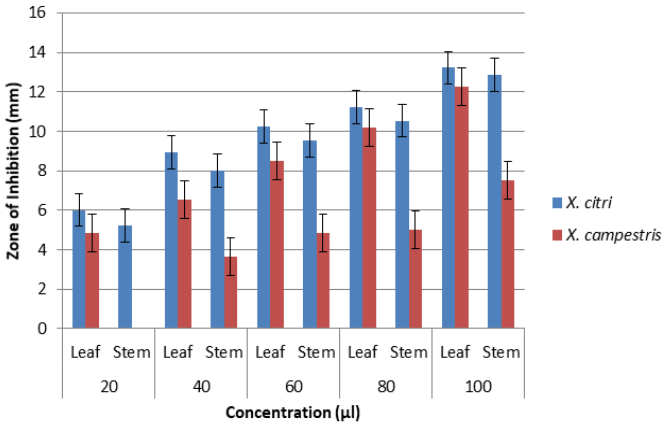

Fig 1. Comparative antibacterial activity of methanol extracts of M. paniculata against *X. citri and X. campestris*

Table 3. Comparative antibacterial activity of aqueous extract of *M. paniculataagainst X. citri and X. campestris*

S. No.	Concentration (µl)	Plant part	Zone of Inhibition (mm)	
			X. citri	*X. campes-tris*
1.	20	Leaf	NI	NI
		Stem	NI	NI
2.	40	Leaf	4.5±0.50*	NI
		Stem	8±0.20*	NI
3.	60	Leaf	6±0.03*	NI
		Stem	10±0.30*	NI
4.	80	Leaf	7±0.60*	NI
		Stem	11±0.80*	NI
5.	100	Leaf	8±0.50*	NI
		Stem	12±0.75*	NI

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($p < 0.05$). NI = No inhibition

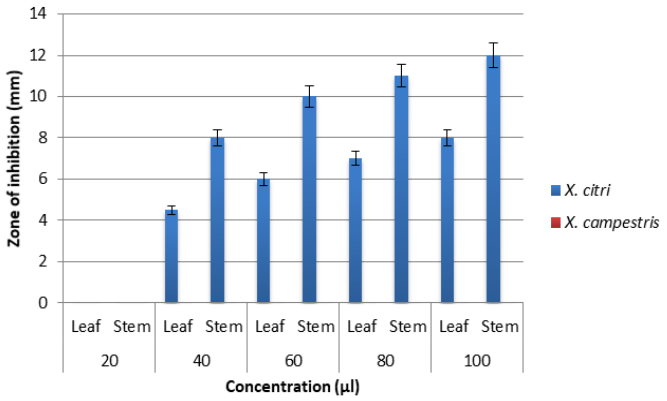

Fig 2. Comparative antibacterial activity of aqueous extract of *M. paniculata* against *X. citri and X. campestris*

Discussion

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. The degradable character, low cost and safe for the environment, medicinal plant extracts will be serving as a good substitute of chemical pesticides Highest ZOI was observed at 100% concentration followed by 80% and 60% whereas minimum ZOI was recorded at 40% and 20% concentrations. During this investigation different concentrations of methanolic and aqueous extracts of the samples were tested to control the growth of *X.citri* and *X. campestris*, by Agar well diffusion method. Terblanche *et al*., (2017) evaluated the extraction yield of *Cotyledon orbiculata* in aqueous and methanol solvent extract. The methanol extracts of *Ceiba pentandra* showed the highest extraction yield

(17.5%) rather than other solvent extracts (Ibrahim *et al*., 2017). *Allamanda cathartica*, *Allium sativum*, *Citrus limon*, *Tamarindus indica*, *Prunus domestica*, *Averrhoa carambola*, *Piper betle* and *Terminalia arjun* showed significant ZOI against *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis* (Chowdhury *et al*., 2013). Narendhiran *et al*., (2014) reported the antimicrobial activity of *Thespesia populnea* by using agar well diffusion method. *Murraya paniculata* exhibited good antibacterial activity against both the tested bacteria this may be due to the presence of various secondary metabolites. Compean and Ynalbez, (2014) revealed that the biological activities of plants is attributed to the presence of different active components like alkaloids, flavonoids, tannins, saponins.

Xanthomonas axonopodis pv.*punicae* which is responsible for 'bacterial blight' in pomegranate, exhibited the maximum ZOI of inhibition against *Mentha spicata, Murraya koeninngi, Allium sativum* and *Tridax procumbens* (Alane and Swami, 2016). Korpe *et al*., (2012) have evaluated antibacterial activities of methanol and aqueous extracts of *Urtica dioca* and *Urtica pilulifera* against *Clavibacter michiganesis* and *Xanthomonas vesicatoria*. Both test plant extracts exhibited the maximum inhibitory concentration of 256 and 1024 µg/ ml for *C. michiganesis* and 512 and 1024 µg/ ml for *X. vesicatoria*. Selvamohan *et al*., (2012) evaluated the antibacterial potential of methanol, ethanol and aqueous extracts of *Aloe vera, Phyllanthus emblica, Phyllanthus niruri, Cynodon dactylon, Murrya koenigii, Lawsonia inermis* and *Adhathoda vasica* against *Staphylococcus* species*, Escherichia coli, Klebsiella* species and *Pseudomonas* species by the method of agar well diffusion and disc diffusion. Methanol extract showed highest ZOI as compare to other extracts. Mita *et al*., (2013) also worked on inhibitory activity of *Murraya paniculata* using different solvent extracts like methanol, aqueous, petroleum ether, carbon tetra chloride and chloroform.

CONCLUSION

From this research work it was observed that the efficacy of methanolic leaf extract of *M. paniculata* is effective against *X. citri*. Hence, the leaf extract is eco-friendly, economic, easily degradable and compatible for management of various diseases of *X. citri*.

REFERENCES

Alane, S. K. and Swami, C. S (2016) Antibacterial activity of plant extracts against *Xanthomonas axonopodis* pv. *punicae* causing bacterial blight of Pomegranate (*Punica granatum* L.). *Bioscience Discovery*. 7(1): 70-73.

Alvarez, A. M (2000) Black rot of crucifers. In Mechanisms of resistance to plant diseases (eds. A.J. Slusarenko *et al*.). Kluwer Academic Publications, Dordrecht, Netherlands, 21-52.

Balandrin, M. F., J. A. Klocke, E. S. Wurtele and W. H. Bollinger (1985) Natural plant chemicals: Sources of industrial and medicinal materials. *Science*. 228: 1154-1160.

Brittie, G. A (2006) Plant- associated bacteria. Survey, Molecular phylogeny, Genomics and Recent advances. *Springer*.12: 1-56.

Chowdhury, J. U., Bhuiyan, M. N. I. and Yusuf, M (2008) Chemical composition of the leaf essential oils of *Murraya koenigii* (L.)Spreng and *Murraya paniculata* (L.) Jack. *Bangladesh Journal Pharmacological Society*. 3: 59-63.

Chowdhury, M. A. N., Ashrafuzzaman, M., Ali, M. H., Liza, L. N. and Zinnah, K. M. A (2013) Antimicrobial activity of some medicinal plants against multi drug resistant human pathogens. *Advances in Bioscience and Bioengineering*. 1(1): 1-24.

Compean, K. L. and Ynalvez, R. A (2014) Antimicrobial activity of plant secondary metabolites: A review. *Research Journal of Medicinal Plant*. 8(5): 204-213.

Dye, D., Bradbury, W., Goto, J. F., Hayward, M., Lelliott, A. C. and Schroth, M. N (1980) International standards for naming pathovars of phytopathogenic bacteria and a list of pathovar names and pathotype strains. *Review of Plant Pathology*. 59: 153-168.

Gottwald, T. R. and Graham, J. H (2000) Compendium of Citrus Diseases. American Phytopathological Society, St. Paul, 5-7.

Harborne, J. B (1984) Phytochemical methods-A guide to modern techniques of plant analysis. Chapman and Hall, London, 238.

Ibrahim, R., Abubakar, E. M., Modibbo, S. M. and Lamaran, B. G (2017) Percentage yield and acute toxicity of the plant extracts of *Ceiba pentandra* grown in Bauchi State, North Eastern Nigeria. *Journal of Pharmacognosy and Phytochemistry*. 6(5): 1777-1779.

Korpe, D. A., Iseri, O. D., Sahin, F. I., Cabi, E. and Haberal, M (2012) High-antibacterial activity of Urtica spp. seed extracts on food and plant pathogenic bacteria. *International Journal of Food Sciences and Nutrition*. 1-8.

Magaldi, S., Mata-Essayag, S. and Hartung de Capriles, C (2004) Well diffusion for antifungal susceptibility testing. *International Journal of Infectious Diseases*. 8: 39–45.

Mita, T. A., Shihan, M., Rahman, H., Sharmin,M., Maleque,T., Alvi, M., Razi-Ul-Hasan, M. and Chowdhury, S. R (2013) *In vitro* antioxidant, cytotoxic, thrombolytic, antimicrobial and membrane stabilizing activities of *Murraya paniculata. American Journal of Research Communication.* 1(5): 226-237.

Narendhiran, S., Mohanasundaram, S., Arun, J., Rannjith, R. V., Saravanan, L., Catherine, L. and Subathra, M (2014) Phytochemical screening and antimicrobial activity of Thespesia populnea linn. *International Journal of Pharmacognosy and Phytochemical Research*. 6(1): 7-10.

Ng, N. K. and Abdulhadi - Noaman, Y (2012) Bioactivity studies and chemical constituents of *Murraya paniculata* (linn) Jack. *International food research journal*. 19(4): 1307-1312.

Roopashree, T. S., Dang, R., Rani, R. H. S. and Narendra, C (2008) Antibacterial activity of antipsoriatic herbs: *Cassia tora*, *Momordica charantia* and *Calendula officinalis*. *International Journal Applied Research in Natural Products.* 1(3): 20–28.

Seidemann, J (2005) World Spice Plants**:** Economic Usage, Botany, Taxonomy; *Springer*. Berlin, Germany.

Selvamohan, T., Ramadas, V. and Kishore, S. S. S (2012) Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. *Advances in Applied Science Research*. 3(5): 3374-3381.

Swings, J. G. and Civerolo, E. L (1993) *Xanthomonas*. Chapman & Hall, London.

Terblanche, U., Semakalu, C. C. S., Mtunzi, F. and Pillay, M (2017) Screening of variables influencing extraction yield of *Cotyledon orbiculata*: 2^3 Full Factorial Design. *International Journal of Pharmacognosy and Phytochemical Research*. 9(3): 303-312.