Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Spl Issue [3] 2022 : 09-13

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Ecofriendly Disease Management of *Vicia Faba* Disease Incited by *Pseudomonas viridiflava*

Manju Meena* and Ashwani Kumar Verma
Dept. of Botany, Raj Rishi Govt. College, Alwar
*E-mail: 26manjumeena@gmail.com

ABSTRACT
In the present study antibacterial activity of seven Sariska medicinal plant parts extracts namely *Emblica officinalis* fruits, *Moringa oleifera* leaves, *Azadirachta indica* leaves, *Tinospora cordifolia* leaves and *Pongamia pinnata* leaves, *Terminalia arjuna* bark, and *Desmodium gangeticum* resin was carried out against *Vicia faba* pathogen *Pseudomonas viridiflava* by using well-diffusion methods. Antibacterial activity was detected at two 50% and 100% (w/v) concentrations of methanol extracts. All experimental medicinal plants showed more effective bacterial inhibitory activity against *P. viridiflava*. Among them, bark extract of *T. arjuna* gave maximum inhibition annulus (IA) that was 1271.1 mm^2 at 100% and 1142.96 mm^2 at 50% concentration. Thereafter leaf extract of *M.oblifera* and *T.cordifolia* showed 1142.96 mm^2 IA at 100% concentration. The results concluded that *E. officinalis*, *M. oleifera*, *A.indica*, *T.cordifolia*, *P.pinnata*, *T. arjuna,* and *D. gangeticum* have antibacterial activity against *Pseudomonas viridiflava* pathogen. Hence these medicinal plants can be used for eco-friendly disease management of *Vicia faba* pathogenic bacterial disease.
Keywords: Antibacterial activity, *Pseudomonas viridiflava*, *Vicia faba*, Inhibition annulus

Received 05.08.2022 Revised 23.09.2022 Accepted 11.10.2022

INTRODUCTION

Vicia faba L. legume crop has significant value in human foods because it has valuable nutritional contents including phenolic compounds, carbohydrates, protein, fatty acids, lipids, and minerals such as nitrogen, calcium, phosphate, Sulphur, potassium, and magnesium [5, 8, 28, 25]. It has nitrogen fixation potential and provides residual N for subsequent crops [23]. Its root traits are responsible for the improvement of soil textures [27]. Based on crop production fava bean has the fourth rank after beans, pea, and chickpea. A total of 7.5% of production is related to fava bean worldwide [12]. Moreover, its productivity was reduced progressively due to bacterial infections.

The infections in plants can be detected easily by morphological characterization like tumors, blights, fruit and leaf spots, rots, cankers, and vascular wilts [18]. *Pseudomonas* species led to cause various symptomatic infections in different plant species such as blight, galls, cankers, soft rot, and spots on the stem and leaves parts. Plant pathogenic micro-organisms cause various diseases in several plants by penetration in plant tissue [9]. *Pseudomonas syringae* pv. syringae causes brown spot diseases in fava bean which is the maximum economically significant disease [28].

Pseudomonas viridiflava is a multi-host pathogenic bacteria responsible to cause severe diseases in *Solanum lycopersicum* [1, 16], *Cucumis melo* [3, 4, 15, 16], *Amaranthus blitum* (blite), *Arabidopsis thaliana* [14], *Chrysanthemum morifolium* (chrysanthemum), *Solanum melongena* (eggplant) [16]. The symptomatic infections such as yellowing to brown-black spots at the nodes and wilting are the symptoms of *P. viridiflava* infected tomato plant as well as soft rot and brown discoloration are also visualized in the inner part of the vascular system. In the Aegean islands and eastern Mediterranean region, *Pseudomonas* species cause 50% and 12%, respectively stem necrosis diseases [32, 4].

During the last few decades, the interest of researchers has been focused on several medicinal plants having many anti-oxidant and anti-bacterial compounds. The use of herbal drugs is the best alternative to synthetic drugs due to their less expensive and less detrimental effects [10]. Furthermore, higher antibiotic resistance activity of pathogens also increased interest in the use of medicinal plants worldwide [13]. Among them, Sariska Tiger Reserve is more famous for Phyto-diversity with medicinal values [17]. In Sariska, approximately 110 plant species belonging to 88 genera under 43 families have been reported

to cure numerous diseases including diabetes, dysentery, jaundice, fever, skin problems, rheumatism, diarrhea, etc. [19]. Accordingly the present study aimed to identify antimicrobial properties in methanol extract of *Emblica officinalis* fruits, *Moringa oleifera* leaves, *Azadirachta indica* leaves, *Tinospora cordifolia* leaves, and *Pongamia pinnata* leaves, *Terminalia arjuna* bark and *Desmodium gangeticum* resin available in Sariska National Park against *Pseudomonas viridiflava* pathogen causing infection in *Vicia faba* plant.

MATERIAL AND METHODS

Collection of Plant Samples

Fresh fruits (*Emblica officinalis*), leaves (*Moringa oleifera*, *Azadirachta indica*, *Tinospora cordifolia,* and *Pongamia pinnata*), Bark (*Terminalia arjuna*), and Resin (*Desmodium gangeticum*) free from the disease were collected from Sariska National Park, Alwar, Rajasthan.

Samples Preparation

Fresh plant parts were carefully cleaned with tap water and then dried in shade. Dried plant parts were crushed by mortar pestle in pine powder. The powdered plant parts (5gm) extracted with methanol (50ml) by using the Soxhlet extraction apparatus for 10-12 h, followed by the extract filtration from Whatman No. 1 filter paper. The filtrate was dried at 90°C by a Rotary vacuum evaporator to obtain the concentrated plant extract. An extract can be stored at -18°C in a freeze condition until used for further studies [24].

Test microorganism

The anti-bacterial potential of methanol extract of Sariska medicinal plant was assessed against *Pseudomonas viridiflava* isolated from infected seeds, leaves, and fruit parts of *Vicia faba*.

Antibacterial screening of extracts

In vitro, anti-bacterial properties of the methanol extract of Sariska plants were determined by using well diffusion method against *Pseudomonas viridiflava* (Perez *et al.,* 1990). The 100% concentration was prepared from crude methanol extract according to 10mg/ml with dimethyl-sulphoxide (DMSO). Further dilution of 50% conc. was made with DMSO [30]. Both 100% and 50%concentrationwere used for the experiment. The Mueller Hinton agar was used as the test medium that was prepared and sterilized by autoclaving at 15 lbs pressure (121°C) for 15mins. After sterilized media preparation, Mueller Hinton agar plates were prepared in Laminar Air Flow. The inoculum of the tested pathogen was poured into sterile Petri plates and remained stand for 30 seconds. Upon incubation, the remaining non-adhere pathogenic bacteria culture was decanted. Further, 6mm wells were prepared by using gel puncture. Both 100% and 50% plant extract (90 µl) were introduced individually and then plates were incubated at 37ºC for 24hrs. The zone of inhibition prepared around well was measured in cm by scale as anti-bacterial potential of the methanol extract. The diameter of inhibitory bacterial activity was compared with negative control (DMSO). The inhibition annulus (IA) of each plant extract was calculated using the formula [29, 31, 19, 20]: $\pi (R_1\text{-}R_2) (R_1 + R_2)$, Where R_1 is the radius of inhibition zone + radius of well, R_2 is the radius of well and the value of π is 3.14.

RESULT AND DISCUSSION

Medicinal plants are widely used to treat several disorders including atherosclerosis, neurosis, duodenal ulcers, cardiac insufficiency, asthenia etc. Different medicinal plants have more than one therapeutic potential because these plants have numerous bio-active compounds and trace elements [22]. Hence, herbal drugs are widely used throughout the world to treat several diseases due to more safety purposes, cost-effective and higher medicinal values [35]. As the antimicrobial results shown in Table 1, the highest inhibition annulus was displayed at 100% concentration of *Terminalia arjuna* bark methanol extracts (1271.1mm^2) against *P. viridiflava*, followed by1142.96 mm^2 IA was observed with the leaf extract of *Moringa oleifera* and *Tinospora cordifolia* at 100 % conc. and bark extract of *T. arjuna* at 50% conc. The methanol extract of *D. gangeticum* resin showed 1020.5 mm^2 IA at 100 % conc. As a consequence, the relative sequence of IA against *P. viridiflava* at 100% concentration was found as follows: *Terminalia arjuna* bark>*Moringa oleifera* leaf>*Tinospora cordifolia* leaf>*Desmodium gangeticum* resin>*Azadirachta indica* leaf>*Pongamia pinnata* leaf>*Emblica officinalis* fruit. The methanolic leaf extract of *Aesculus hippocastanum* and *Tilia cordata* gave maximum antibacterial activity against *Pseudomonas aeruginosa* with disc diffusion method and *Listeria ivanovii* with micro-broth dilution method (Vatľák *et al.,* 2021). Arulmozhi *et al.,* 2018 evaluated the anti-microbial activity of leaves from ethyl acetate, aqueous, methanol, and petroleum ether extracts of *Tribulus terrestris, Sonchus asper, Capparis zeylanica*. Among them, extract of *C. zeylanica* leaves ethyl acetate (EtOAc) showed more significant anti-bacterial and anti-fungal properties against *S. dysenteriae, S. paratyphi, S. epidermidis*, *E. faecallis,* and *C. albicans*. The extracts of hydro-methanol of *Zingiber officinale*, *Rhus tripartata*, *Wisteria frutescens*, *Nigella sativa*,

Punica granatum, *Cistus monspeliensis, Beta vulgaris* and *Curcuma angustifolia* exhibited a more effective inhibition zone with the range of 6 to 23 mm against gram +ve and gram –ve pathogens (Bereksi*et al.,* 2018). *Azadirachta indica, Allium cepa, Vernonia anthelmentica, Tamarix aphylla, Tagetes erecta* and *Plumbago zelanicum* extracts exhibited consequential antibacterial effect at 50% concentration towards *Xanthomonas campestris* [11]. Verma and Agrawal, [34] studied anti-microbial potential on six aqueous extracts of medicinal plants that were leaves part of *Azadirachta indica* and *Withania somnifera*, fruits part of *T. chebula* and *E. officinalis*, the rhizome of *Z. officinalis* and bulb of *A. sativum* at 100% concentration towards bacterial blight pathogen (*P. syringae*) of *Pea sativum* plant. The highest anti-bacterial potential was recognized by aqueous extract of *Allium sativum* bulbs with 455.98mm^2 IA thereafter fruit extract of *T.chebula* with 415.25mm^2 IA. The antibacterial effects of aqueous extracts of 29 medicinal plants were estimated against *Pseudomonas syringae* caused bacterial speck disease in tomatoes. From them, *Allium sativum, Coriandrum sativum,* and *Eucalyptus camaldulensis* extract significantly revealed a zone of inhibition ranging from 3mm to 13.6mm [21]. A study conducted by Belgüzar *et al.,* [6] observed prominent antibacterial activity with stems and leaves extracts of *Viscum album* at 5% and 10% concentrations against *Clavibacter michiganensis* causing tomato bacterial cancer and wilt disease, *Xanthomonas axonopodis* causing bacterial leaf spot disease in pepper and tomato and *P. syringae* causing bacterial leaf spot disease in tomato.

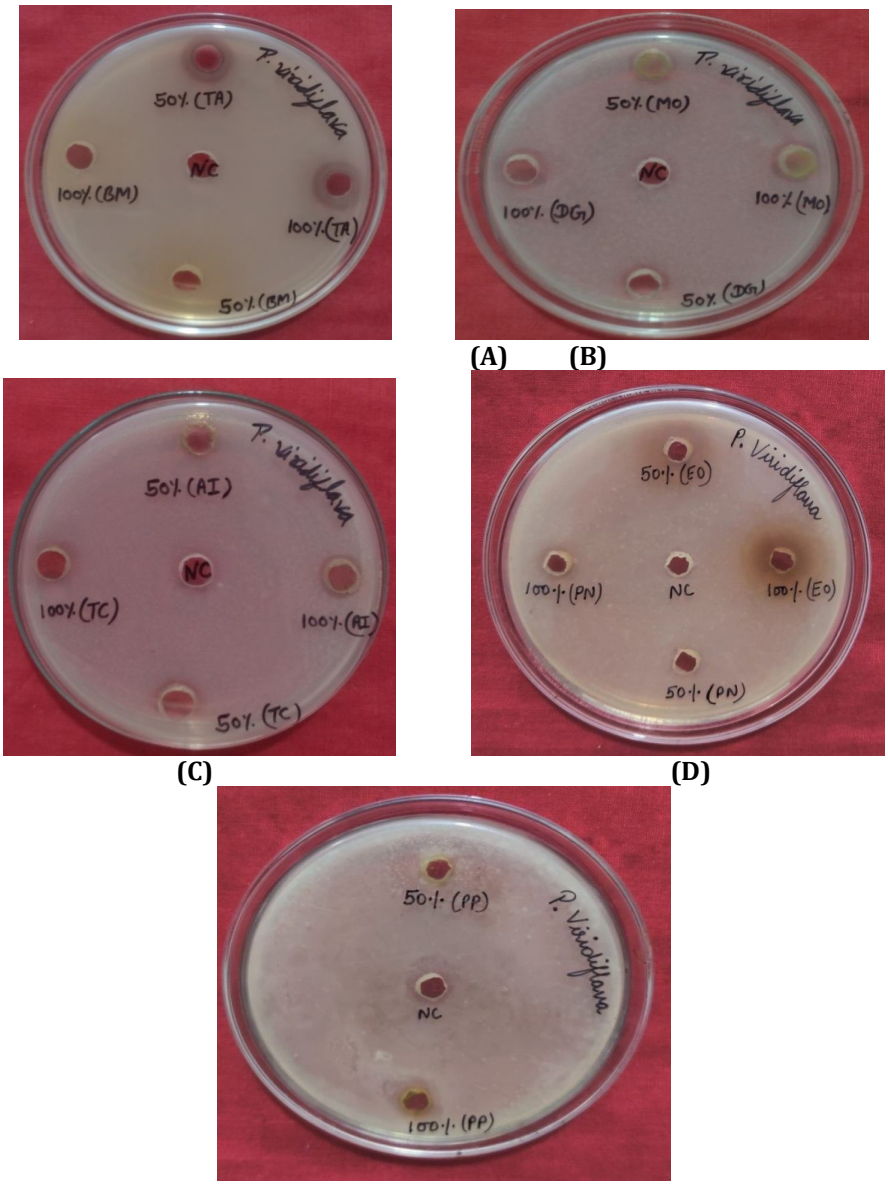

Figure 1: Disease management of *Pseudomonas viridiflava* by methanol extract of SariskaPlant (A) *T. arjuna* (B) *M. oleifera*, *D. gangeticum*(C) *A. indica*, *T. cordifolia* (D) *E. officinalis* (E) *P. pinnata*

Table 1: Antimicrobial activity of methanol plant extracts for inhibition of *Pseudomonas viridiflava*

Methanol extract	Part used	Percent Concentration (w/v)	
		Pseudomonas viridiflava	
		50% IA(mm²)	**100% IA(mm²)**
Desmodium gangeticum	Resin	904.32	1020.5
Moringa oleifera	Leaf	904.32	1142.96
Terminalia arjuna	Bark	1142.96	1271.1
Azadirachta indica	Leaf	794.42	904.32
Tinospora cordifolia	Leaf	904.32	1142.96
Emblica officinalis	Fruit	Nil	794.42
Pongamia pinnata	Leaf	Nil	904.32

CONCLUSION

In conclusion, consequential inhibition activity of methanol extracts of *E. officinalis* fruits, *M. oleifera* leaves, *A. indica* leaves, *T. cordifolia* leaves, *P. pinnata* leaves, *T. arjuna* bark, and *D. gangeticum* resin was detected against *P. viridiflava* pathogen causing infection in *V. faba* plant. Due to significant results in the future, a phytochemical analysis should be performed to isolate the active metabolites from these Sariska plants that show antimicrobial potential. Accordingly, these antimicrobial agents can be used to prepare novel herbal fertilizers that can prove to be effective to control plant pathogenic bacterial infections with the highest yield.

ACKNOWLEDGMENT

The authors are highly thankful to the Principal, Raj Rishi Govt. College, Alwar for providing facilities and infrastructure and moreover his support to conduct the study. We also extend our thanks to the faculty members and staff of Department of Botany for their support in many ways.

REFERENCES

1. Alivizatos, A.S. (1986). Tomato pith necrosis caused by *Pseudomonas viridiflava*. Ann Inst PhytopatholBenaki., 15, 41–47.
2. Arulmozhi, P., Vijayakumar, S., Kumar, T. (2018). Phytochemical analysis and antimicrobial activity of some medicinal plants against selected pathogenic microorganisms. Microbial pathogenesis, 123, 219-226.
3. Aysan, Y. andCinar, O. (2001).Dispersion of bacterial stem necrosis on tomato grown in greenhouses in the eastern Mediterranean region of Turkey and observations in these greenhouses; Tekirdag, Turkey.51–56.
4. Aysan, Y., Mirik, M., Ala, A., Sahin, F., Cinar (2003).First report of *Pseudomonas viridiflava* on melon in Turkey. New Disease Reports 7.
5. Baseony, A. and Abdel Moneim, M. (2012). Nutritional values of faba bean and its utilization. In Hamdy AH (ed) The fava bean. Agricultural Research Center, Giza, Egypt, 381–416.
6. Belgüzar, S., Şin, B., Eroğlu, Z., Kadıoğlu, İ., Yanar, Y. (2020). The Effect of Common Mistletoe (*Viscum album* L.) Extracts, Collected from Different Host Plants, on Some Plant Pathogenic Bacteria. Turkish Journal of Agriculture-Food Science and Technology, 8(1), 80-84.
7. Bereksi, M.S., Hassaïne, H., Bekhechi, C., Abdelouahid, D.E. (2018). Evaluation of Antibacterial Activity of Some Medicinal Plants Extracts Commonly Used in Algerian Traditional Medicine against Some Pathogenic Bacteria.Pharmacog J., 10(3), 507-12.
8. Brown, G.D. (1977). Field bean (*Vicia faba* L) as a potential human food. In: Proceedings Symposium on the Production, Processing and Utilization of the Field Bean (*Viciafaba* L.). 80–87
9. Buonaurio, R. (2008). Infection and plant defense responses during plant–bacterial interaction. Plant-Microbe Interactions, 169–197.
10. Chew, A.L., Jessica, J.J., Sasidharan, S. (2012). Antioxidant and antibacterial activity of different parts of *Leucasaspera*. Asian Pac J Trop Biomed., 2, 176-180.
11. Didwania, N., Sadana, D., Trivedi, P.C. (2013). Antibacterial activity of a few medicinal plants against *Xanthomonascampestris*pv. campestris. Int. J. Res. Pharm. Sci., 4(2), 177-182.
12. FAOSTAT (2016).Statistical database of The United Nation Food and Agriculture Organization (FAO), Rome. http://www.Fao.org/faostat/en/#data/QC
13. Farjana, A., Zerin, N., Kabir, M.S. (2014). Antimicrobial activity of medicinal plant leaf extracts against pathogenic bacteria. Asian Pac J Trop Dis., 4(2), S920-S923.
14. Goss, E.M., Kreitman, M., Bergelson, J. (2005).Genetic diversity, recombination and cryptic clades in *Pseudomonas viridiflava* infecting natural populations of Arabidopsis thaliana. Genetics 169: 21–35.
15. Goumas, D.E. andChatzaki, A.K. (1998). Characterization and host range evaluation of *Pseudomonas viridiflava* from melon, blite, tomato, chrysanthemum and eggplant. Eur. J. Plant Pathol., 104, 181–188.
16. Goumas, D.E., Malathrakis, N.E., Chatzaki, A.K. (1999). Characterization of *Pseudomonas viridiflava* associated with a new symptom on tomato fruit. Eur J Plant Pathol., 105, 927–932.
17. Goyal, P.K. and Jeyabalan, G. (2020).Traditional Medicinal Plants of Sariska Tiger Reserve Used In Diabetes. International Journal of Pharmacognosy, 7(2), 36-43.

18. Höfte, M. and De Vos P. (2007).Plant pathogenic *Pseudomonas* species. In: Gnanamanickam S.S. (eds) Plant-Associated Bacteria. 507-533. Springer, Dordrecht.
19. Jain, R. and Agrawal, K. (2011).Bio-efficacy of plant extracts against *Pseudomonas syringae*pv. syringae causing leaf spot of cluster bean. Ann. Pl. Protec. Sci., 19(1), 106-112.
20. Jain, S.C., Jain, R., Singh, R. (2009). Ethno botanical survey of Sariska and Siliserh Regions from Alwar District of Rajasthan, India.Ethno Botanical Leafle, 13, 171-88.
21. Karabüyük, F. and Aysan, Y. (2019).Antibacterial Effects of Some Plant Extracts against Tomato Bacterial Speck Disease Caused by *Pseudomonas syringae*pv. tomato. Journal of Tekirdag Agricultural Faculty, 16(2), 231-243.
22. Kurmukov, A.G. (2013). Phytochemistry of medicinal plants. In Medicinal Plants of Central Asia: Uzbekistan and Kyrgyzstan, 13-14. Springer, New York, NY.
23. Maluk, M., Ferrando-Molina, F., Lopez delEgido, L., Langarica-Fuentes, A., Yohannes, G. G., Young, M. W., James, E. K. (2022). Fields with no recent legume cultivation have sufficient nitrogen-fixing rhizobia for crops of fava bean (*Viciafaba* L.). Plant and Soil, 1-24.
24. Nahak, G. and Sahu, R.K. (2017). Bio-controlling effect of leaf extract of *Tagetespatula* L. (Marigold) on growth parameters and diseases of tomato. Pak. J. Biol. Sci., 20, 12-19.
25. Omar, S.A.M. (2021). The Importance of Faba Bean (*Viciafaba* L.) Diseases in Egypt.In Mitigating Environmental Stresses for Agricultural Sustainability in Egypt, 371-388.Springer, Cham.
26. Perez, C., Paul, M. and Bazerque, P. (1990).An antibiotic assay by the agar-well diffusion method.Acta. Biol. Med. Exp., 15, 113-115.
27. Roughley, R.J., Sprent, J.I., Day, J.M. (1983).Nitrogen fixation. In: PD Hebblethwait (ed) The Faba Bean (*Vicia bean* L.). Butterworth, London, 233–260.
28. Singh, D., Sangle, U.R., Kumar, B., Tripathi, H.S., Singh, K.P., Gupta, A.K. (2012). Integrated Disease Management of Fava Bean (*Viciafaba* L.).279-301.
29. Smale, M.J. and Keil, H.C. (1966).A biochemical study of the intervarietal resistance of *Pyruscommunis* to fire blight.Phytochemistry, 5, 1113-1120.
30. Soltania, J. and Aliabadib, A.A. (2013). Antibacterial effects of several plant extracts and essential oils on *Xanthomonasarboricola*pv. Juglandis*in vitro*. J. of Essential Oil Bearing Plants, 16(4), 461-468.
31. Thornberry, H. (1959). A paper-disc plate method for the quantitative evaluation of fungicides and bactericides.Phytopathol., 40, 950-954.
32. Ustun, N. andSaygili, H. (2001). Pith necrosis on greenhouse tomatoes in Aegean region of Turkey; Evora, Portugal. 70–73.
33. Vatľák, A., Kolesárová, A., Vukovič, N., Rovná, K., Petrová, J., Vimmerová, V., Kačániová, M. (2021).Antimicrobial activity of medicinal plants against different strains of bacteria. Journal of Microbiology, Biotechnology and Food Sciences, 2021, 174-176.
34. Verma, A.K. and Agrawal, K. (2015).Bio-Efficacy of some medicinal plant extracts against *Pseudomonas syringae*Pv. Pisicausing Bacterial Blight of Pea. International Journal of Pharmacology & Toxicology, 5(1), 67-70.
35. Yudharaj, P., Shankar, M., Sowjanya, R., Sireesha, B., Ashok Naik, E., Jasmine Priyadarshini, R. (2016).Importance and uses of medicinal plants – An Overview. International Journal of Preclinical and Pharmaceutical Research, 7(2), 67-73.

CITATION OF THIS ARTICLE

M Meena and A K Verma. Ecofriendly Disease Management of *Vicia Faba* Disease Incited by *Pseudomonas viridiflava.* Bull. Env.Pharmacol. Life Sci., Vol Spl Issue [3] 2022: 09-13