International Journal of Current Advanced Research
ISSN: O: 2319-6475, ISSN: P: 2319-6505, Impact Factor: SJIF: 5.995
Available Online at www.journalijcar.org
Volume 7; Issue 3(A); March 2018; Page No. 10458-10461
DOI: http://dx.doi.org/10.24327/ijcar.2018.10461.1773

Research Article

INVITRO STUDIES OF SOME MEDICINAL PLANT EXTRACTS AGAINST PAPAYA ROOT ROT DISEASE

Mahadevi M*., Thavaselvi A[2]., Latha V[1] and Panneerselvam A[2]

[1]Kundavai Naachiyaar Government Arts College for Women (Autonomous), Thanjavur - 613 007, Tamil Nadu, India
[2]Department of Botany and Microbiology, A.V.V.M Sri Pushpam College (Autonomous), Poondi - 613 503, Thanjavur, Tamil Nadu, India

A R T I C L E I N F O

Article History:

Received 6th December, 2017
Received in revised form 21st January, 2018 Accepted 05th February, 2018
Published online 28th March, 2018

Key words:

Biocontrol, *V. negundo* L. , Antifungal activity,Plant extracts.

A B S T R A C T

The antifungal activity of Benzene, Diethyl ether, Distilled water extract and Ethyl acetate extracts of selected medicinal plants such as *A. indica* A. Juss., *C. roseus* (L.) G. Don, *J.adhatoda* L., *O. tenuiflorum* L. and *V. negundo* L. against the *P. palmivora* (Butl.) revealed that *V. negundo* L. plant extracts exhibited maximum antifungal activity. The antifungal activity of the *V. negundo* L. ethyl acetate extract was found to be comparable to the standard fungicides Copper oxychloride and Bordeaux mixture tested. The results indicated that the *V. negundo* L. plant extracts are good antifungal agents. Totally 29 prominent peaks were obtained in the mass spectrum of *V. negundo* L. plant extract. The major phytocompounds were 2-Butanol, 4-(2,2-dimethyl-6-methylenecyclohexylidene)- (18.115 %) and 4,8,13-Cyclotetradecatriene-1,3-diol,1,5,9-trimethyl-12-1-met (15.226 %). Present study showed that *V. negundo* L. plant extracts are potential biocontrol agents for root rot disease in Papaya plant.

INTRODUCTION

Higher plants are much more important in the production of economically important organic compounds, pharmaceuticals, pesticides and fungicides (Aslam *et al*., 2010). The antifungal activity from the plant extracts have been shown to be effective against plant pathogen and ecofriendly to the environment (Daru and Onyedeneke, 2010). Previously, the various plant extracts that have been reported as a source of biofungicide because of the substances of plant extract inhibited the growth of plant pathogens and reduced the hazard to human health and environment. The presence of antifungal compounds in higher plants has long been recognized as important factors for controlling some plant diseases (Tapwal *et al*., 2011).

Natural plant products can be alternatives to currently used synthetic fungicides, since they provide unlimited opportunities for the discovery of new fungicides because of their rich bioactive chemical constituents. Plants produce secondary metabolites such as flavonoids, saponins, alkaloids, tannins, and phenols that are important for survival. These metabolites allow plants to defend themselves from herbivory effects, pathogens and from other plants, and also provide protection from adverse physical effects, such as damaging UV-radiation, water loss, and low temperatures (Salehan *et al*., 2013).

P. palmivora infects multiple hosts that hold an economic significance including cacao, coconut, papaya, mango, and black pepper making this a pathogen of great concern. Hence, in the present investigation to evaluate the five selected medicinal plants against *P. palmivora* (Butl.). Identify the bioactive compounds of potential biocontrol plant *V. negundo* L. by GC-MS analysis.

Corresponding author:* **Mahadevi M
Kundavai Naachiyaar Government Arts College for Women (Autonomous), Thanjavur - 613 007, Tamil Nadu, India

MATERIALS AND METHODS

In vitro biological control of P. palmivora (Butl.)by using some medicinal plants

Collection of plant leaves

The medicinal plant leaves such as*Azadirachta indica* A. Juss., *Catharanthus roseus* (L.) G. Don, *Justiciaadhatoda* L., *Ocimum tenuiflorum* L. and *Vitex negundo* L. were collected from Perumalagaram village, Thiruvarur Dt**.** and identified with the help of regional floras (Gamble, 1935; Matthew, 1983; Nair and Henry, 1983). Herbarium specimens were prepared by following Jeffrey (1982). Specimen was further confirmed with reference to Herbarium sheets available in the Department of Botany, Tiruchirappalli, Tamil Nadu, India and a voucher herbarium specimen was deposited in the Herbarium, Tiruchirappalli.

Sterilization of plant leaves

The disease free and fresh plant leaves were selected and they were washed with distilled water for three times. Then surface sterilized with 0.1% mercuric chloride for 20 seconds. Again the leaves were washed thoroughly with distilled water (three times). Then kept in the shade for 10 days to dry and then crushed using mortar and pestle, then further reduced to powder using electric blender. The powder were passed through 80 µm sieve mesh and preserved in air tight containers and used for further studies.

Preparation of extracts

Plant extracts were prepared according to the methodology of Indian Pharmacopoeia (Anonymous, 1996).

Preparation of aqueous extract

Five grams of leaf powdered sample of each plant was macerated separately with 50 mL of sterile distilled water for 10 min; the macerate was filtered through double layered muslin cloth and then centrifuged at 4000 rpm for 30 min. The supernatant were collected and preserved in airtight containers for further analysis.

Preparation of organic solvents extracts

Five grams of powdered sample of each plant was extracted separately with the 50 mL selected solvents *viz.*Benzene, Diethyl ether and Ethyl acetate. The leaf powder was filled in the thimble and extracted with the solvents by using a soxhlet extractor for 24 hrs. The obtained extracts were concentrated by using a rotary flash evaporator. The plant extracts were well preserved in airtight containers for further analysis.

Antifungal activity of selected medicinal plants against P. palmivora (Butl.)

The antifungal activity of benzene, diethyl ether, distilled water and ethyl acetate extracts of *A. indica* A. Juss., *C. roseus* (L.) G. Don, *J.adhatoda* L., *O. tenuiflorum* L. and *V. negundo* L. were evaluated by well agar method against *P. palmivora* (Butl.) (Perez *et al*., 1990).

Freshly prepared and sterilized potato dextrose agar medium was poured into each petriplate and allowed to solidify. One percent Streptomycin sulphate solution was added to the medium before pouring into petriplates for preventing bacterial growth. The test fungal culture *P. palmivora* (Butl.) was evenly spread over the media by using sterile cotton swab. Then wells (5 mm) were made in the medium by using sterile cork borer, 200 µl of the benzene, diethyl ether, distilled water and ethyl acetate extracts of selected medicinal plants were transferred into separate wells. Then these plates were incubated at 27 ± 2°C for 48-72 hrs. After incubation period, the results were observed and measured the diameter of inhibition zone around each well. All the tests were performed in three replicates and the activity was expressed as the mean of inhibition diameters (mm) produced by the plant extracts.

Effect of commercial fungicide & solvents on P. palmivora (Butl.) (Positive & Negative control)

The commercial fungicides *viz*., Mancozeb, Copper oxychloride and Bordeaux mixture (mg/ml) was tested against *P. palmivora* (Butl.)by well agar method.The effect of solvents such as benzene, diethyl ether, distilled water and ethyl acetate on *P. palmivora* (Butl.)was studied by well agar method.

Gas Chromatography and Mass Spectrometry (GC – MS) analysis of V. negundo L.

Based on the results of antifungal efficacy of medicinal plants, the potential *V. negundo* L. plant extract was subjected to GC –MS analysis. The GC –MS analysis of plant extract was evaluated by the method of Merlin *et al.* (2009).

Sample preparation

20 g powdered plant material (leaves) was soaked in 50 ml of methanol overnight and then filtered through Whatman filter paper No.41 along with 2 gm sodium sulphate to remove the sediments and traces of water in the filtrate. Before filtering, the filter paper along with sodium sulphate was wetted with absolute alcohol. The filtrate was concentrated by bubbling nitrogen gas into the solution and reduces the volume to 1 ml. The extract contains both polar and non-polar phytocomponents of the plant material used.

Assay

The GC –MS analysis was done according to the method previously described in this chapter.

RESULTS AND DISCUSSION

In vitro biological control of P. palmivora (Butl.) by some medicinal plants

In vitro biological control of *P. palmivora* (Butl.) was evaluated by using some medicinal plants. The results of antifungal efficacy of benzene, diethyl ether, distilled water and ethyl acetate extracts of selected medicinal plants such as *A. indica* A. Juss., *C. roseus* (L.) G. Don, *J.adhatoda* L., *O. tenuiflorum* L. and *V. negundo* L. were summarized in Table-1. Among the five plants tested, *V. negundo* L. plant extracts exhibited maximum antifungal activity against *P. palmivora* (Butl.) followed by *J.adhatoda* L. and *A. indica* A. Juss extracts. *O. tenuiflorum* L. and *C. roseus* (L.) G. Don plant extracts exhibited least antifungal activity against the tested plant pathogen. The ethyl acetate extract of all the plants showed the maximum antifungal activity and the distilled water extract showed minimum activity against the tested pathogen. The results indicated that all plant extracts had different levels of antifungal activity against the tested plant pathogenic fungus.

Table 1 *In vitro* biological control of *P. palmivora* (Butl.) by some Indian medicinal plants

S. No.	Name of the plant	Zone of inhibition (Diameter in mm)			
		Benzene extract	Diethyl ether extract	Distilled water extract	Ethyl acetate extract
1.	*A. indica* A. Juss.	11.7 ± 1.53	13.3 ± 0.58	9.7 ± 2.89	15.7 ± 1.53
2.	*C. roseus* (L.) G. Don	9.3 ± 2.08	11.7 ± 3.06	9.3 ± 0.6	12.3 ± 1.12
3.	*J.adhatoda* L.	12 ± 1	16.7 ± 1.53	10 ± 1	19 ± 1
4.	*O. tenuiflorum* L.	10.3 ± 1.53	11 ± 1.73	8.7 ± 1.53	12.7 ± 2.52
5.	*V. negundo* L.	17 ± 2.65	18 ± 3.61	13 ± 1	22.7 ± 2.08

Results expressed as Mean ± Standard Deviation (n-3)

The results of antifungal activity of *V. negundo* L. were in concurrence with results of Siva *et al*. (2008) who reported that *V. negundo* L.plant extracts exhibited significant antifungal activity against *Fusarium oxysporum* causing wilt disease of *Solanum melogena* L. by *in vitro.* Plant bio-

fungicides are cheap, locally available, non toxic, and are easily degradable. There is a great demand for them as alternative agents to control plant pathogenic fungi (Hadizadeh *et al*., 2009; Singh and Srivastava, 2013). Bapat *et al*. (2016) reported significant antifungal activity of ethanolic and petroleum ether extracts of *V. negundo* L. against phytopathogenic fungus *Sclerotium rolfsii* Sacc.

Effect of commercial fungicides & solvents on P. palmivora (Butl.) (Positive &Negative control)

The effect of commercial fungicides *viz*., Mancozeb, Copper oxychloride and Bordeaux mixture (mg/ml) was tested against *P. palmivora* (Butl.) to compare the potentials of extracts. The results of effect of commercial fungicides were presented. The fungicide Mancozeb exhibited the highest antifungal activity against *P. palmivora* (Butl.) (25mm) followed by Copper oxychloride (21 mm) and Bordeaux mixture (19 mm). The antifungal activity of ethyl acetate extract of *V. negundo* L. was found to be comparable to the standard fungicides tested.The results of antifungal effect of four solvents such as Benzene, Diethyl ether, Distilled water, Ethyl acetate revealed no activity against*P. palmivora* (Butl.).

Das *et al*., (2014) reported the efficacy of fungicides *viz.,* Carbendazim, Captaf, Copper oxychloride, Mancozeb and Ridomil MZ- 72 against *Phomopsis vexans* showed complete inhibition of mycelial growth by Carbendazim followed by other fungicides. However, Chemical fungicide application has resulted in the accumulation of residual toxicity in soil and vegetables, increase environmental pollution and alter the biological balance in the soil by decimating non-target and beneficial microorganisms. Adverse effects of chemical fungicides on the environment and human health are burning issues and there is a need to search for a new fungicides eco-friendly in nature.

Phytoconstituents analysis of ethyl acetateextract of V. negundo L. by GC -MS

Gas Chromatography - Mass *Spectrometry* (GC-MS) analysis was used to determine the phytochemical constituents present in *V. negundo* L. leaf extract. The active principles of ethyl acetate extract of *V. negundo* L. with their retention time (RT), molecular formula, molecular weight and peak area (%) were presented. The chromatogram of the analysis is shown in Fig.1. Totally 29 prominent peaks were obtained in the mass spectrum of *V. negundo* L. plant extract. The major phytocompounds were 2-Butanol, 4-(2,2-dimethyl-6-methylenecyclohexylidene)- (18.115%) and 4,8,13-Cyclotetradecatriene-1,3-diol, 1,5,9-trimethyl-12-1-met (15.226%).

Derbalah *et al*., (2011) reported the plant extracts are a bio fungicide control agent that is crucial for two particular reasons. The first reason is its safe usage for people and environmental accumulation. The second is its ability to control the pathogens and to prevent the pathogens from developing resistance to fungicide. Other researcher attributed the inhibitory effect of these plant extracts to hydrophobicity characters of these plant extracts and their components. This enables them to partition in the lipids of the fungal cell wall membrane and mitochondria disturbing their structure and rendering them more permeable. Leaking of ions and other cell contents can then causing cell death (Al-Rahmah *et al*., 2013). Similar work was done by Thakur and Pandey (2016) who identified 11 chemical compounds in leaf extract of *V. negundo* (L.). The antifungal potential of *V. negundo* L. might be due to the presence of the phytoconstituents identified by GC-MS.

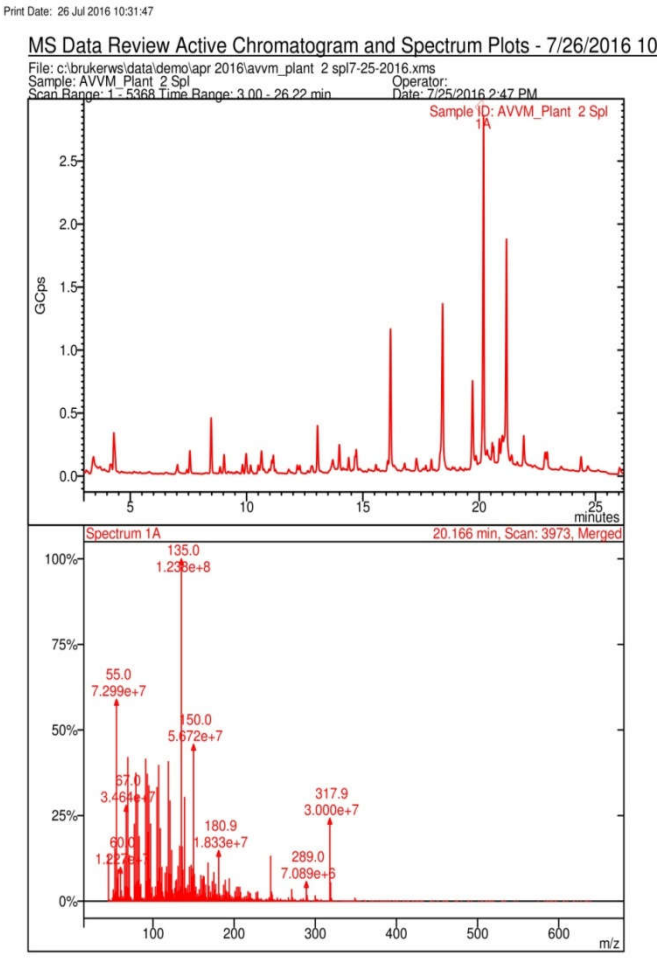

Fig 1 GCMS spectrum of *V. negundo* L.

CONCLUSION

The conclusion of this study suggested that *V. negundo* L. plant extracts possessing significant broad spectrum antifungal activity against *P. palmivora* (Butl.). This potential may be developed into an ecofriendly treatment to control root rot disease of Papaya plant. In addition, *V. negundo* L. plant extracts could be used to control the growth of plant pathogenic fungi and may be applied as agro-eco-friendly fungicides for various crops cultivation fields.

References

Al-Rahmah A. N., Mostafa A. A., Abdel-Megeed A., Yakout S. M. and Hussein S. A., 2013. Fungicidal activities of certain methanolic plant extracts against tomato phytopathogenic fungi. *Afr. J. Microbiol. Res*., 7(6): 517-524.

Anonymous, 1996. Indian pharmacopoeia. Ministry of Health Govt. of India Publication. New Delhi.

Gamble, J. S., 1935. Flora of the presidency of Madras. Botanical Survey of India, Calcutta, India.

Hadizadeh, I., Peivastegan, B. and Kolahi, M., 2009. Antifungal activity of nettle (*Urticad ioica* L.), colocynth (*Citrullus colocynthis* L. Schrad), oleander (*Nerium oleander* L.) and konar (*Ziziphus spina-christi*

L.) extracts on plants pathogenic fungi. *Pakistan J. Biol. Sci.*, 12: 58-63.

Jeffrey, C., 1982. Introduction to plant taxonomy, Cambridge University Press, Cambridge, United Kingdom.

Matthew, K. M., 1983. The flora of Tamil Nadu carnatic: Parts I – III. Diocesan Press, Madras.

Merlin, N. J., Parthasarathy, V., Manavala, R. and Kumaravel, S., 2009. Chemical investigation of aerial parts of *Gmelina asiatica* Linn by GC-MS. *Pharmacog. Res.,* 1(3): 152-156.

Nair, N. C. and Henry, A. N., 1983. Flora of Tamil Nadu, BSI, Southern Circle, Coimbatore, India.

Perez, C., Pauli, M. and Bazerque, P., 1990. An antibiotic assay by the well agar method. *Acta. Biol. Med. Exper.,* 15: 113-115.

Siva, N., Ganesan, S., Banumathy, N. and Muthuchelian, 2008. Antifungal effect of leaf extract of some medicinal plants against *Fusarium oxysporum* causing wilt disease of *Solanum melogena* L. *Ethnobot. Leaflets,* 12: 156-163.

Thakur, M.K. and Pandey, S. 2016. GC-MS analysis of phytochemical constituents in leaf extract *Vitex negundo* (L.). *World J. Pharm. Pharm. Sci.,* 5(8): 672-675.

Aslam, A., Naz, F., Arshad, M., Qureshi, R. and Rauf, C. A. 2010. *In Vitro* antifungal activity of selected medicinal plant diffusates against *Alternaria solani, Rhizoctonia solani* and *Macrophomina phaseolina. Pak. J. Bot.,* 42: 2911-2919.

Bapat U.C., Smrity Prabha and Jyoti Kumar, 2016. Antifungal activity of ethanolic and petroleum ether extracts of some medicinal plants against the plant pathogenic fungus *Sclerotium rolfsii* sacc. *Int. J. Bioassays*, 5(7): 4714-4719.

Daru, C. M. and Onyedeneke, N. E. 2010. *In vitro* study on the antimicrobial activity and phytochemical analysis of ethanolic extracts of the mesocarp of *Voacanga africana*. *Am. J. Pl. Physiol.*, 5: 163-169.

Derbalah, A. S., El-Mahrouk, M. S. and El- Sayed, A. B., 2011. Efficacy and safety of some plant extracts against tomato early blight disease caused by *Alternaria solani*. *Plant Pathol. J.*, 10(3): 115-121.

Salehan, N.M., S. Meon and I.S. Ismail, 2013. Antifungal activity of *Cosmos caudatus* extracts against seven economically important plant pathogens. *Int. J. Agric. Biol*., 15: 864-870.

Singh, P. and Srivastava, D. 2013. Phytochemical screening and *in vitro* antifungal investigation of *Parthenium hysterophorus* extracts against *A. alternata*. *Int. Res. J. Pharm.*, 4: 190-193.

Tapwal, A., Nisha, Garg, S., Guatam, N. and Kumar, R. 2011. *In vitro* antifungal potency of plant extracts against five phytopathogens. *Braz. Arch. Biol. Technol.*, 54(6): 1093- 1098.

How to cite this article:

Mahadevi M *et al* (2018) 'Invitro Studies of Some Medicinal Plant Extracts against Papaya Root Rot Disease', *International Journal of Current Advanced Research,* 07(3), pp. 10458-10461. DOI: http://dx.doi.org/10.24327/ijcar.2018.10461.1773

****