Bioscience Discovery, 13(1):41-44, Jan. - 2022
Open Access, Peer Reviewed, Referred Research Journal

Print & Online available on https://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)
Research Article

Antibacterial activity of *Vitex negundo* Linn. Against *Xanthomonas axonopodis* pv. *Punicae.*

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Article Info
Received: 16-11-2021,
Revised: 29-12-2021,
Accepted: 30-12-2021
Keywords: Bacterial blight, Antibacterial, Pomegranate, *Xanthomonas axonopodis* pv. Punicae. *Vitex negunda.*

Abstract
The Pomegranate crop is affected by 'Bacterial blight' caused by *Xanthomonas axonopodis* pv. Punicae, responsible for severe planting failure. It results in the fall of leaves and fruits. It is very difficult to treat the disease with chemicals and antibiotics and farmers are suffering from severe economic losses. In the present study, ethanol, methanol and water extracts of *Vitex negundo* Linn. were analyzed qualitatively for biochemical composition and used against *Xanthomonas axonopodis* pv. Punicae in vitro. The plant extracts have shown anti-bacterial activity and caused growth inhibition of *Xanthomonas axonopodis* pv. Punicae. It was found that higher concentration (10%) ethanol, methanol and water extracts of *Vitex negundo* Linn, are showing to be effective antibacterial activity than lower concentration (5%) against *Xanthomonas axonopodis* pv. Punicae

INTRODUCTION

Vitex negundo Linn., belongs to the Verbenaceae family, comprises 75 genera and nearly 2500 species and locally known as Nirgudi, native from tropical Eastern and Southern Africa and Asia, it is also found in Afghanistan, Bangladesh, India, Bhutan, Pakistan, Myanmar, Malaysia and Nepal. *Vitex negundo* Linn. a woody, aromatic deciduous shrub growing to a height of 2-5 m, slender tree with quadrangular branch lets. The leaves have five leaflets in a palmately arrangement, which are lanceolate, 4-10 cm long, hairy beneath and pointed at both ends. This plant is commonly found near fresh water bodies, disturbed land, grasslands, and mixed open forests. (Raghavendra *et al.*, 2010, Rahman and Bhattacharya, 1982). It is grown commercially as a crop in parts of Asia, Europe, North America and West Indies, also finds use as a food crop and a source of timber (Yenjerappa *et al.*, 2014)

Vitex negundo Linn. contains distinctive class of auxiliary metabolites, as polyphenolic compounds, terpenoids, glycosidiciridoids and alkaloids. Its leaves comprise of viridiflorol, sabiene, 4-terpineol, and gamma-terpiene (Singh *et al.*, 1999), butanoic corrosive, p-hydroxy benzoic corrosive, oleanolic corrosive (Surveswaran *et al.*, 2007) angusid, nutrient C, nishindine, sitosterol (Khare, 2004). This plant likewise comprises of vetugnoside, negundoside, 5 hydroxy-7,4'- dimethoxy flavones (Gautam *et al.*, 2008) To the polyphenolic intensifies present in this plant, a high cancer prevention agent potential has been proposed, investigated by applying different perceived in vitro frameworks (Mondal and Sharma, 2009). p-Hydroxybenzoic corrosive and β-sitosterol have been the most regularly detached phenolic compounds from *Vitex negundo* Linn. Pomegranate (*Punica granatum* L.) is belonging to the smallest botanical family *punicaceae.* India is largest pomegranate producer in the world sharing about 36 per cent of the world's production and above 30 per cent of the international Trade.

The average of total production is 8 lakh tons per annum in India. The crop is affected by 'Bacterial blight' caused by *Xanthomonas axonopodis* pv. *Punicae*, which is responsible for the failure of crop. Studies were conducted in different parts of the world identified *Xanthomonas auxonopodis* Pv. *Punicae* as the causal organism of bacterial blight, which is a gram-negative short rod bacterium. The pathogen infects all the cultivated varieties nevertheless of age of the plants. The infection appears as yellowish water-soaked circular spots on the plant part and later converted to irregular injuries. In advanced stages of infection, tissue necrosis occurs on the leaves and twigs. In the case of fruits, the disease develops into cracks and later the fruit became completely back and dries off. This is one of the most damaging diseases of pomegranate. Due to bacterial blight of pomegranate, the yield loss was noted up to 90 per cent (Chowdappa *et al.,* 2018**).** It results in the falling of leaves as well as fruits. It is very hard to manage the infection with chemicals as well as antibiotics and farmers suffer from heavy economic losses. The pathogen can infect in any stage of growth of the plant. The damage is observed on fruits which develop black oily spots later become completely black cracking and dries off. In advanced stage of infection tissue necrosis occurs on leaves and twigs. Use of chemicals in agriculture causes several opposing and environmental dangers (Singh *et al.,* 1999). The primary disease management includes spraying bleaching powder, farmyard manure, urea, Bordeaux mixture to control bacterial blight of pomegranate (Yenjerappa *et al.,* 2014) Regular use of chemicals in agriculture land causes killing of flora and fauna of the soil, increase in development of resistance in plant pathogen against chemicals and residual toxicity remains in plant and animals. To overcome this problem, there is growing interest worldwide in the utilization of sustainable material for pathogen control (Arangale *et al.,* 2020). In the present study, ethanol, methanol and water extracts of *Vitex negundo* Linn. were analyzed qualitatively for biochemical composition and used against *Xanthomonas axonopodis* pv. *Punicae* in vitro.

MATERIALS AND METHOD

Collection of diseased plant parts of Pomegranate plant: Disease infected Pomegranate fruits were collected from field located at village Belhekarwadi Tal. Newasa district Ahmednagar, Maharashtra, India.

Isolation and Identification of pathogen from lesions on diseased fruit : The disease infected portion of fruits were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for one minute and washed three times with sterile distilled water and mixed gently with sterile scalpel in sterile saline. The presence bacteria in fruit lesion were confirmed by performing ooze test. The suspension was serially diluted and plated on sterile Petri plate with Nutrient Glucose Agar medium with composition: Beef extract-0.3%, peptone-0.5%, glucose-0.25%, agar-2%, pH 6.8 (Raghavendra *et al.,* 2010). The inoculated plates were incubated at 30ºC for72 hours. After the incubation typical mucoid yellow color colonies were selected and screened for morphological and bio-chemical characteristics according to Bergy's manual of determinative Bacteriology and identified as *Xanthomonas*.

Preparation of aqueous plant extracts: Leaves of the *Vitex negundo* Linn. were collected from Sonai, Tal- Newasa, Ahmednagar (M.S.). The plant material was wash tap water remove soil and dust particles and then dry in shady place temperature (25 ± 2°C). Plants leaves were crush in mixture and to make fine powder. Fifty grams of leaves powder was crush in 50ml of sterile distilled water using mortar and pestle. The extracts were filter double layered cheese cloth, and then through Whatman filter paper No.1. The Filter extracts centrifuged at 5000 rpm for 20 minutes supernatant was stored in sterilized bottle and labeled properly. 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 (Kulshrestha *et al.,* 2015).

Preparation of ethanol /methanol plant extract: 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/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 Whatman 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 (Digvijay *et al.,* 2014, Arangale *et al.,* 2020)

In vitro Antimicrobial activities of the extract against Xanthomonas axonopodispv. Punicae

Forty-eight hours old bacterial culture of *Xanthomona saxonopodis*pv. *Punicae* was seeded into the nutrient agar medium at lukewarm temperature (40°C), mixed well and poured into sterile Petri plates. Wells of 4 mm diameter was prepared using a cork borer and plant extracts (0.02 ml) were filled in wells. Three replicates were maintained for each extract at 5 per cent and 10 per cent separately. Plates containing nutrient agar with bacterial suspension without leaf extract were maintained as control. All these Petri plates were incubated at room temperature (28 + 20^0C) for 48 hrs. (Bonyadi *et al.,* 2009).

Phytochemical Analysis

Phytochemical screening of ethyl acetate extract for the presence of these secondary metabolites: Alkaloids (Draggendorff's), flavonoids (Shibat'as reaction), Saponins (Frothing test), tannins (5% ferric chloride), Terpenoids (2, 4-dinitro-phenyl hydrazine), glycosides (fehling's solution), steroids (Liebermann's Burchard test) were evaluated according to the methods described by Edeoga *et al.* 2005.

RESULTS AND DISCUSSION

The results of the present study show that all the plant extract inhibited the pathogen. Higher concentration of aqueous and methanol plant extract shows high inhibited compared lower concentration in all three replications. The phytochemical analysis of Ethanol and Methanol extract shows the presence of Alkaloids, Flavonoids, Tannin, Saponins and Terpenoids.

Table 1: Antibacterial activity of different solvent extracts of *V. negundo* Linn against *Xanthomonas axonopodis* pv. *Punicae*.

Sr. No.	Common Name	Botanical Name	Inhibition Zone (cm)* (Aqueous plant Extract)		Inhibition Zone (cm)* (Ethanol plant Extract)		Inhibition Zone (cm)* (Methanol plant Extract)	
			5%	10%	5%	10%	5%	10%
1.	Control (Sterile Distilled Water)	_	0.00	0.00	0.00	0.00	0.00	0.00
2.	caste tree (Nirgudi)	*Vitex negundo* Linn.	**2.80**	**4.30**	**3.70**	**5.50**	**3.40**	**6.20**

*- Mean of three replications.

Aqueous extract had showed the presence of Alkaloids, Flavonoids, and Saponins. In previous findings flavonoids were found to be effective antimicrobial substances against a wide range of microorganisms, probably due to their ability to form a complex with extra cellular, soluble protein and bacterial cell wall: In addition, more lipophilic flavonoids may also disrupt microbial membrane. Secondary metabolites of plant origin appear to be one of the alternatives for the control of these antibiotic resistant human pathogens. The most important of their bioactive compounds of plants are such as alkaloids, flavonoids, tannins and phenolic compounds. This antibacterial activity may be due to the presence of secondary metabolites.

CONCLUSION

In the present study, ethanol, methanol and water extracts of *Vitex negundo* Linn. were analyzed qualitatively for biochemical composition and used against *Xanthomonas* axonopodis pv. *Punicae* in vitro. The plant extracts have shown anti-bacterial activity and caused growth inhibition of *Xanthomonas axonopodis* pv. *Punicae*. It was found that higher concentration (10%) ethanol, methanol and water extracts of *Vitex negundo* Linn, are showing to be effective antibacterial activity than lower concentration (5%) against *Xanthomonas axonopodis* pv. *Punicae*. The vast research on this plant proves significance as an antibacterial source which can be an answer to various unsolved infections.

Table 2: Phytochemical analysis of different solvent extracts of V. negundo Linn.

Plants	Extract	Alkaloids	Flavonoids	Tannin	Saponins	Terpenoids	Glycosides	Steroids
Vitex negundo Linn.	Ethanol	+	+	+	+	+	+	+
	Methanol	+	+	+	+	+	+	+
	Aqueous	+	+	-	+	-	-	-

ACKNOWLEDGEMENT

The author would like to express their deep gratitude to Principal of Mula Education Society's, Arts, Commerce and Science College, Sonai, Ahmednagar (M.S.) India for providing laboratory facilities for research work.

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