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Plant Extracts as Bio-protectant Against *Pseudomonas syringae* Infection in Peach

SURENDER KUMAR BHARDWAJ

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ABSTRACT

In continuation of our ongoing efforts to search for plant based eco-friendly bio-protectant against disease of fruit crops, 37 plants samples of thirty plant species were bio-assayed by agar diffusion methods against Pseudomonas syringae pv. syringae, a causal organism of bacterial canker of peach. The fruit extracts of Terminalia chebula has shown a very strong inhibitory activity against Pseudomonas syringae pv. syringae. The various parts of Terminalia belerica (bark, fruit, leaf and stem) have also shown more or less equal inhibitory effect against the test bacterium. The combined extracts of Terminalia belerica back and fruits of Terminalia chebula in general showed a strong enhancement in activities over these individual extracts of bark extracts. Some of the other plants such as Acacia arabicae, Caesalpinia bonducella, Rosa damascena and Terminalia arjuna also showed the inhibitory effect against the test bacteria.

INTRODUCTION

In discriminate use of synthetic chemicals to control plants microbial diseases is in extensive scrutiny these days as they are causing health discomforts in people who consume these crops. Fruits crops are in particular focus because danger of consuming residual chemicals is maximum as they are mostly consumed raw.

Pseudomonas syringae pv. syringae Van Hall is known to cause serious losses in almost all stone fruit crops like cherry, plum, peach and apricot etc. [10]. Bacterial canker disease caused by *P. syringae* in peach crop is a very serious problem in Europe [9]. Many countries have restricted the application of chemical antibiotics to control bacterial canker of peach [16]. Our laboratory is engaged in searching a bio-control option to address this issue. We have earlier reported the activity of 20 different phyto extracts on *P. syringae* [5]. In this communication, we present data pertaining to extent of inhibitory potential of 37 more plant extracts prepared using different plant parts of 30 common plants available in the state of Haryana in northern India.

MATERIALS AND METHODS

In all, 37 different types of extracts were prepared using 30 medicinal herbs that presently grow in Haryana & neighboring areas. These plant species & their traditional uses [11] are listed in Table-1. All plant parts were treated for extract preparation as previously reported by me [5].

*Corresponding author; E-mail: skbmdu67@gmail.com, Department of Botany, M. D. University, Rohtak-124 001, Haryana, India

Table 1 Common names, families and traditional medicinal uses of plants used in the present study.

Sr. No.	*Botanical Name*	*Common Name*	*Name of Family*	*Distribution*	*Traditional Uses of Plants*
1.	***Acacia arabicae* Willd.**	Kikar	Mimosaceae	India and Tropical Africa	Used for making furniture's, tanning, dyeing fabrics yellow, stem yields gum while seeds are fermented with dates to give beverages
2.	***Anthocephalus cadamba* (Mig.)**	Kadam	Rubiaceae	Tropical Asia	The bark is used as a tonic and reduces fever
3.	***Caesalpinia bonducella* (Flem.)**	Karanjua	Caesalpiniaceae	Tropics	Seeds are mixed with black pepper to make a tonic and to reduce fevers
4.	***Cassia siamea* (Lam.)**	Siama	Caesalpiniaceae	India to Indonesia	This plant contains a compound named Barakol. The leaves, tender pods and seeds are edible
5.	***Mimosa hamata* (Willd.)**	Aill	Mimosaceae	Tropical Asia	Tonic, in urinary complaints, glandular swellings, blood-purifier
6.	***Nerium oleander* (L.)**	White Kaner	Apocynaceae	Tropical Asia	Root is used against ringworm; flowers are used for perfume and produce good honey
7.	***Ocimum sanctum* (L.)**	Tulsi	Labiatae	Old World Tropics	The plant is sacred to the Hindus and is grown in front of temples; the leaves are used as a condiment
8.	***Phoenix rupicola* (L.)**	Khajur	Palmae	California, N. America, Minor Asia	Fruits are eaten fresh or dried, mixed with milk or fermented to make alcoholic beverages
9.	***Physalis minima* (L.)**	Papotan	Solanaceae	Tropics	The fruits are eaten as a vegetable
10.	***Physalis maxima* (L.)**	Papotan (Large)	Solanaceae	Tropics	The berries are used in sauces and preserves
11.	***Plumeria alba* (L.)**	Pagoda	Apocynanceae	C America & Caribbean, S Asia.	The heart of the wood is part of a traditional medical preparation taken as a vermifuge or as a laxative
12.	***Pongamia pinnata* (L. Mirr.)**	Papari	Papilionaceae	Asia	The oil of seeds are used to treat skin diseases & for burning
13.	***Psidium guajava* (L.)**	Amrood	Myrtaceae	Mexico, Peru, W Indies	The fruit is a good source of vitamin C, used in jellies and preserves, especially Guava Cheese which is sold commercially
14.	***Pterospermum acerifolium* (Willd.)**	Kanak Champa	Sterculiaceae	E India, Malaysia	Local Hindu people employ the plant for religious purposes. Its bark is also supposed to be used in case of scabies and topical preparation in lipsticks
15.	***Ricinus communis* (L.)**	Arand	Euphorbiaceae	Tropical Africa and Asia	Castor Oil is extracted. Medicinally it is used as a laxative; an insecticide is extracted from the leaves
16.	***Rosa damascena* (Mill.)**	Gulab	Rosaceae	N Temperate, Tropical mountains, Balkans & Asia Minor	The oil extracted from the flowers/ buds contains citronella, geranol, nerol, linalool, used in perfumery and for flavouring

17.	***Salvadora persica* (Garc.)**	Jal/ Pillu	Salvadoraceae	Tropical Africa, Asia	The fruits and bark are used in local medicines, twigs are used as toothbrushes
18.	***Sida cordifolia* (L.)**	Kanghi	Malvaceae	Native to India, Warm especially America	The Hindus use a decoction of the roots to treat stomach complaints, asthma and heart conditions
19.	***Solanum nigrum* (L.)**	Makoi	Solanaceae	Cosmopolitan	The fruits (Wooderberries) are eaten in pies etc., shoots and leaves are used as a vegetable
20.	***Strebelus asper* (Lour.)**	Sohra	Moraceae	Tropical Asia	A decoction of the bark is used in India to treat dysentery, diarrhea and fevers
21.	***Tagetes erecta* (L.)**	Gendha	Compositae	Mexico, Old & New World	More widely a decoction of the flowers and leaves is used to treat intestinal worms, stomach upsets and to control menstruation
22.	***Tamarix gallica* (L.)**	Jhau	Tamaricaceae	N India, W Himalayas	Galls (Sakum) on the plant are used for tanning
23.	***Tectona grandis* (L.f.)**	Teak	Verbenaceae	E India into Malyaysia	A very valuable timber, a yellow dye from the bark is used locally for dying basket work
24.	***Terminalia arjuna* Wight. and Arn.**	Arjun	Combretaceae	E. India into Malyaysia	The wood is valuable; a decoction of the bark is used as a heart stimulant
25.	***Terminalia belerica* (Roxb.)**	Baheda	Combretaceae	E India into Philippines	The fruits are eaten raw; a decoction of the fruits is used as an eye-lotion
26.	***Terminalia chebula* (Retz.)**	Harad	Combretaceae	Central Asia	The fruits are used for tanning and as a tonic to stimulate the appetite
27.	***Thevetia nereifolia* (Juss.)**	Pili Kaner	Apocynaceae	Tropical America, W Indies	The bark is used medicinally to reduce fevers
28.	***Tribulus terrestris* (L.)**	Bhakhri	Zygophyllaceae	Old and New World Tropics	The fruits are used as a diuretic in the Sudan
29.	***Vernonia anthelmintica* (Willd.)**	Kali Jiri	Compositae	Tropical Asia	The leaves are used locally in India as a salve for leprosy and skin diseases and in a decoction as an abortive [11].
30.	***Ziziphus jujuba* (Mill.)**	Ber	Rhamnaceae	E India, Malaysia, China, Japan	Fruits are eaten; mucilage from the fruits was used to make bronchial pastilles

Antibacterial efficacy of plant extracts against *P. syringae* was assayed by agar diffusion method. For this, 15% (w/v) aquous extracts of a given plant part (dried & powdered) was prepared by boiling for 15 min, followed by centrifugation at 1200 rpm for 15 min. The supernatant was than autoclaved at 121°C and the pH wa adjusted to 7.0 with 2.5 ml/ l NaOH or 2.7 ml/l Hcl.

For testing against bacterial growth, bacterial cultures were raised as per our earlier reported paper [5]. Wells (8mm diameter size) were cut in agar plates and these were filled with 0.1 ml of 15% plant extracts prepared as above. The plates were incubated at 37°C$\pm$2°C and zone of inhibition were measured after 24hr of incubation. Each treatment was replicated three times. The treatment that caused a clearance zone of inhibition >12 mm (including 8mm well size) were considered as effective. Minimum inhibitory concentration (MIC) of extracts was determined using sample concentration ranged from 0.25% to 3.0 % [11] and was defixed as lowest concentration required for preventing visible bacterial growth even after 40 hrs of incubation at 37°C. For assaying the antibacterial activity of combined plant samples, the selected

plant extracts were combined in the ration 1:1.

The various parts of each plant were collected from different region of Haryana and its neighboring states on the basis of their traditional values [11] as shown in Table 1. The plant materials were prepared and bio-assayed for their antibacterial activity as well as minimum inhibitory concentration of plant samples against tested bacterium as explained in my earlier research paper published in Journal of Medicinal and Aromatic Plant Sciences [5].

RESULTS

The activity of the 13 plants extracts treatments against the bacterial growth of *Pseudomonas syringae* pv. *syringae* is summarized in Table 2. It was observed that out of 37 plants parts extracts tested the bark, fruit, leaf and stem samples of *Terminalia belerica* have shown significant inhibitory effect against the test bacterium. The fruit extracts of *Terminalia belerica* (26.5 mm) showed highest inhibitory effect against the bacterial growth. The strong inhibitory activity was also showed by various parts of *Terminalia belerica* i.e. bark (24.5 mm), stem (23.5 mm), leaf (22.5 mm) and fruit (21.5 mm). The appreciable inhibitory effect was also depicted by seeds and stem extracts of *Acacia arabicae* (18.5 mm), petal extracts of *Rosa damascena* (18.0 mm), seed extracts of *Terminalia arjuna* (17.0 mm) and seed extracts of *Caesalpinia bonducella* (16.5 mm). The test bacterium was less inhibited by petal extracts of *Mimosa hamata* (10.0 mm) and leaf extracts of *Vernonia anthelmintica* (11.5 mm). The twenty six plants samples tested i.e. *Anthocephalus cadamba* (Leaf), *Cassia siamea* (Seed), *Mimosa hamata* (Leaf & Stem), *Mimosa hamata* (Stem), *Nerium oleander* (Leaf), *Ocimum sanctum* (Seed & Stem), *Phoenix rupicola* (Seed), *Physalis minima* (Leaf), *Physalis maxima* (Leaf), *Plumeria alba* (Leaf), *Pongamia pinnata* (Seed), *Psidium guajava* (Fruit), *Pterospermum acerifolium* (Seed), *Ricinus communis* (Leaf), *Salvadora persica* (Root), *Sida cordifolia* (Seed), *Solanum nigrum* (Leaf), *Strebelus asper* (Leaf), *Tagetes erecta* (Petal), *Tamarix gallica* (Inflorescence), *Tectona grandis* (Leaf), *Thevetia nereifolia* (Leaf & Pod), *Tribulus terrestris* (Whole Plant), *Vernonia anthelmintica* (Leaf) & *Ziziphus jujuba* (Leaf) did not show any appreciable antibacterial effect against *P. syringae*. However, the combined extracts of fruit extracts *Terminalia chebula* + bark extracts of *Terminalia belerica* showed an enhancement in activities (30.5 mm) over the individual extracts (Table- 2). The MIC of two plants samples i.e. *Mimosa hamata* and *Vernonia anthelmintica* was observed to be 2.0% for the test bacteria and *Acacia arabicae*, *Caesalpinia bonducella*, *Rosa damascena* and *Terminalia arjuna* showed 1.0% MIC. *Terminalia belerica* (bark, fruit, leaf, stem) and *Terminalia chebula* including combined extracts of *Terminalia belerica and Terminalia chebula* showed a MIC of 0.5%.

DISCUSSION

Considering the need to develop alternative for an alternative eco-friendly approach to control phytopathogens, it was through logical to screen the antibacterial effects of locally available flora. The obtained results showed differential activities of the plant extracts against the bacterial growth of *Pseudomonas syringae* pv. *Syringae*. The response varied from no effect to very strong inhibition against the bacterial growth and a definite potential for new effective bactericides. Among different plants, the fruit extracts of *Terminalia chebula* showed highest inhibitory activity followed by bark, fruit, leaf and stem parts extracts of *Terminalia belerica*, which could be due to the presence of some antimicrobial phytochemicals [1, 2, 3, 10]. Hence, the spray of the extracts of *Terminalia chebula* and *Terminalia belerica* could be used for protecting plants against pathogenic bacteria as good substitute of synthetic chemicals. The stem extracts of *Acacia arabicae*, petal extracts of *Rosa damascena*, seed extracts of *Terminalia arjuna*, seed extract of *Caesalpinia bonducella*, petal extracts of *Mimosa hamata* and leaf extracts of *Vernonia anthelmintica* also showed inhibitory effect against the bacterial growth because of their phyto-constituent that are also reported in literature to possess various medicinal properties [3, 4, 6, 7, 8].

Table 2 Anti-bacterial properties and minimum inhibitory concentrations (MIC) of plant extracts effective against *Pseudomonas syringae* pv. *syringae*

Sr. No.	Name of Plant	Part Used	Zone of Inhibition (mm)*	Minimum Inhibitory Concentrations (%)				
				0.25	0.5	1.0	2.0	3.0
1.	*Acacia arabicae* (Willd.)	Stem	18.0 ± 1.76	+	+	-	-	-
2.	*Caesalpinia bonducella* (Flem.)	Seed	16.5 ± 1.62	+	+	-	-	-
3.	*Mimosa hamata* (Willd.)	Petal	10.5 ± 1.88	+	+	+	-	-
4.	*Rosa damascena* (Mill.)	Petal	18.0 ± 1.28	+	+	-	-	-
5.	*Terminalia arjuna* Wight. and Arn.	Seed	17.0 ± 1.12	+	+	-	-	-
6.	*Terminalia belerica* (Roxb.)	Bark	24.5 ± 0.28	+	-	-	-	-
7.	*Terminalia belerica* (Roxb.)	Fruit	21.5 ± 1.62	+	-	-	-	-
8.	*Terminalia belerica* (Roxb.)	Leaf	22.5 ± 0.85	+	-	-	-	-
9.	*Terminalia belerica* (Roxb.)	Stem	23.5 ± 0.96	+	-	-	-	-
10.	*Terminalia chebula* (Retz.)	Fruit	26.5 ± 0.42	+	-	-	-	-
11.	*Vernonia anthelmintica* (Willd.)	Leaf	11.50 ± 2.14	+	+	+	-	-
	Terminalia belerica (Bark) *Terminalia chebula* (Fruit) (Combine Extracts)		30.50 ± 0.22	+	-	-	-	-

The combined samples of bark extracts of *Terminalia belerica* + fruit extracts of *Terminalia chebula* showed an enhancement in activities over these two individual extracts. Possible reasons for enhancement may be due to additive or synergestic effect of phytotoxic chemicals present in the two extracts. Therefore, the spray of the combined extracts of bark extracts of *Terminalia belerica* and fruit extracts of *Terminalia chebula* can be a strong recommendation for protecting bacterial canker of peach crops caused by *Pseudomonas syringae* pv. *syringae*.

Since the extracts of *Acacia arabicae*, *Caesalpinia bonducella, Mimosa hamata*, *Rosa damascena*, *Terminalia arjuna*, *Terminalia belerica*, *Terminalia chebula* and *Vernonia anthelmintica* used in this study have not been tested before as inhibitor of phytopathogenic bacteria of *Pseudomonas syringae* pv. *syringae*, therefore, they are the new addition to this field of study.

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