National Journal of Life Sciences, **Vol. 17(2) 2020 : 101-105** ISSN : 0972-995X (Print), 2321-7960 (Online)

POTENTIAL USE OF SOME TRADITIONAL PLANTS-EXTRACTS AS BIO-PROTECTANT AGAINST *XANTHOMONAS CITRI* SUB SP *CITRI*

SURENDER KUMAR BHARDWAJ[1*] and JITENDER SINGH LAURA[2]

[1]*Botanical/Herbal Garden, M. D. University, Rohtak (Haryana)*
[2]*Department of Environmental Sciences, M. D. University, Rohtak (Haryana)*

ABSTRACT : The thirty seven plants samples were bio-assessed by agar diffusion methods against *Xanthomonas citri* sub sp *citri*, a causal organism of citrus canker. The various parts of *Terminalia belerica* (bark, fruit, leaf and stem) have shown more or less equal marvelous inhibitory effect against the test bacterium followed by leaf extracts of *Mimosa hamata* against the bacterium growth. The fruit extracts of *Terminalia chebula* has shown strong inhibitory activity against *Xanthomonas citri*. The combined extracts of bark extracts of *Terminalia belerica* and leaf extracts of *Mimosa hamata* in general showed a strong enhancement in activities over the individual extracts of bark extracts of *Terminalia belerica* and leaf extracts of *Mimosa hamata* against the bacterium growth respectively. Some of the other plants such as *Cassia siamea*, *Nerium oleander* and *Thevetia nereifolia* also showed the inhibitory effect against the test bacteria.

Key words : *Xanthomonas citri, Antibacterial activity, Plants-extracts, Phytochemicals.*

INTRODUCTION

Citrus fruits are one of the world's most important staple human diets as it has good nutritive value and mostly consumed as raw. For the ever increasing world population require the production of huge quantities of citrus fruits but our efforts are hampered due to biotic as well as abiotic factors. Among the biotic factors various diseases caused due to phytopathogens are major hindrance in desired production of citrus fruits. The bacterial canker disease caused by *Xanthomonas citri* sub sp. *citri* is one of them (Gottwald *et al.*, 2001). Attempts have been made to manage the disease by treating with chemical compounds *i.e.* Streptocycline, copper sulphate, Bordex mixture (Behlau *et al.*,2011 and Graham *et al.*,2008). In discriminate use of synthetic chemicals to control the bacteria is in extensive scrutiny these days as they ultimately added in the natural environment, resulting serious affect in the human health (Ramachandra and Nagarathna, 2003). So there is an urgent need to develop sustainable methods for these horrible diseases. The screening and testing of the efficacy of these potential plants based sources for antibacterial activity could be an important step towards the assessment of the degree of variability among the diverse natural flora of a particular region. Therefore, the present study was undertaken to widen the spectrum of plants having antibacterial activity.

MATERIAL AND METHODS

The various parts of each plant were collected from different region of Haryana and its neighboring states on the basis of their traditional values (Usher,1971) as shown in Table.1. The collected plant materials were thoroughly washed with tap water, followed with distilled water and then kept in dark under the filter papers at room temperature till completely dry. Each sample was individually grounded into powder form for preparation of extract. The bacteria *Xanthomonas citri* used for the study was procured from the cultures stocks of our Department. The culture was maintained at 4ºC on Nutrient Agar medium with periodic subculturing.

Antibacterial tests : Fifteen percent plants parts extracts was prepared (15 g/100 ml water) by brewing in boiling water for 15 minutes followed by centrifugation at 12000 rpm for 15 minutes. The extracts were autoclaved for 15 minutes at 121ºC and the pH was adjusted to 7.0 with 2.5 mol/1 NaOH or 2.7 mol/1 HCl (Toda *et al.*,1989).

The antibacterial activity was tested by agar diffusion method with slight modification of Toda *et al.* (1989). Bacterial suspensions were cultured in peptone water for 6-8h and 0.2 ml of this culture was spread on Mueller-Hinton agar in Petri dishes. Wells (8 mm diameter in size) were cut in agar plates and were filled 0.1 ml of 15% plants extracts. The plates inoculated with Xanthomonas citri were incubated at 37±2ºC. The resulting zone of inhibition was measured after 24 h. Each combination of isolates and antimicrobial agent was repeated three times. The isolate which showed clear zone of inhibition more than 12 mm including the 8 mm well size were considered sensitive and those with less than 12 mm as resistant.

Minimum Inhibitory Concentration (MIC) was determined by the agar dilution method (Koneman *et al.*,1988) where plants samples concentration ranged from 0.25-3.0% and defined as the lowest concentration that prevented visible growth of microorganisms after incubation for 40 hours at 37±2ºC.

Assay for antibacterial activity of combined plant samples : The sample of each plant was prepared as explained earlier. The selected plants extracts were combined in the ratio 1:1.

*Corresponding author (email : skbmdu67@gmail.com) Received 14.08.2020 Accepted 15.10.2020

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RESULTS AND DISCUSSION

The activity of the plants extracts against the bacterial growth of *Xanthomonas citri* is presented in Table.2. It is commonly observed that out of 37 plants parts extracts tested the bark, fruit, leaf and stem samples of *Terminalia belerica* have show inhibitory effect against the test bacterium. The leaf extracts of *Terminalia belerica* (29.5 mm) showed marvelous inhibitory effect against the bacterial growth of *Xanthomonas citri* followed by leaf extracts of *Mimosa hamata* (26.0 mm). The strong inhibitory activity was also showed by fruit extract of *Terminalia chebula* (24.5 mm) including various parts of *Terminalia belerica i.e.* bark (22.5), stem (21.0 mm) and fruit (21.5 mm) against the test bacterium. The appreciable inhibitory effect was showed by seed extracts of *Cassia siamea* (22.5 mm), leaf extracts of *Nerium oleander* (23.0 mm), leaf extracts of *Thevetia nereifolia* (21.0 mm), petal extracts of *Mimosa hamata* (19.5 mm), stem extracts of *Acacia arabicae* (16.5 mm), leaf extract of *Anthocephalus cadamba* (17.5 mm), petal extracts of *Rosa damascena* (16.5 mm), leaf extracts of *Physalis maxima* (17.5mm), leaf extracts of *Physalis maxima* (16.5 mm) against the test bacteria. The intermediate inhibitory effect was showed by whole plant extracts of *Tribulus terrestris* (14.5 mm), leaf extracts of *Plumeria alba* (13.5 mm), petal extracts of *Tagetes erecta* (13.5 mm), stem extracts of *Ocimum sanctum* (13.5 mm), seed extracts of *Ocimum sanctum* (13.0 mm), stem extracts of *Mimosa hamata* (12.5 mm) and root extracts of *Salvadora persica* (12.5 mm). The test bacterium was less inhibited by pod extracts of *Thevetia nereifolia* (10.5 mm). The fourteen plants samples *i.e. Caesalpinia bonducella* (Seed), *Phoenix rupicola* (Seed), *Pongamia pinnata* (Seed), *Psidium guajava* (Fruit), *Pterospermum acerifolium* (Seed), *Ricinus communis* (Leaf), *Sida cordifolia* (Seed), *Solanum nigrum* (Leaf), *Strebelus asper* (Leaf), *Tamarix gallica* (Inflorescence), *Tectona grandis* (Leaf), *Terminalia arjuna* (Seed), *Vernonia anthelmintica* (Leaf) & *Ziziphus jujuba* (Leaf) did not show any antibacterial effect against the test bacteria. However, the combined extracts of leaf extracts *Terminalia belerica* + leaf extracts of *Mimosa hamata* showed an enhancement in activities (32.0 mm) over the individual extracts (Table.2).

Minimum Inhibitory Concentrations (MIC) : The MIC of *Tribulus terrestris*, *Plumeria alba*, *Tagetes erecta*, *Ocimum sanctum*, *Mimosa hamata*, *Salvadora persica*, *Thevetia nereifolia* showed 2.0% while *Acacia arabicae*, *Anthocephalus cadamba*, *Cassia siamea*, *Mimosa hamata*, *Nerium oleander*, *Rosa damascena* and *Thevetia nereifolia* showed 1.0% Minimum Inhibitory Concentrations. The *Terminalia belerica* (bark, fruit, leaf, stem) and *Terminalia chebula* showed 0.5% Minimum Inhibitory Concentrations for the test bacteria *Xanthomonas citri* as presented in Table.2.

Table. 1 Common names and families of plants used in experiment.

S.	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 (Usher,1971).
2.	*Anthocephalus cadamba* (Mig.)	Kadam	Rubiaceae	Tropical Asia	The bark is used as a tonic and reduces fever (Usher, 1971).
3.	*Caesalpinia bonducella* (Flem.)	Karanjua	Caesalpiniaceae	Tropics	Seeds are mixed with black pepper to make a tonic and to reduce fevers (Usher,1971).
4.	*Cassia siamea* (Lam.)	Siama	Caesalpiniaceae	India to Indonesia	This plant contains a compound named Barakol. The leaves, tender pods and seeds are edible (Usher,1971).
5.	*Mimosa hamata* (Willd.)	Aill	Mimosaceae	Tropical Asia	Tonic, in urinary complaints, glandular swellings, blood-purifier (Usher,1971).
6.	*Nerium oleander* (L.)	White Kaner	Apocynaceae	Tropical Asia	Root is used against ringworm; flowers are used for perfume and produce good honey (Usher,1971).
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 (Usher,1971).
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 (Usher,1971).
9.	*Physalis minima* (L.)	Papotan	Solanaceae	Tropics	The fruits are eaten as a vegetable (Usher,1971).
10.	*Physalis maxima* (L.)	Papotan (Large)	Solanaceae	Tropics	The berries are used in sauces and preserves (Usher,1971).
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 (Usher,1971).
12.	*Pongamia pinnata* (L. Mirr.)	Papari	Papilionaceae	Asia	The oil of seeds are used to treat skin diseases & for burning (Usher,1971).

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 (Usher,1971).
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 (Usher, 1971).
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 (Usher,1971).
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 (Usher,1971).
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 (Usher,1971).
19. *Solanum nigrum* (L.)	Makoi	Solanaceae	Cosmopolitan	The fruits (Wooderberries) are eaten in pies etc., shoots and leaves are used as a vegetable (Usher,1971).
20. *Strebelus asper* (Lour.)	Sohra	Moraceae	Tropical Asia	A decoction of the bark is used in India to treat dysentery, diarrhea and fevers (Usher,1971).
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 (Usher,1971).
22. *Tamarix gallica* (L.)	Jhau	Tamaricaceae	N. India, W. Himalayas	Galls (Sakum) on the plant are used for tanning (Usher,1971).
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 (Usher,1971).
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 (Usher,1971).
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 (Usher,1971).
26. *Terminalia chebula* (Retz.)	Harad	Combretaceae	Central Asia	The fruits are used for tanning and as a tonic to stimulate the appetite (Usher,1971).
27. *Thevetia nereifolia* (Juss.)	Pili Kaner	Apocynaceae	Tropical America, W. Indies	The bark is used medicinally to reduce fevers (Usher, 1971).
28. *Tribulus terrestris* (L.)	Bhakhri	Zygophyllaceae	Old and New World Tropics	The fruits are used as a diuretic in the Sudan (Usher,1971).
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 (Usher,1971).
30. *Ziziphus jujuba* (Mill.)	Ber	Rhamnaceae	E. India, Malaysia China, Japan	Fruits are eaten; mucilage from the fruits was used to make bronchial pastilles (Usher,1971).

Having the need for an alternative eco-friendly approach to control the phytopathogens, it was believed to be worthwhile to screen the antibacterial effects of locally available flora. The results obtained are indicating of the differential activities of the plant extracts against the bacterial growth of *Xanthomonas citri* because many of these extracts have shown very strong inhibition against the bacterial growth of test bacteria (Table 2) and a definite potential for new effective bactericides. Among the different plants, the leaf extracts of *Terminalia belerica* showed strong inhibitory activity followed by bark, fruit and stem parts extracts including fruit extracts of *Terminalia chebula* against *Xanthomonas citri*, which could be due to the presence of some antimicrobial phytochemicals (Ahmad & Beg,2001; Aswal *et al.*,1996; Bakhru,1997 and Pandey,1993), hence, the spray of the extracts of *Terminalia belerica* and *Terminalia chebula* could be used for protecting plants against pathogenic bacteria and a good substitute of synthetic chemicals.

The seed extracts of *Cassia siamea*, leaf extracts of *Nerium oleander*, *Thevetia nereifolia*, *Anthocephalus cadamba*, *Physalis maxima* and *Physalis maxima*, petal extracts of *Mimosa hamata* and *Rosa damascena* showed inhibitory effect against the bacterial growth of test bacteria, which might be due to the presence of some antimicrobial secondary metabolites in the plant sample, some phytochemicals have also been reported in literature to possess various medicinal properties (Bakhru,1997; Bhakuni *et al.*,1990; Dhawan *et al.*, 1977; Dilip & Bikash,2004 and Dixit *et al.*,1975).

The combined samples of leaf extracts *Terminalia chebula*+leaf extracts of *Mimosa hamata* showed an enhance-

ment in activities over the individual extracts of leaf extracts of *Terminalia belerica* and leaf extracts of *Mimosa hamata* respectively. Possible reasons for enhancement may be due to : (a) Greater concentration of the various groups of botano-chemicals than in case of individual extracts due to additive effect of the extracts. (b) Greater diversity of the various groups of botano-chemicals due to supplementation by one or the other plant extracts. (c) The possibility of synergistic effect of the botano-chemicals in the cocktail cannot be ruled out. Therefore, the spray of the combined extracts of leaf extracts *Terminalia chebula* and leaf extracts of *Mimosa hamata* could strongly be recommended for protecting bacterial canker of peach crops caused by *Xanthomonas citri* and a strong substitute of synthetic chemicals.

Tribulus terrestris, *Plumeria alba*, *Tagetes erecta*, *Ocimum sanctum*, *Mimosa hamata*, *Salvadora persica* and *Thevetia nereifolia* were observed 2.0% for the test bacteria and *Acacia arabicae*, *Anthocephalus cadamba*, *Cassia siamea*, *Mimosa hamata*, *Nerium oleander*, *Rosa damascena* and *Thevetia nereifolia* showed 1.0% Minimum Inhibitory Concentrations while, *Terminalia belerica* (bark, fruit, leaf, stem) and *Terminalia chebula* showed 0.5% Minimum Inhibitory Concentrations for the test bacteria *Xanthomonas citri* as presented in Table.2.

Minimum Inhibitory Concentration : In general the MIC of various plants samples ranges from 0.5-2.0% as presented in Table.2. The test bacterium *Xanthomonas citri* was observed sensitive at very low concentrations of the aqueous extracts of *Terminalia belerica* (bark, fruit, leaf, stem) and *Terminalia chebula* including combined extracts of *Terminalia belerica*

Table. 2 Anti-bacterial activity and Minimum Inhibitory Concentrations (MIC) of plant extracts against *Rathyibacter tritici*.

S.	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	16.5±1.64	+	+	-	-	-
2.	*Anthocephalus cadamba* (Mig.)	Leaf	17.5±1.92	+	+	-	-	-
3.	*Caesalpinia bonducella* (Flem.)	Seed	---	NT	NT	NT	NT	NT
4.	*Cassia siamea* (Vahl.)	Seed	22.5±1.16	+	+	-	-	-
5.	*Mimosa hamata* (Willd.)	Leaf	26.0±0.82	+	+	-	-	-
6.	*Mimosa hamata* (Willd.)	Petal	19.5±1.52	+	+	+	-	-
7.	*Mimosa hamata* (Willd.)	Stem	12.5±2.18	+	+	+	+	-
8.	*Nerium oleander* (L.)	Leaf	23.0±0.88	+	+	-	-	-
9.	*Ocimum sanctum* (L.)	Seed	13.0±1.78	+	+	+	-	-
10.	*Ocimum sanctum* (L.)	Stem	13.5±1.98	+	+	+	-	-
11.	*Phoenix rupicola* (L.)	Seed	---	NT	NT	NT	NT	NT
12.	*Physalis minima* (L.)	Leaf	16.5±1.46	+	+	+	-	-
13.	*Physalis maxima* (L.)	Leaf	17.5±1.42	+	+	+	-	-
14.	*Plumeria alba* (L.)	Leaf	13.5±1.86	+	+	+	-	-
15.	*Pongamia pinnata* (L.Mirr.)	Seed	---	NT	NT	NT	NT	NT
16.	*Psidium guajava* (L.)	Fruit	---	NT	NT	NT	NT	NT
17.	*Pterospermum acerifolium* (Willd.)	Seed	---	NT	NT	NT	NT	NT
18.	*Ricinus communis* (L.)	Leaf	---	NT	NT	NT	NT	NT
19.	*Rosa damascena* (Mill.)	Petal	16.5±1.26	+	+	-	-	-
20.	*Salvadora persica* (Garc.)	Root	12.5±2.12	+	+	+	-	-
21.	*Sida cordifolia* (L.)	Seed	---	NT	NT	NT	NT	NT
22.	*Solanum nigrum* (L.)	Leaf	---	NT	NT	NT	NT	NT
23.	*Strebelus asper* (Lour.)	Leaf	---	NT	NT	NT	NT	NT
24.	*Tagetes erecta* (L.)	Petal	13.5±1.46	+	+	+	-	-
25.	*Tamarix gallica* (L.)	Inflores	---	NT	NT	NT	NT	NT
26.	*Tectona grandis* (L.f.)	Leaf	---	NT	NT	NT	NT	NT
27.	*Terminalia arjuna* Wight. and Arn.	Seed	---	NT	NT	NT	NT	NT
28.	*Terminalia belerica* (Roxb.)	Bark	22.5±0.56	+	-	-	-	-
29.	*Terminalia belerica* (Roxb.)	Fruit	21.5±0.78	+	-	-	-	-
30.	*Terminalia belerica* (Roxb.)	Leaf	29.5±0.46	+	-	-	-	-
31.	*Terminalia belerica* (Roxb.)	Stem	21.0±0.88	+	-	-	-	-
32.	*Terminalia chebula* (Retz.)	Fruit	24.5±0.38	+	-	-	-	-
33.	*Thevetia nereifolia* (Juss.)	Leaf	21.0±0.66	+	+	-	-	-
34.	*Thevetia nereifolia* (Juss.)	Pod	10.5±2.64	+	+	+	-	-
35.	*Tribulus terrestris* (L.)	WP	14.5±1.72	+	+	+	-	-
36.	*Vernonia anthelmintica* (Willd.)	Leaf	---	NT	NT	NT	NT	NT
37.	*Ziziphus jujuba* (Mill.)	Leaf	---	NT	NT	NT	NT	NT
	Terminalia belerica (Leaf) *Mimosa hamata* (Leaf)	Combine extracts	32.50±0.42	+	-	-	-	-

The data were subjected to analysis of variance (ANOVA) and pair-wise comparison of the means was done by using Duncan's multiple range test (p=0.05). Analyses were done with GenStat 18 (Payne *et al.*,2015).

and *Terminalia chebula*. Minimum Inhibitory Concentrations was found slightly higher in case of *Acacia arabicae*, *Anthocephalus cadamba*, *Cassia siamea*, *Mimosa hamata*, *Nerium oleander*, *Rosa damascena* and *Thevetia nereifolia* against the test bacterium while *Tribulus terrestris*, *Plumeria alba*, *Tagetes erecta*, *Ocimum sanctum*, *Mimosa hamata*, *Salvadora persica* and *Thevetia nereifolia* were observed to show inhibitory effect against the *Xanthomonas citri* at very higher concentrations as compared to others tested plants samples (Table.2). The variations in the Minimum Inhibitory Concentrations might be due to slight differences in phytochemicals composition. Earlier, various reports were found registered in literature about the variations in Minimum Inhibitory Concentrations of plants samples for phytopathogens (Owuor *et al.*,1986 and Toda *et al*.,1989).

Since the extracts of *Terminalia belerica*, *Terminalia chebula*, *M.hamata*, *N.oleander*, *C.siamea* and *T.nereifolia* used in this study have not been tested before as inhibitor of phytopathogenic bacteria of *Xanthomonas citri*, therefore, they are the new addition to this field of study. The presence of various secondary metabolites such as alkaloids, quaternary alkaloids, coumarins, flavanoids, steroids/terpenoids, phenols etc. have been reported in the various plants extracts (Ahmad & Beg,2001; Aswal *et al.*,1996; Bhakuni *et al.*,1990; Dhawan *et al.*,1977; Dilip & Bikash,2004; Dixit *et al.*,1975 and Pandey,1993), which may be responsible for the antibacterial properties of the plants studied.

The study has shown that some plants namely *Terminalia belerica*, *Terminalia chebula*, *Mimosa hamata*, *Nerium oleander*, *Cassia siamea* and *Thevetia nereifolia* are very effective in inhibiting the bacterial growth of *Xanthomonas citri*. These plants could be further subjected to field trials to access their effectiveness in field conditions and can subsequently be explored for the possibilities towards the identification of the key bioactive agents, through implying modern microbiology and biochemical techniques.

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