Indian J. Agric. Res., **43** (1) : 26-31, 2009

AGRICULTURAL RESEARCH COMMUNICATION CENTRE
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ANTIBACTERIAL ACTIVITY OF SOME PLANT-EXTRACTS AGAINST PLANT PATHOGENIC BACTERIA *XANTHOMONAS CAMPESTRIS* PV. *CAMPESTRIS*

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ABSTRACT

The aqueous extracts of twenty plants were screened by agar diffusion method for their antibacterial activity against *Xanthomonas campestris* pv. *campestris*, a causal organism of black rot of cabbage and cauliflower. *Xanthomonas campestris* pv. *campestris* was found most sensitive to the leaf extract of *Camellia sinensis*. Some of the other plants such as *Acacia arabicae, Aegle marmelos, Acacia catechu, Achyranthus asper, Asparagus racemosus, Azadirachta indica, Callistemon lanceolatus* and *Acacia fernesiana,* also showed the inhibitory effect against the test bacteria.

Key words : Antibacterial activity, Plant extracts, Pathogenic bacteria, Black rot.

INTRODUCTION

In order to maintain the productivity, more and more chemicals are being added in the natural environment, which enter the food chain through water, soil, and air resulting serious harmful affects on human health (Ramachandra and Nagarathna, 2003). According to the survey made by the WHO, more than 50,000 people in developing countries are annually poisoned and 5,000 die as a result of the effects of toxic agents, used in agriculture. In India 35,000 – 40,000 tons of hazardous chemicals are sprayed on the crops every year, instead of helping the poor, these chemicals are causing cancer, sterility and death (Das, 1983). To avoid the use of these horrible diseases causing synthetic chemicals, the plants and their products should be utilized to combat phytopathogens. As plants are known to possess various secondary metabolites, which showed inhibitory effect against the growth of pathogens. Keeping these problems in view, efforts are underway to search economic safe phytochemicals, which could be utilized for disease control. Therefore, the screening and testing the efficacy of plants for antibacterial activity was undertaken to explore their antibacterial activity.

MATERIAL AND METHODS

Plant materials *viz.* flowers, leaves, root, seed and stems were collected from various parts of Haryana and their neighbouring states on the basis of their traditional values (Table 1). The collected palnt materials were throughly washed with tap water and then by distilled water and kept in dark in between the filter papers at room temperature till completely dry. Each plant sample was individually grounded into powder form for preparation of extract. The bacteria *Xanthomonas campestri* pv. *campestris* (MTCC No. : 2286) used for the study was obtained from the IMMTC, Chandigarh. The culture was maintained at 4°C on Nutrient Agar Medium with periodic sub-culturing.

Antibacterial test : The experiment was carried out during 2003-2004 in the laboratory conditions at the Department of Biosciences, M.D. University,

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Rohtak, Haryana. Plant part extract (15% w/v) was prepared by brewing in hot water. 15g dry powder of each plant sample was weighed and put in a cheese cloth bag and suspended in 100ml of boiling distilled water for 15 minutes. The final volume should be same by adding distilled boiled water. The supernatants were collected in screw-capped vials and sterilized by autoclaving for 15 minutes at 121°C and the pH was adjusted to 7.0 (Toda *et al.,* 1989).

The assay for antibacterial activity of each plant part extract was tested by agar diffusion method (Mahajan *et al*., 1991). Bacterial suspensions were cultured in peptone water for 6-8h and 0.2ml of this culture was spread on Mueller – Hinton agar in petri dishes. Wells (8mm diameter) were cut in agar plates and were filled 0.1ml of 15% plants extracts. The plates inoculated with *Xanthomonas campestris* pv. *campestris* were incubated at 30±1ºC. 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 12mm including the 8mm well size were considered sensitive and those with less than 12mm as resistant.

Minimum Inhibitory Concentration (MIC) was determined by the agar dilution method after incubation for 40 hours at 30±1ºC (Koneman *et al*., 1988) where plants extract concentration ranged from 0.25% – 3.0%.

RESULTS AND DISCUSSION

The activity of the plant-extracts against the bacterial growth of *Xanthomonas campestris* pv. *campestris* is presented in Table 2. It was observed that out of 20 plants parts extracts tested, nine plant extracts showed inhibitory effect against the bacterial growth of *Xanthomonas campestris* pv. *campestris*. The maximum inhibitory effect was shown by leaves extracts of *Camellia sinensis* (19.5mm), bark extracts of *Acacia arabicae* (18.0mm) and fruit extracts of *Aegle marmelos* (17.5mm), while the bark extracts of *Acacia catechu* (16.5mm), *Achyranthus asper* (16.5mm), root extracts of *Asparagus racemosus* (16.5mm), leaf extracts of *Azadirachta indica* (16.0mm) exhibited more or less equal inhibitory effect on the bacterial growth. The test bacterium was less inhibited by bark extracts of *Callistemon lanceolatus* (14.5mm) and seed extracts of *Acacia fernesiana* (11.0mm). The rest eleven plants samples did not show antibacterial effect against the test bacteria.

Minimum Inhibitory Concentrations (MIC): In general the MIC of various plants extracts was observed 1.0%. *Acacia fernesiana* showed 2.0% MIC while *Achyranthus asper* showed 3.0% MIC. *Asparagus racemosus* showed 0.5% MIC for the test bacteria *Xanthomonas campestris* pv. *campestris* (Table 2).

Among the different plants screened, leaf extracts of *Camellia sinensis* and bark extracts of *Acacia arabicae* showed maximum inhibitory activity against *Xanthomonas campestris* pv. *Campestris* (Table 2). The antimicrobial activity of *Camellia sinensis* extracts have been attributed to its different components like caffeine, tannins and other polyphenolic compounds particularly gallocatechin (Fukai *et al*., 1991; Kubo *et al*., 1992). The use of tea extracts for protecting plants against pathogenic organisms have earlier been suggested by Dubey, (1991). The inhibitory activity of the bark extracts of *Acacia arabicae* might be due to the presence of some antimicrobial secondary metabolites (Parkash and Garg, 1981; Usher, 1971; Pandey, 1993). The test bacterium was inhibited by the fruit extracts of *Aegle marmelos*, which might be due to the presence of some antimicrobial secondary metabolites present the plant. The plant possesses various medicinal (Usher, 1971; Pandey, 1993) as well as various antimicrobial properties (Ganesan *et al*., 2004).

The bark extracts of *Acacia catechu* showed inhibitory activity against *Xanthomonas campestris* pv. *campestris*, which could be due to the presence of some antimicrobial phytochemicals (Chopra *et al*., 1992; Panday, 1993; Singh and Sharma, 1978). The root extracts of *Asparagus racemosus* was observed effective against the growth of *Xanthomonas campestris pv. campestris*. The plant is reported to possess various medicinal

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

Botanical Name	*Common Name*	*Name of Family*	*Distribution*	*Traditional Uses of Plants*
Acacia arabicae	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).
Acacia catechu	Katha	*Mimosaceae*	East India	Used in the treatment of diarrhea and throat infections (Usher, 1971).
Acacia fernesiana	Ghand Babul	*Mimosaceae*	Tropics	Flowers are a source of essential oil used in perfumery (Usher, 1971).
Achyranthus asper	Chirchita	*Amaranthaceae*	Asia	Pulmonary affections cough asthma and skin diseases (Dastur, 1962).
Adhatoda vasica	Adusa	*Acanthaceae*	Tropical India	A decoction of the leaves is expectorant, and is used to relieve bronchitis (Usher, 1971).
Aegle marmelos	Bael Patter	*Rutaceae*	India	A decoction of the leaves is a febrifuge and expectorant and is particularly used for asthmatic complaints. Also used to treat acute bronchitis, fever and dysentery (Dastur, 1962).
Albizia lebbeck	Siris	*Mimosaceae*	Tropical Asia to Australia	The bark is used to treat boils and the leaves and seeds to treat diseases of the eyes (Usher, 1971).
Aloe vera	Gawar Patha	*Liliaceae*	Mediterranean. Introduces to New World Tropics.	The active principle is aloin which is used to treat intestinal worms, to encourage menstruation and as a cathartic (Usher, 1971).
Alstonia scholaris	Chitvan	*Apocynaceae*	Ceylon to Australia	The dried bark has been used since ancient times as a tonic and to treat intestinal complaints, including worms (Usher, 1971).
Anthocephalus cadamba	Kadam	*Rubiaceae*	Tropical Asia	The bark is used as a tonic and reduces fever (Usher, 1971).
Asparagus racemosus	Satawari	*Liliaceae*	Middle East, India, Australia	The roots are applied to relieve irritations. They are also used to treat dysentery, and are diuretic (Usher, 1971).
Astercantha longifolia	Talamkhana	*Acanthaceae*	India	Decoction of root is diuretic; seeds are given in gonorrhoea, and with milk sugar in spermatorrhoea (Vasishta, 1972).

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Azadirachta indica	Neem	*Meliaceae*	East India, Ceylon	Non-drying oil is extracted from the seeds. It is used for soap-making and to treat skin diseases, locally. The bark and leaf extracts are used as a tonic, and to reduce fevers (Usher, 1971).
Bambusa sapinosa	Bans	*Gramineae*	East India	Boiled young shoots eaten locally as a vegetable. Wood used for general construction work. (Usher, 1971).
Brassicae campestris	Sarson	*Cruciferae*	Temperate Europe, Asia, introduced to N. America. Grown around the Black Sea	The oil (Ravinson Oil), extracted from the seeds. It is used locally as a luminant, Lubricant, and in the manufacture of soap (Usher, 1971).
Bryophyllum calycinum	Patherchat	*Crassulaceae*	Throughout India & N. Temprate	Leaves are useful in vitiated conditions of *pitta* and *vata*, haematemesis, haemorrhoids, menorrhagia, cuts and wounds, discolouration of the skin, boils, sloughing ulcers, burns, scalds, corn, diarrhoea, dysentery, vomiting and acute inflammations (Sala, 1995).
Caesalpinia bonducella	Kamju	*Caesalpiniaceae*	Tropics	In India seeds are mixed with black pepper to make a tonic and to reduce fevers. A tonic is also made from the bark (Usher, 1971).
Callistemon lanceolatus	Bottle Brush	*Myrtaceae*	Australlia , India	Leaves are a Tea substitute and have a delightfully refreshing flavour (Cribb and Cribb, 1976), tan dye is obtained from the leaves (Grae, 1974).
Calotropis procera	Ak	*Ascliapdaceae*	Tropical Africa and India	The root bark is used to treat leprosy in India (Usher, 1971).
Camellia sinensis	Chai	*Theaceae*	India and China	Astrigent, diuretic stimulant (Chopra *et al*., 1992).

(Usher, 1971; Pandey, 1993) and various antifungal properties against phytopathogenic fungi (Mishra and Dixit, 1977; Singh and Sharma, 1978; Chitra and Kannabiran, 2002). The stem extracts of *Achyranthus asper* was found effective against the growth of *Xanthomonas campestris pv. campestris*. The plant is reported to possess various medicinal (Usher, 1971; Pandey, 1993) and various antibacterial properties (Aswal *et al*., 1984; Newton *et al*., 2002). The leaf extracts of *Azadirachta indica* was observed effective against the bacterial growth of *Xanthomonas campestris* pv. *campestris*. It is known to possess various medicinal as well as antimicrobial properties

Table 2. Anti-bacterial activity and Minimum Inhibitory Concentrations (MIC) of Plant-extracts against *Xanthomonas campestris* pv. *campestris*

Plant Species	Part Used	Zone of Inhibition (mm)*	Minimum Inhibitory Concentrations (%)				
			0.25	0.5	1.0	2.0	3.0
Acacia arabicae	Bark	18.0 ± 1.24	+	+	-	-	-
Acacia catechu	Bark	16.5 ± 2.15	+	+	-	-	-
Acacia fernesiana	Seed	11.0 ± 1.78	+	+	+	-	-
Achyranthus asper	Stem	16.5 ± 1.88	+	+	+	+	-
Adhatoda vasica	Flower	-	NT	NT	NT	NT	NT
Aegle marmelos	Fruit	17.5 ± 1.13	+	+	-	-	-
Albizia lebbeck	Seed	-	NT	NT	NT	NT	NT
Aloe vera	Stem	-	NT	NT	NT	NT	NT
Alstonia scholaris	Leaf	-	NT	NT	NT	NT	NT
Anthocephalus cadamba	Bark	-	NT	NT	NT	NT	NT
Asparagus racemosus	Root	16.5 ± 1.16	+	-	-	-	-
Astercantha longifolia	Seed	-	NT	NT	NT	NT	NT
Azadirachta indica	Leaf	16.0 ± 0.84	+	+	-	-	-
Bambusa sapinosa	Seed	-	NT	NT	NT	NT	NT
Brassicae campestris	Seed	-	NT	NT	NT	NT	NT
Bryophyllum calycinum	Leaf	-	NT	NT	NT	NT	NT
Caesalpinia bonducella	Leaf	-	NT	NT	NT	NT	NT
Callistemon lanceolatus	Bark	14.5 ± 1.46	+	+	-	-	-
Calotropis procera	Leaf	-	NT	NT	NT	NT	NT
Camellia sinensis	Leaf	19.5 ± 1.25	+	+	-	-	-

*Mean ± SD NT = Not Tested

(Sharma and Nanda, 2000; Newton *et al*., 2002). The bark extracts of *Callistemon lanceolatus* was found effective against the test bacterium. The plant possesses various antimicrobial properties (Dubey et al., 1990). The seed extracts of *Acacia fernesiana* was found effective. The plant is reported to possess various traditional properties (Usher, 1971).

The test bacteria *Xanthomonas campestris* pv. *campestris* was observed sensitive to a very low concentration (0.5%) of the aqueous extracts of *Asparagus racemosus*. The Minimum Inhibitory Concentrations was found slightly higher in case of *Acacia arabicae*, *Acacia catechu*, *Aegle marmelos*, *Azadirachta indica*, *Callistemon lanceolatus* and *Camellia sinensis* against the test bacterium while *Acacia fernesiana* and *Achyranthus asper* were observed to show inhibitory effect against the *Xanthomonas campestris* pv. *campestris* at higher concentrations as compared to others tested plants samples (Table 2). The variations in the Minimum Inhibitory Concentrations might be due to differences in phytochemicals composition (Owuor, *et al*., 1986; Toda *et al*., 1989).

Since the extracts of *Acacia arabicae* and *Acacia fernesiana*, *Achyranthus asper* and *Callistemon lanceolatus* used in this study have not been tested before as inhibitor of phytopathogenic bacteria, therefore, this is a new report. 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 (Aswal *et al*., 1984; Abraham *et al*., 1986; Chopra *et al*., 1992) which may be responsible for the antibacterial properties of the plants studied.

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