Biosciences, Biotechnology Research Asia Vol. **4**(2), 693-698 (2007)

Antibacterial properties of some plant extracts against plant pathogenic bacteria *Rathyibacter tritici*

S.K. BHARDWAJ* and J.S. LAURA

Department of Biosciences, M. D. University, Rohtak, Haryana (India)

(Received: July 12, 2007; Accepted: September 17, 2007)

ABSTRACT

The aqueous extracts of twenty plants were screened by agar diffusion methods for their antibacterial activity against *Rathyibacter tritici*, a causal organism of tundu diseases of wheat. The *Rathyibacter tritici* was found most sensitive to the leaves extracts of *Camellia sinensis*. Some of the other plants showed the activity against the test bacteria in the following order *Aegle marmelos> Azadirachta indica> Callistemon lanceolatus> Calotropis procera> Acacia arabicae> Brassica campestris> Adhatoda vasica*.

Key words: *Rathyibacter tritici*, antibacterial activity, plants extracts, phytochemicals.

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 as a result it seriously affect the human health[1]. According to the WHO survey, 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[2]. So there is an urgent need to develop sustainable methods for these horrible diseases. As plants and their product are known to possess various secondary metabolites, which showed inhibitory effect against the growth of pathogens, therefore, the plants and their product should be utilized to combat the diseases causing pathogens. Keeping these problems in view, efforts are underway to search economic safe phytochemicals, which could be utilized for disease control. Thus the object of present study was to exploit the potential of different plants extracts against *Rathyibacter tritici* for selecting the most potent plant possessing antibacterial activities.

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 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 *Rathyibacter tritici* used for the study was procured from cultures stocks of our Department. The culture was maintained at 4°C on Nutrient Agar medium with periodic sub-culturing.

Antibacterial tests

Fifteen percent plants parts extracts was prepared by brewing in boiling water for 15 minutes followed by centrifugation at 12000 rpm for 15 minutes. 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[3].

The assay for antibacterial activity of each plant part extract was tested by agar diffusion method[4]. 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 diam) were cut in agar plates and were filled 0.1ml of 15% plants extracts. The plates inoculated with *Rathyibacter tritici* were incubated at 37°C. 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[5] 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 40hours at 37°C.

RESULTS

The activity of the plants extracts against the bacterial growth of *Rathyibacter tritici* is presented in Table 2 and Fig. 1. It is commonly observed that out of 20 plants parts extracts tested the leaves extracts of *Camellia sinensis* (22.0mm) showed marvelous inhibitory effect against the bacterial growth of *Rathyibacter tritici*. The strong inhibitory effect was shown by fruit extracts of *Aegle marmelos* (17.5mm) and appreciable inhibitory effect was showed by leaves extracts of *Azadirachta indica* (14.5mm) and bark extracts of *Callistemon lanceolatus* (14.0mm) against the test bacteria, while two plants samples also showed more or less equal inhibitory effect on the bacterial growth i.e. bark extracts of *Acacia arabicae* (12.5mm) and leaves extracts of *Calotropis procera* (13.0mm). The test bacteria was observed resistant to flower extracts of *Adhatoda vasica* (10.0mm) seed extracts of *Brassicae campestris* (10.5mm). The rest twelve plants samples did not show antibacterial effect against the test bacteria.

Minimum Inhibitory Concentrations (MIC)

The MIC of four plants samples i.e. *Acacia arabicae Adhatoda vasica Brassicae campestris* and *Callistemon lanceolatus* were observed 2.0% for the test bacteria. *Aegle marmelos* and *Calotropis procera* showed 1.0% Minimum Inhibitory Concentrations while *Azadirachta indica* and *Camellia sinensis* showed 0.5% Minimum Inhibitory Concentrations for the test bacteria *Rathyibacter tritici* as presented in Table 2.

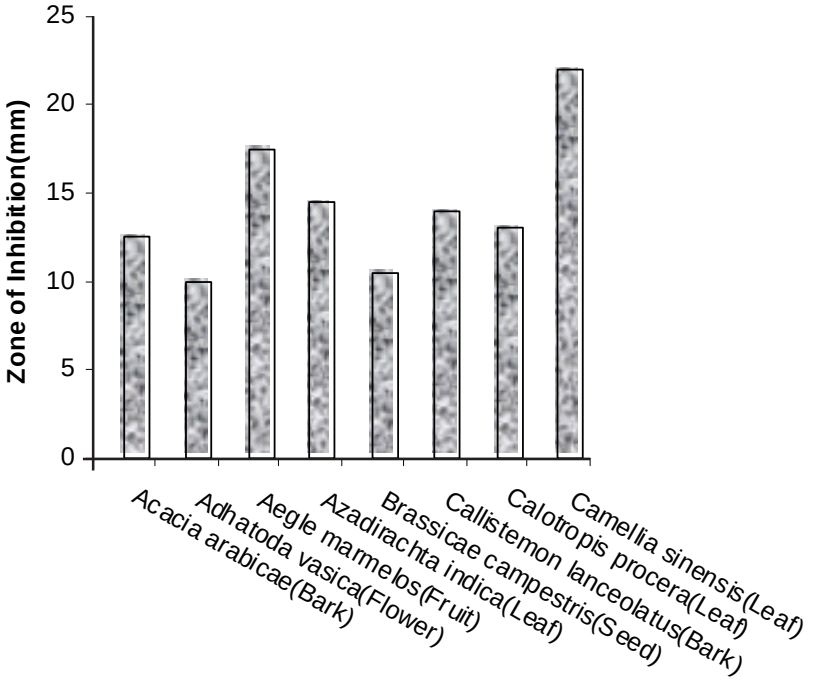

Fig. 1. Effect of various plant samples on the growth of *Rathyibacter tritici*

Table 1: Details of plants used in the experiment with their traditional uses

S. No.	Botanical Name	Common Name	Family	Distribution	Traditional Uses of Plants
1.	*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 (9).
2.	*Acacia catechu*	Katha	*Mimosaceae*	East India	Used in the treatment of diarrhea and throat infections (9).
3.	*Acacia fernesiana*	Ghand Babul	*Mimosaceae*	Tropics	Flowers are a source of essential oil used in perfumery (9).
4.	*Achyranthus aspera*	Chirchita	*Amaranthaceae*	Asia	Pulmonary affections cough asthma & skin diseases (23).
5.	*Adhatoda vasica*	Adusa	*Acanthaceae*	Tropical India	A decoction of the leaves is expectorant, & is used to relieve bronchitis (9).
6.	*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 (23).
7.	*Albizia lebbeck*	Siris	*Mimosaceae*	Tropical Asia to Australia	The bark is used to treat boils and the to Australia leaves and seeds to treat diseases of the eyes (9).
8.	*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 (9).
9.	*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 (9).
10.	*Anthocephalos. cadamba*	Kadam	*Rubiaceae*	Tropical Asia	The bark is used as a tonic and reduces fever (9).
11.	*Asparagus racemosus*	Satawari	*Liliaceae* India vs Australia	Middle East,	The roots are applied to relieve irritations. They are also used to treat dysentery, and are diuretic (9).
12.	*Astercantha longifolia*	Talamkhana	*Acanthaceae*	India	Decoction of root is diuretic; seeds are given in gonorrhoea, and with milk sugar in spermatorrhoea (24).
13.	*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 (9).
14.	*Bambusa sapinosa*	Bans	*Gramineae*	East India	Boiled young shoots eaten locally as a vegetable. Wood used for general construction work (9).
15.	*Brassica 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 (9).
16.	*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 (25).
17.	*Caesalpinia bonducella*	Karnju	*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 (9).
18.	*Callistemon lanceolatus*	Bottle Brush	*Myrtaceae*	Australlia , India	Leaves are a Tea substitute and have a delightfully refreshing flavour (26); tan dye is obtained from the leaves (27).
19.	*Calotropis procera*	Ak	*Ascliapdaceae*	Tropical Africa & India	The root bark is used to treat leprosy in India (9).
20.	*Camellia sinensis*	Chai	*Theaceae*	India and China	Astrigent, diuretic stimulant (22).

DISCUSSION

Considering the need for an alternative eco-friendly approach to control the phyto pathogens, 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 *Rathyibacter tritici* 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 whose extracts were found to be effective are leaves extracts of *Camellia sinensis* and fruit extracts of *Aegle marmelos* Fig. 1. The test bacteria *Rathyibacter tritici* was observed most sensitive to leaves extracts of *Camellia sinensis* and showed maximum potential, because the antimicrobial activity of *Camellia sinensis* extracts have been attributed to its different components like caffeine, tannins and other polyphenolic compounds particularly gallocatechin[6,7]. The use of tea sprays could be used for protecting plants against pathogenic organisms. Such applications have also been suggested earlier[8]. The test bacterium was observed sensitive to the fruit extracts of *Aegle marmelos* Fig. 1, which might be due to presence of some bioactive agent in the plant samples as plant possesses various medicinal[9,10] as well as various antimicrobial properties[11]. The leaf extracts of *Azadirachta indica* was observed effective against the bacterial growth of *Rathyibacter tritici* Fig. 1. Earlier, *Azadirachta indica* was found in literatures to possess various medicinal as well as antimicrobial properties by several workers [12,13]. The bark extracts of *Acacia arabicae* showed inhibitory effect against the bacterial growth of test bacteria Fig. 1, which might be due to the presence of some antimicrobial secondary metabolites in the plant sample, some

Table 2: Antibacterial activity and Minimum Inhibitory Concentrations (MIC) of Plant extracts against *Rathyibacter tritici*

S No.	Name of the Plant	Part Used	Zone of Inhibition (mm)	Minimum inhibitory concentrations (%)				
				0.25	0.5	1.0	2.0	3.0
1.	*Acacia arabica*	Bark	12.5 ± 1.39	+	+	+	-	-
2.	*Acacia catechu*	Bark	-	NT	NT	NT	NT	NT
3.	*Acacia fernesiana*	Seed	-	NT	NT	NT	NT	NT
4.	*Achyranthus aspera*	Stem	-	NT	NT	NT	NT	NT
5.	*Adhatoda vasica*	Flower	10.0 ± 3.25	+	+	+	-	-
6.	*Aegle marmelos*	Fruit	17.5 ± 0.74	+	+	-	-	-
7.	*Albizia lebbeck*	Seed	-	NT	NT	NT	NT	NT
8.	*Aloe vera*	Stem	-	NT	NT	NT	NT	NT
9.	*Alstonia scholaris*	Leaf	-	NT	NT	NT	NT	NT
10.	*Anthocephalus cadamba*	Bark	-	NT	NT	NT	NT	NT
11.	*Asparagus racemosus*	Root	-	NT	NT	NT	NT	NT
12.	*Astercantha longifolia*	Seed	-	NT	NT	NT	NT	NT
13.	*Azadirachta indica*	Leaf	14.5 ± 1.65	+	-	-	-	-
14.	*Bambusa sapinosa*	Seed	-	NT	NT	NT	NT	NT
15.	*Brassicae campestris*	Seed	10.5 ± 2.45	+	+	+	-	-
16.	*Bryophyllum calycinum*	Leaf	-	NT	NT	NT	NT	NT
17.	*Caesalpinia bonducella*	Leaf	-	NT	NT	NT	NT	NT
18.	*Callistemon lanceolatus*	Bark	14.0 ± 1.95	+	+	+	-	-
19.	*Calotropis procera*	Leaf	13.0 ± 1.73	+	+	-	-	-
20.	*Camellia sinensis*	Leaf	22.0 ± 0.82	+	-	-	-	-

*Mean ± SD NT = Not Tested

phytochemicals have also been reported in literature[14] and possess various medicinal properties[9,10] hence, the spray of the extracts of *Acacia arabicae* could be used for protecting plants against pathogenic organisms instead of synthetic chemicals.

Minimum Inhibitory Concentration

In general the MIC of various plants samples ranges from 0.5% - 2.0% as presented in Table 2. The test bacteria *Rathyibacter tritici* was observed sensitive at very low concentrations of the aqueous extracts of *Azadirachta indica* and *Camellia sinensis*. Minimum Inhibitory Concentrations was found slightly higher in case of *Acacia arabicae*, *Aegle marmelos* and *Calotropis procera* against the test bacterium while *Acacia arabicae, Adhatoda vasica, Brassicae campestris* and *Callistemon lanceolatus* were observed to show inhibitory effect against the *Rathyiabacter tritici* 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[3,15].

The antimicrobial activities of plants studied have also been found registered in various literature i.e *Adhatoda vasica*[16], *Brassicae campestris*[17], *Callistemon lanceolatus*[18], *Calotropis procera*[19].

Since the extracts of *Acacia arabicae* used in this study have not been tested before as inhibitor of phytopathogenic bacteria, 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[20,21,22] which may be responsible for the antibacterial properties of the plants studied.

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