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Antibacterial activity of some plant extracts against *Rathyibacter tritici*

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ABSTRACT

The aqueous extracts from 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 seed extracts of *Acacia arabicae* and seed extracts of *Casuarinae equisetifolia*. Some of the other plants showed the activity against the test bacteria in the following order *Cuscuta reflexa* > *Anthocephalus cadamba*> *Azadirachta indica*> *Capparis decidua* > *Cassia fistula* > *Cannavis sativa* > *Brassicae jaunca*> *Clerodendron inerme*.

Key words: *Rathyibacter tritici*, Antibacterial Activity, Plants-extracts, Phytochemicals.

INTRODUCTION

The ever expanding world population requires the production of huge quantities of food, but our efforts are hampered due to various diseases caused by phytopathogens. There is an urgent need for a quantum jump in productivity, particularly of the major crop wheat, that too with the present trend of declining per capita availability of arable land. 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 in continuations of previous paper[3], the object of present study was to exploit the potential of some more 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.

Table 1: Common names and families of plants used in experiments

S. No.	Botanical Name	Common Name	Name of Family	Distribution	Traditional Uses of Plants
1.	*Acacia arabica* 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[8].
2.	*Acacia catechu* Willd.	Katha	*Mimosaceae*	East India	Used in the treatment of diarrhea and throat infections[8].
3.	*Adhatoda vasica* Nees.	Adusa	*Acanthaceae*	Tropical India	A decoction of the leaves is expectorant, and is used to relieve bronchitis[8].
4.	*Aegle marmelos* (L.)	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[19].
5.	*Albizia stipulata* Benthm.	Siris	*Mimosaceae*	Tropical Asia to Australia	The bark is used to treat boils and the leaves and seeds to treat diseases of the eyes[8].
6.	*Anthocephalus cadamba* (Mig.)	Kadam	*Rubiaceae*	Tropical Asia	The bark is used as a tonic and reduces fever[8].
7.	*Azadirachta indica* (A.) Juss.	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[8].
8.	*Brassica juncea* (L.)	Sarson	*Cruciferae*	Temperate Europe Asia, introduced to N. America, Black Sea	The oil (Ravinson Oil), extracted from the seeds. It is used locally as a luminant, Lubricant, and in the manufacture of soap[8].
9.	*Cannabis sativa* (L.)	Bhang	*Cannabidaceae*	Central Asia	Fibres used for cordage, sailcloth and caulking boat, seeds used in manufacture of paints, varnishes and soap, drug (bhang, hashish, ganja and marihuana) is produced. Its use is illegal in many countries[8].
10.	*Capparis decidua* (Roth.)	Karil	*Capparidaceae*	Sahara	Fruits eaten locally[8].
11.	*Carissa carandus* (L.)	Kraundha	*Apocynaceae*	India to Malaysia	The red, plum-like berries are eaten locally and made into jellies and preserves[8].

12.	*Cassia fistula* (L.)	Amaltash	*Leguminosae*	Tropical Africa	The pulp of pods is used as a laxative[8].
13.	*Casuarina equisetifolia* (L.)	Chok/Jhau	*Casuarinaceae*	New South Wales, Queensland, India	Wood is used for roof shingles and posting[8].
14.	*Cedrela toona* (Roxb.)	Toon	*Meliaceae*	India to Australia	Flowers are source of a red and yellow dye, wood is used for furniture, house building, tea chests, oil casks and cigar box[8].
15.	*Ceiba pentandra* (Benth.)	Kapok Tree	*Bombraceae*	South America, India	The fibres are insect repellent; gum is laxative and in bowel complaints, juice from its roots is a cure for diabetes[20].
used					
16.	*Citrus limon* (Burmann.)	Nimbu	*Rutaceae*	Sub Tropical Asia, Greeks and Romanas , Azores, California and Italy	Fruits are good source of Vitamin C and B_1, carotene, Juice is extracted and used for fruit drinks, confectionery and flavouring, also a commercial of citric acid. Lemon oil is used in perfumery, flavouring foods, flavouring liqueurs[8].
source					
17.	*Clerodendron inerme* (Gaertn.)	Lanjai	*Verbenaceae*	Tropical and Sub Tropical, India	Used as blood purifier[20].
18.	*Colvillea wallichii* (L.)	Losara	*Boraginaceae*	India	Fruits are demulcient, expectorant and useful in bronchial affections and in irritation of urinary passages[20].
19.	*Curcuma domestica* (L.)	Haldi	*Zingiberaceae*	South Asia, India, China, East Indies and West Indies	Rhizome is a source of yellow dye. In India and Far East the juice is used for treating stomach complaints, bruises; fumes from the burning rhizome relieve colds and catarrh, and a paste of the rhizome accelerates the formation of scabs caused by smallpox and chickenpox[8].
20.	*Cuscuta reflexa* (L.)	Amar Bel	*Convolvulaceae*	Tropical and Temperate, India, Western Peninsula and Baluchistan	Seeds are carminative and anthelmatic; plant used externally against itch, internally in protracted fevers; Infusion of the plant is used to wash sores[20].

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[4].

The assay for antibacterial activity of each plant part extract was tested by agar diffusion method[5]. 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[6] 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 seed extracts of *Acacia arabicae* (22.5mm) and seed extracts of *Casuarinae equisetifolia* (22.0mm) showed marvelous inhibitory effect against the bacterial growth of *Rathyibacter tritici*. The strong inhibitory effect was shown by whole plant extracts

Table 2: Antibacterial activity and minimum inhibitory concentrations (MIC) of plant extracts against *Rathyibacter tritici*

S. 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 arabica* Willd.	Seed	22.5±0.55	+	-	-	-	-
2.	*A. catechu* Willd.	Root	-	NT	NT	NT	NT	NT
3.	*Adhatoda vasica* Nees.	Leaf	-	NT	NT	NT	NT	NT
4.	*Aegle marmelos* (L.)	Leaf	-	NT	NT	NT	NT	NT
5.	*Albizia stipulata* (Benthm.)	Seed	-	NT	NT	NT	NT	NT
6.	*Anthocephalus cadamba* (Mig.)	Leaf	17.5±1.15	+	+	-	-	-
7.	*Azadirachta indica* (A.) Juss.	Seed	17.0±1.35	+	-	-	-	-
8.	*Brassica juncea* (L.)	Seed	11.5±3.25	+	+	+	-	-
9.	*Cannabis sativa* (L.)	Leaf	14.5±2.20	+	+	-	-	-
10.	*Capparis decidua* (Roth.)	Seed	16.5±1.25	+	+	+	-	-
11.	*Carissa carandus* (L.)	Fruit	-	NT	NT	NT	NT	NT
12.	*Cassia fistula* (L.)	Seed	16.5±1.11	+	+	+	-	-
13.	*Casuarina equisetifolia* (L.)	Seed	22.0±1.55	+	+	+	-	-
14.	*Cedrela toona* (Roxb.)	Leaf	-	NT	NT	NT	NT	NT
15.	*Ceiba pentandra* (Benth.)	Seed	-	NT	NT	NT	NT	NT
16.	*Citrus limon* (Burmann.)	Seed	-	NT	NT	NT	NT	NT
17.	*Clerodendron inerme* (Gaertn.)	Leaf	11.0±3.65	+	+	+	+	-
18.	*Colvillea wallichii* (L.)	Leaf	-	NT	NT	NT	NT	NT
19.	*Curcuma domestica* (L.)	Rhizome	-	NT	NT	NT	NT	NT
20.	*Cuscuta reflexa* (L.)	Whole Plant	18.5±1.65	+	+	+	-	-

*Mean ± SD NT = Not Tested

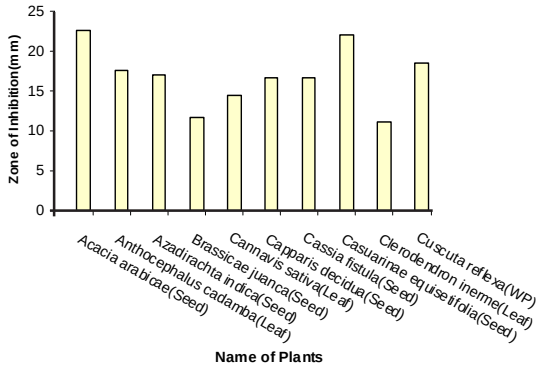

Fig. 1: Antibacterial activity of plants extracts against *Rathyibacter tritici*

of *Cuscuta reflexa* (18.5mm), leaf extracts of *Anthocephalus cadamba* (17.5mm), seed extracts of *Azadirachta indica* (17.0mm), seed extracts of *Capparis decidua* (16.5mm) and seed extracts of *Cassia fistula* (16.5mm) and appreciable inhibitory effect was showed by leaves extracts of *Cannabis sativa* (14.5mm) against the test bacteria. The test bacteria was observed resistant to seed extracts of *Brassicae juanca* (11.5mm) and leaves extracts of *Clerodendron inerme* (11.0mm). The rest ten plants samples did not show antibacterial effect against the test bacteria.

Minimum inhibitory concentrations (MIC)

The MIC of *Clerodendron inerme* was observed 3.0% while the MIC of four plants samples i.e. *Brassicae juanca*, *Capparis decidua*, *Cassia fistula* and *Cuscuta reflexa* were observed 2.0% for the test bacteria. *Anthocephalus cadamba*, *Cannabis sativa* and *Casuarinae equisetifolia* showed 1.0% Minimum Inhibitory Concentrations while *Acacia arabicae* and *Azadirachta indica* showed 0.5% Minimum Inhibitory Concentrations for the test bacteria *Rathyibacter tritici* as presented in Table 2.

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 plants whose extracts were found to be effective are *Acacia arabicae*, *Casuarinae equisetifolia* and *Cuscuta reflexa*.

The seed extracts of *Acacia arabicae* showed strong 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 phytochemicals have also been reported in literature[7] and possess various medicinal properties[8, 9], hence, the spray of the extracts of *Acacia arabicae* could be used for protecting plants against pathogenic organisms instead of synthetic chemicals.

The seed extracts of *Casuarinae equisetifolia* also found strong effective against the bacterial growth of test bacteria, which might be due to the presence of some antimicrobial agents. Various reports were available about the medicinal, biological and economical properties[8,10,11] ; hence, the spray of the extracts of *Casuarinae equisetifolia* could be used for protecting plants against pathogenic organisms.

The antimicrobial activities of plants studied have also been found registered in various literature i.e. *Azadirachta indica*[12, 13], *Cannabis sativa*[14], *Capparis decidua*[15], *Cassia fistula*[16].

Minimum inhibitory concentration

In general the MIC of various plants samples ranges from 0.5% - 3.0% as presented in table 2. The test bacteria *Rathyibacter tritici* was observed sensitive at very low concentrations of the aqueous extracts of *Acacia arabicae* and *Azadirachta indica*. Minimum Inhibitory Concentrations was found slightly higher in case of. *Brassicae juanca*, *Capparis decidua*, *Cassia fistula* and *Cuscuta reflexa* against the test bacterium while *Clerodendron inerme* was 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[4, 17].

Since the extracts of *Anthocephalus cadamba*, *Brassicae juanca*, *Clerodendron inerme* and *Cuscuta reflexa* 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[10,15,18]which may be responsible for the antibacterial properties of the plant studied.

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