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Potential Use of Some Plant Extracts Against Tundu Disease of Wheat (*Triticum aestivum* L.)

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Abstract: The aqueous extracts of twenty plants were screened using agar diffusion method for assessing the antibacterial activity against *Rathyibacter tritici*, the causal organism of tundu disease of wheat. The combined extracts of leaf extracts of *Musa paradisiaca* and *Nerium indicum* in general showed a strong enhancement in activity over the individual extracts of each *Musa paradisiacal* or *Nerium indicum* against the bacterium growth respectively. Some of the other plant extracts such as *Acacia arabicae*, *Acacia catechu*, *Lawsonia alba* and *Mimosa hamata* also showed the inhibitory effect against the tested bacterium in a lower extent.

Key words: *Triticum aestivum* · *Rathyibacter tritici* · Antibacterial Activity · Plants-extracts · Phytochemicals

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second most important winter cereal after rice in India. India holds its stand in wheat production, globally and the production was lower by 2.42 mt (2.55 %) in comparison to previous year's historic production of 94.88 mt as per the estimate of the Directorate of Economics and Statistics, Government of India [1]. Nearly 99% of human population all over the world depends upon wheat for diet and the world still faced a fundamental food security challenge, since agricultural production accounted for less than five percent of the gross world product (an aggregate of all gross domestic products) and the percent of the human population working in agriculture has decreased over time [2]. For the ever increasing world population require the production of huge quantities of wheat 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 wheat. The tundu disease caused by *Rathyibacter tritici* is one of them and is considered a minor disease problem worldwide which is quite common in north-west regions of India. Attempts have been made to manage the disease by treating with chemical compounds [3], which ultimately added in the natural environment, resulting serious affect in the human health [4]. So there is an urgent need to develop sustainable methods for these horrible diseases. Screening and testing of the efficacy of potential plants which are based on the 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.

MATERIALS 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 [5] 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 *Rathyibacter tritici* used for the study was procured from the cultures stocks of our Biosciences 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 (15g/100ml water) by brewing in boiled distilled water for 15 minutes followed by centrifugation

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Table 1: Common Names and Families of Plants used in Experiment

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 [5].
2.	*Anthocephalus cadamba* (Mig.)	Kadam	*Rubiaceae*	Tropical Asia	The bark is used as a tonic and reduces fever [5].
3.	*Cassia nodusa* (Ham.)	Gulabi Amaltash	*Caesalpiniaceae*	West Malaysia	The wood is used for posts and tool handles while roots are used as soap for washing clothes [5].
4.	*Dedonia viscosa* (L.)	Ailyer	*Sapindaceae*	Tropics & Subtropics, Australia	Fruits used as hops in manufacture of beer, bark as an astringentand a poultice [5].
5.	*Jacranda mimosaefolia* (D.Don.)	Nili Gulmohar	*Bignoniaceae*	Tropical South America	The wood is used in general carpentry [5].
6.	*Lagerstroemia flos-reginae* (Retz.)	Jarul	*Lythraceae*	Malaysia	The wood is insect resistant and used for house building, flooring, bridges and railways sleepers [5].
7.	*Lantana camera* (L.)	Ghaneri	*Verbenaceae*	Tropical America	A decoction of the leaves is used locally as a tonic and stimulant [5].
8.	*Lantana macrophylae* (Mart.)	Ghaneri	*Verbenaceae*	South America	A decoction of leaves is used in Brazil to treat rheumatism and the fruits are used to make a tonic [5].
9.	*Lawsonia alba* (L.)	Mahendi	*Lythraceae*	Old World Tropics, N. Africa, Arabia to India.	The bark used to treat jaundice and nervous complaints, flowers yield a scented oil, dried leaves yield a green powder used to dye hair, palm and nails orange brown (Henna) and to dye horses coats and fabric [5].
10.	*Melia azadirachta* (L.)	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 [5].
11.	*Mimosa hamata* (Willd.)	Aill	*Mimosaceae*	Tropical Asia	Tonic, in urinary complaints, glandular swellings, blood-purifier [5].
12.	*Murraya koenigii* (Kurz.)	Kadi Pata	*Rutaceae*	East Asia, Pacific Islands, Himalayas.	A decoction of the bark leaves and root is used locally as a tonic [5].
13.	*Musa paradisiaca* (L.)	Kela	*Musaceae*	Tropical Asia	The high starch content of the fruits, flour from the fruit is an excellent invalid food [5].
14.	*Nerium indicum* (Mill.)	Red Kaner	*Apocynaceae*	Tropical Asia	A poultice of the root is used against ringworm, to induce abortion and for suicide, flowers are used for perfume and produce good honey [5].
15.	*Nicotiana tabocum* (L.)	Tamakhu	*Solanaceae*	Tropical America	The cured and dried leaves are used to make tobacco, snuff ans a source of nicotine for the manufacture of insecticides and nicotine sulphate [5].
16.	*Nychtenthus arbor-tristis* (L.)	Har Sringar	*Verbenaceae*	India	The leaves yield a bright yellow dye [5].
17.	*Ocimum basilicum* (L.)	Ban Tulsi	*Labiatae*	India, S.E. Asia, N.E. Africa	The plant is cultivated for the essential oil used in perfumery, soap making, to flavour liqueurs and sauces [5].
18.	*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 [5].
19.	*Onosoma echinoids* (L.)	Inderjo	*Boraginaceae*	Central Europe To Himalayas	The roots yield a red dye (Orsanette) used in India to dye fats and wool, in place of Alkanna [5].
20.	*Phoenix dactylifera*	Khajur	*Palmae*	California, N. America, Minor Asia	Fruits are eaten fresh or dried, mixed with milk or fermented to make alcoholic beverages [5].

at 12000 rpm for 15 minutes. The extracts were sterilized by autoclaving at 15 lb pressure for 15 minutes and the pH was adjusted to 7.0 with 2.5 mol/ 1 NaOH or 2.7 mol/ 1 HCl [8].

The antibacterial activity was tested by agar diffusion method with slight modification [8]. 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 in size) were cut in agar plates and were filled 0.1ml of 15% plants extracts. The plates inoculated with *Rathyibacter tritici* were incubated at 37° ± 2°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 [9] 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° ± 2°C.

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. Assay for the antibacterial activity was tested by agar diffusion method with slight modification [8].

RESULTS

The activity of the plants extracts against the bacterial growth of *Rathyibacter tritici* is presented in Table 2. It is commonly observed that out of 20 plants parts extracts tested the leaves extracts of *Musa paradisiaca* (24.0 mm) showed marvelous inhibitory effect against the bacterial growth of *Rathyibacter tritici*. The strong inhibitory effect was shown by leaves extracts of *Nerium indicum* (23.0 mm) and appreciable inhibitory effect was showed by leaves extracts of *Acacia arabicae* (19.0 mm), petals extracts of *Mimosa hamata* (18.5 mm) and leaves extracts of *Lawsonia alba* (18.0 mm) against the test bacteria, while seven plants samples also showed more or less equal inhibitory effect on the bacterial growth i.e. and leaves extracts of *Ocimum sanctum* (14.5 mm) and *Lantana macrophylae* (14.0 mm), seed extracts of *Dedonia viscosa* (13.0 mm), petals extracts of *Lantana camera* (13.0 mm), leaves extracts of *Nicotiana tabocum* (13.0 mm), leaves extracts of *Ocimum basilicum* (12.0 mm) and stem extracts of *Phoenix dactylifera* (12.0 mm). The rest eight plants samples did not show antibacterial effect against the test bacteria. The combined extracts of leaves extracts of *Musa paradisiaca* + leaves extracts of *Nerium indicum* showed an enhancement in activities (26.5 mm) over the individual extracts (Table 2).

Minimum Inhibitory Concentrations (MIC): The MIC of six plants samples *i.e*. *Dedonia viscosa*, *Lantana camera*, *Lantana macrophylae*, *Nicotiana tabocum*, *Ocimum basilicum* and *Phoenix dactylifera* were observed 2.0% for the test bacteria and *Acacia arabicae* and *Ocimum sanctum* showed 1.0% Minimum Inhibitory Concentrations while *Lawsonia alba*, *Mimosa hamata*, *Musa paradisiaca* and *Nerium indicum* including combined extracts of *Musa paradisiaca* and *Nerium indicum* 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 different plants, the leaf extracts of *Musa paradisiaca* showed strong inhibitory activity against *Rathyibacter tritici*, which could be due to the presence of some antimicrobial phytochemicals [7, 10], hence, the spray of the extracts of *Acacia arabicae* could be used for protecting plants against pathogenic organisms instead of synthetic chemicals. The leaves extracts of *Nerium indicum* showed inhibitory activity against *Rathyibacter tritici*, which could be due to the presence of some antimicrobial phytochemicals [6, 7, 10, 11], hence, the spray of the extracts of *Nerium indicum* could be used for protecting plants against pathogenic organisms instead of synthetic chemicals. The leaves extracts of *Acacia arabicae*, *Mimosa hamata* and *Lawsonia alba* 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 [6, 7, 12, 13].

Table 2: Anti-bacterial Activity and Minimum Inhibitory Concentrations (MIC) of Plant Extracts against *Rathyibacter tritici*

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.	Leaf	19.0 ± 0.85	+	+	-	-	-
2.	*Anthocephalus cadamba* (Mig.)	Stem	---	NT	NT	NT	NT	NT
3.	*Cassia nodusa* (Ham.)	Seed	---	NT	NT	NT	NT	NT
4.	*Dedonia viscosa* (L.)	Seed	13.0 ± 1.92	+	+	+	-	-
5.	*Jacranda mimosaefolia* (D.Don.)	Seed	---	NT	NT	NT	NT	NT
6.	*Lagerstroemia flos-reginae* (Retz.)	Seed	---	NT	NT	NT	NT	NT
7.	*Lantana camera* (L.)	Petal	13.0 ± 2.75	+	+	+	-	-
8.	*Lantana macrophylae* (Mart.)	Leaf	14.0 ± 2.64	+	+	+	-	-
9.	*Lawsonia alba* (L.)	Leaf	18.0 ± 1.85	+	-	-	-	-
10.	*Melia azadirachta* (L.)	Seed	---	NT	NT	NT	NT	NT
11.	*Mimosa hamata* (Willd.)	Petal	18.5 ± 1.85	+	-	-	-	-
12.	*Murraya koenigii* (Kurz.)	Leaf	---	NT	NT	NT	NT	NT
13.	*Musa paradisiaca* (L.)	Leaf	24.0 ± 0.60	+	-	-	-	-
14.	*Nerium indicum* (Mill.)	Leaf	23.0 ± 0.35	+	-	-	-	-
15.	*Nicotiana tabocum* (L.)	Leaf	13.0 ± 1.80	+	+	+	-	-
16.	*Nychtenthus arbor-tristis* (L.)	Leaf	---	NT	NT	NT	NT	NT
17.	*Ocimum basilicum* (L.)	Leaf	12.0 ± 2.85	+	+	+	-	-
18.	*Ocimum sanctum* (L.)	Leaf	14.5 ± 1.75	+	+	-	-	-
19.	*Onosoma echinoids* (L.)	Pod	---	NT	NT	NT	NT	NT
20.	*Phoenix dactylifera*	Stem	12.0 ± 1.45	+	+	+	-	-
Musa paradisiaca (Leaf) + Combine Extracts *Nerium indicum* (Leaf)			26.5 ± 0.40	+	-	-	-	-

*Mean ± SD NT = Not Tested

The combined samples of leaves extracts of *Musa paradisiaca* + leaves extracts of *Nerium indicum* showed an enhancement in activities over the individual extracts of leaf extracts of *Musa paradisiaca* and *Nerium indicum* 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 leaves extracts of *Musa paradisiaca* + leaves extracts of *Nerium indicum* could be used for protecting wheat crops against pathogenic organisms *Rathyibacter tritici* and a strong substitute 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 while *Lawsonia alba*, *Mimosa hamata*, *Musa paradisiaca* and *Nerium indicum*. Minimum Inhibitory Concentrations was found slightly higher in case of *Acacia arabicae* and *Ocimum sanctum* against the test bacterium while *Dedonia viscosa*, *Lantana camera*, *Lantana macrophylae*, *Nicotiana tabocum*, *Ocimum basilicum* and *Phoenix dactylifera* 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 [8, 14].

The antimicrobial activities of plants studied have also been found registered in various literature i.e. *Dedonia viscosa* [7, 12], *Lantana camera*, *L. macrophylae* and *Phoenix dactylifera* [15], *Nicotiana tabocum* [6, 7] and *Ocimum basilicum*, *O.sanctum* [16].

Since the extracts of *Acacia arabicae*, *Dedonia viscosa*, *Lantana camera*, *Lantana macrophylae*, *Lawsonia alba*, *Mimosa hamata*, *Musa paradisiaca*, *Nerium indicum*, *Nicotiana tabocum*, *Ocimum basilicum*, *Ocimum sanctum* and *Phoenix dactylifera* used in this study have not been tested before as inhibitor of phytopathogenic bacteria *Rathyibacter tritici*, 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, 12, 17], which may be responsible for the antibacterial properties of the plants studied.

CONCLUSION

The study has shown that some plants namely *Musa paradisiaca*, *Nerium indicum*, *Acacia arabicae*, *Mimosa hamata* and *Lawsonia* are very effective in inhibiting the bacterial growth of *Rathyibacter tritici*. 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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