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RESEARCH ARTICLE

Studies on antibacterial activity of some aqueous plant extracts against *Ralstonia solanacearum* causing bacterial wilt and brown rot of potato

■ **G. BISWAL**

SUMMARY

The aqueous leave/flower/seed extracts were prepared from locally grown twenty plants in Depatment of Plant Pathology, Orissa University of Agriculture and Technology in 2009-10 and were tested following inhibition zone technique *in vitro* against *Ralstonia solanacearum* causing bacterial wilt and brown rot of potato. The result indicated that all the aqueous plant extracts exhibited antibacterial property. Maximum zone of inhibition (10mm) was observed in leaf extract of *Musa paradisica* and flower extract of *Nerium indicum* while lowest zone of inhibition (6.68mm) was observed in leaf extract of *Ocimum sanctum* and flower extract of *Ixora coccinea*

Key Words : Aqueous plant extracts, Antibacterial activity, Bacterial wilt, Brown rot

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Potato is third important food crop in the world after outstripping all other food crops. It is a good source of carbohydrates and provides substantial amount of high quality protein, essential vitamins, minerals and trace elements. Due to high protein to calorie ratio, it is more nutritive food than most other food crops and cereals.

The world wide occurrence of potato diseases revealed the presence of 30 fungal diseases, 7 bacterial diseases and 36 viral diseases causing heavy loss individually or collectively to the potato crop. In India, a major loss has been estimated due to late blight (10-70%), bacterial wilt (30-70%), black scurf (10-20%) diseases in potato (Kadian *et al.*, 2007). The bacterial wilt caused by *Ralstonia solanacearum* (E.F.Smith) Yabuuchi *et al.* has been reported from Odisha in 1978 and it is endemic in coastal plains of Odisha, due to prevailing of high water table, warm and humid condition (Shekhawat *et al.*, 1978). The bacterium is primarily tuber borne and can also survive in soil and also have a wide host range affecting brinjal and chilli, mostly grown throughout the year and also tomato preferably in winter season in the coastal plains of the state.

One of the current areas of plant disease management is through the use of plant products which are bio-degrade able, non-hazardous and eco-friendly. During last three decades several reports has been documented on botanical products producing antimicrobial property (Pradhanang *et al.*, 2003). Keeping these in view in the present studies 20 aqueous plant extracts were used to test for their antibacterial property against *Ralstonia solanacearum.*

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MATERIAL AND METHODS

For the evaluation aqueous leave extracts of *Rauvolfia*

serpentina, Punica granatum, Ocimum sanctum, Aerva lanata, Hibsicus rosa–sinensis, Moringa oleifera , Aloe vera, Euphorbia hirta, Polyalthia longifolia, Musa paradisica, flower extracts of Adhatoda vasica, Bauhinia recimosa, Cassia fistula, Impatiens balsamina, Ixora coccinea, Nerium indicum, Psidium guajava, Quisqualis indica and seed extracts of *Cassia tora* and *Cleome viscosa* were used (Table A). The healthy plant parts were collected washed several times in sterilized water and air dried. Fifty grams from each selected plant part along with 50ml of double distilled water were taken and pounded with the help of a mortar and pestle to a fine pulp. The paste was then filtered through two layers of muslin cloth with the application of gentle external pressure to collect maximum filtrate. The filtrate from each plant part was collected separately in different sterile specimen tubes. Each filtrate was then centrifuged at 1500 rpm for 15 minutes to separate out the suspended coarse plant parts. Only the supernatant liquid was drawn carefully into a 5ml syringe and then passed through a membrane filter unit of 0.45nm size to filter sterilize the extract. The filter sterilized extract each pant part thus, collected in a sterilized specimen tube with screw cap and stored in deep freeze maintained at -20^{0}C. Each plant extract was properly labelled with regards to plant species, date of collection and extraction and the part of the plant before storing. The extracts were tested for their antibacterial property following inhibition zone technique (Srilatha and Dhal, 2010).

In this technique, two drops of bacterial suspension of each test bacterium was transferred on to the Petriplate containing NSA medium and spreaded over the surface of the medium with the help of a sterilized glass spreader. Three sets of Hi-media discs (5mm), soaked for one minute in each plant extracts were placed on the media surface of each Petriplate at the equidistance from the centre. In each set four numbers of discs were used to hold sufficient quantity of the plant extract. Two sets of Petridishes were used for testing each plant extract and Petriplates were incubated at 27±1^{0}C for 24 hours in a BOD incubator. After the incubation period, the Petriplates were examined for development of inhibition zone around the discs. The diameters of each zone of inhibition was measured and recorded to assess the antimicrobial properties of plant extracts against each test bacterium. In control the paper discs were soaked in sterilized distilled water and placed over the bacterial smear. The statistical analysis of the data was done

Table A : Local, common and scientific names and traditional uses of test plants

Sr. No	Local name	Common name	Scientific name	Family	Traditional uses of plants
1.	Patalgaruda	Snake root	*Rauvolfia serpentina*	Apocynaceae	Hypertension, Epilepsy, fever, cardiac problem, fever, palpitation (Bhatia,1944)
2.	Dalimba	Pomegranet	*Punica granatum*	Punicaceae	Bactericidal, analgestis, anticoagulants, antiulcer (Morton, 1987)
3.	Tulsi	Sacred basil	*Ocimum sanctum*	Lamiaceae	Fever, asthma, bronchistic, cough (Prakash and Gupta,2005).
4.	Chaya	Aerva plant	*Aerva lanata*	Amaranthaceae	Hypertension, epilepsy, fever(Anita and Malar Retna,2013)
5.	Mandar	Shoe flower	*Hibscus rosa-sinensis*	Malvaceae	Cosmetic and aroma therapy (Kumar *et al*.,2012)
6.	Sajana	Drum stick	*Moringa oleifera*	Moringaceae	Reduces blood pressure(Anwar,2007)
7.	Gheekuanri	Indian aloe	*Aloe vera*	Liliaceae	Treatment of intestinal worms, cosmetic(Usher,1971)
8.	Basanga	Malabarnut tree	*Adhatoda vasica*	Acanthaceae	Treatment of bronchitis (Usher,1971)
9.	Kanchan	Geranium tree	*Bauhinia recimosa*	Caesalpiniaceae	Chemoprotectant (Latha and Panikkar,2001), used against asthma (Nirmal *et al*.,2011)
10.	Hari harika	Euphorbia plant	*Euphorbia hirta*	Caesalpiniaceae	Antimalarial activity(Kumar *et al*.,2010)
11.	Deodaru	False Asohka tree	*Polyalthia longifolia*	Anonaceae	Prevents noise pollution (Katkar *et al*.,2010)
12.	Kadali	Banana	*Musa paradisica*	Musaceae	used against dysentery and diarrhea(Best *et al*.,1984)
13.	Sunari	Indian laburanum	*Cassia fistula*	Caesalpiniaceae	Relieve pain, rduces fever, reduces cholesterol and lower blood pressure (Danish *et al*.,2011)
14.	Haragoura	Garden balsum	*Impatiens balsamina*	Balsaminaceae	Scent and aroma therapy (Ding *et al*.,2008)
15.	Katha Rangani	Jungle geranium	*Ixora coccinia*	Rubiaceae	Roots and barks used against dysentery and diarrhea (Baliga and Kurian,2011)
16.	Karabira	Indian oleander	*Nerium indicum*	Apocynaceae	Flowers were grounded well and along with coconut oil used against scabies, eczema and other skin diseases (Ahmad *et al*., 1991)
17.	Pijuli	Guava	*Psidium guajava*	Myrataceae	Used against fever and diarrhea (Gutierrez *et al*.,2008)
18.	Madhumalati	Rangoon creeper	*Quisqualis indica*	Combretaceae	Ulcers, skin diseases, cut wounds(Sahu *et al*.,2012)
19.	Bana sorisha	Wild mustard	*Cloeme viscosa*	Brassicaceae	Used against fever and diarrhea, cardiac stimulant, carminative (Perumal Samy *et al*.,1999)
20.	Chota Chakunda	Senna tora	*Cassia tora*	Caesalpiniaceae	Used against leprosy, bronchitisc and cardiac disorders, Antimalarial activity (Maity *et al*.,1998)

as per the procedure obtained by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The results revealed that all the extracts had antibacterial property against *R. solanacearum* (Table 1 and Fig.1). Among them leaf extract of *Musa* paradisica (Fig.2) and flower extract of *N. indicum* (Fig.3) exhibited maximum zone of inhibition (10 mm) followed by leaf extract (Fig.4) *of M. oleifera* (9.30 mm) and flower extract of *B. recimosa* (9.30 mm). The flower extract of *A. vasica* produced 8.99 mm zone of inhibition followed

Table 1 : *In-vitro* testing of aqueous leaf/flower /seed extracts of some local available plants

Sr. No.	Scientific name	Plant parts used	Diameter of inhibition zone (mm) against *R. solanacearum*
1.	*Rauvolfia serpentina*	Leaf	7.01 (2.74)
2.	*Punica granatum*	Leaf	8.32 (2.97)
3.	*Ocimum sanctum*	Leaf	6.68 (2.68)
4.	*Aerva lanata*	Leaf	7.68 (2.86)
5.	*Hibscus rosa-sinensis*	Leaf	8.03 (2.92)
6.	*Moringa oleifera*	Leaf	9.30 (3.13)
7.	*Aloe vera*	Leaf	7.01 (2.74)
8.	*Euphorbia hirta*	Leaf	8.32 (2.97)
9.	*Polyalthia longifolia*	Leaf	8.03 (2.92)
10.	*Musa paradisica*	Leaf	10.00 (3.24)
11.	*Adhatoda vasica*	Flower	8.99 (3.08)
12.	*Bauhinia recimosa*	Flower	9.30 (3.24)
13.	*Cassia fistula*	Flower	8.03 (2.92)
14.	*Impatiens balsamina*	Flower	8.03 (2.92)
15.	*Ixora coccinia*	Flower	6.68 (2.68)
16.	*Nerium indicum*	Flower	10.0 (3.24)
17.	*Psidium guajava*	Flower	8.03 (2.92)
18.	*Quisqualis indica*	Flower	8.03 (2.92)
19.	*Cleome viscose*	seed	8.03 (2.92)
20.	*Cassia tora*	seed	8.68 (3.03)
21.	Sterilzed distilled water	S.E.±	0.08
		C.D.(P=0.05)	0.22

$\sqrt{x}$+0.5 transformed values

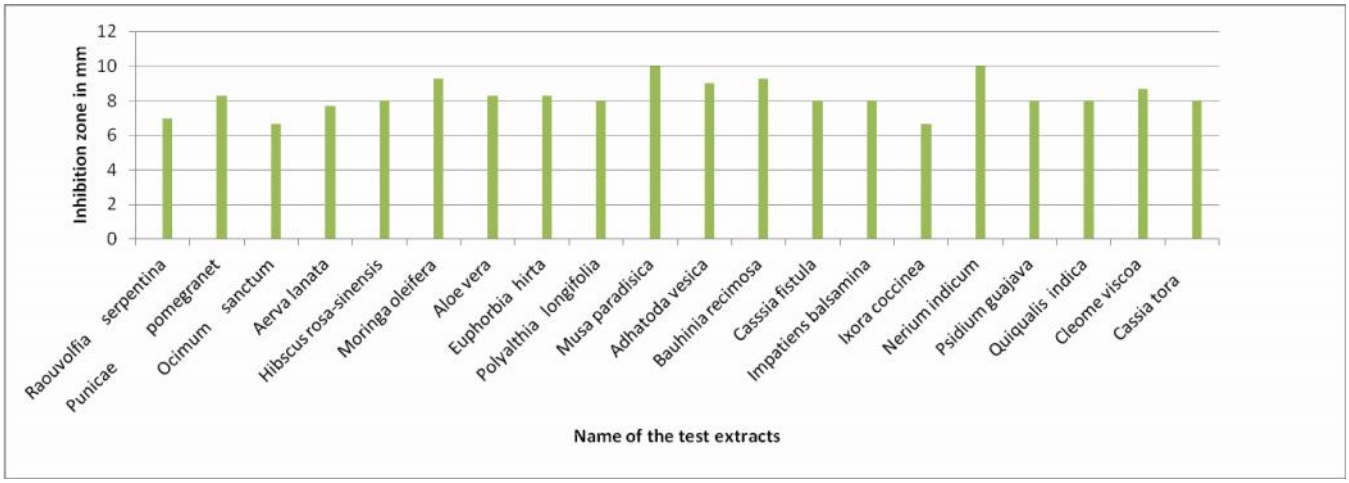

Fig. 1: Histogram showing zone of inhibition of growth of *R. Solanacearum* due to effect of aqueous leaves, flowers and seed extracts of different plant species

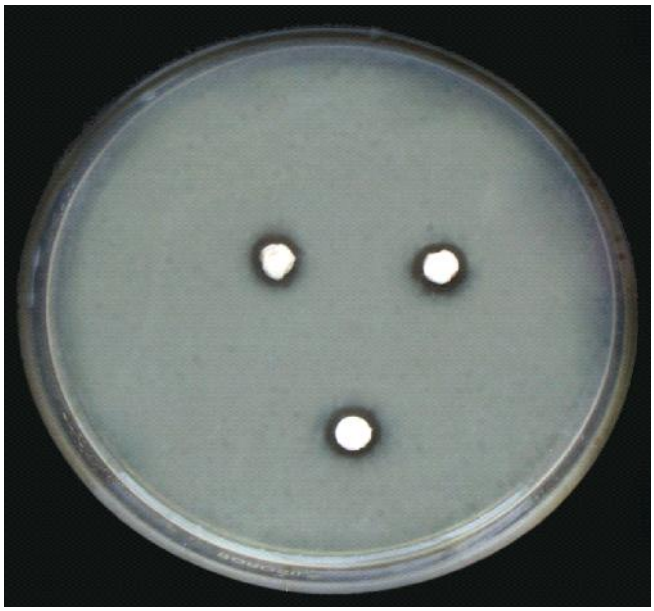

Fig. 2: Inhibition zone produced by aqueous extract of *M. paradisica*

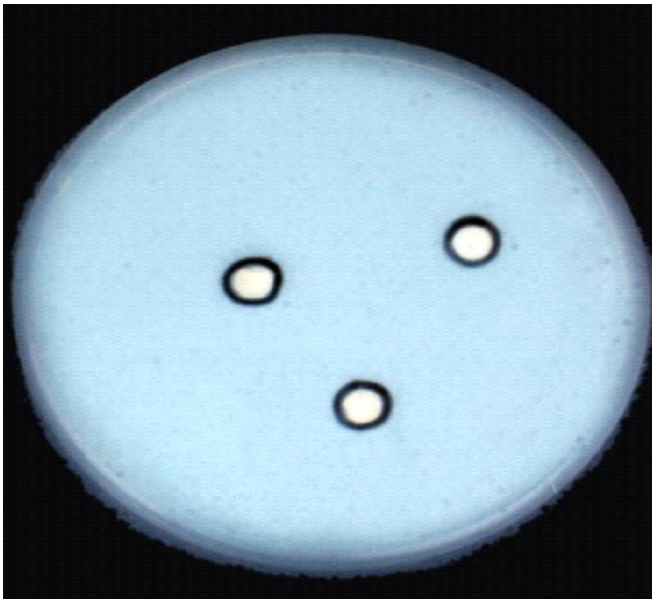

Fig. 3: Inhibition zone produced by aqueous extract of *N. indicum*

Fig. 4: Inhibition zone produced by aqueous extract of *M. oleifera*

by seed extact of *C.viscosa* and the leaf extract both *P. pomegranet* and *E. hirta (*8.32mm). The flower extract of *P. guajava,* the leaf extract *of P. longifolia, H. Rosa-sinensis. A. vera,* the flower extract of *Q. indica, C. fistula, I. balasamina,* the seed extract of *C. tora* exhibited the same zone of inhibition (8.03mm) followed by *A. lanata* (7.68mm) and *R. serpentina* (7.01mm). Among them the flower extract of *I. coccinea* and leaf extact of *O. sanctum* produced lowest zone of inhibition (6.68mm*).* In case of control no inhibition zone was observed (Fig.4). Pradhanang *et al.* (2003) reported the antibacterial property of some plant essential oils against *R. solanacearum* in tomato. The antibacterial property of *A. vera* and *O. sanctum* against *R. solanacearum* in brinjal have already been reported by Srilatha and Dhal (2010). The antibacterial activity of *R. serpentina* and *O. sanctum* has been reported by Upadhyay (2009) and Baskaran (2008). Antimicrobial with cytotoxicity activity has been recorded in *A.lanata* (Chowdhury *et al.*, 2002). Food plant with medicinal uses has been noticed in *M. oleifera* (Anwar, 2007). Kumar *et al.* (2010) cited medicinal uses of *E.hirta. P. betle* leaf extract also exhibited anti–oxidant activity (Dasgupta and De, 2004). Karadi *et al.* (2011) observed antimicrobial activity in *M. paradisica*. Rahim *et al.*(2010) recorded antibacterial activity of *P.guajava* leaf and bark against multi drug resistant *Vibrio cholera*. Antibacterial activity of flower extract of *Adhatoda* (Usher,1971), *N. indicum* (Ramya *et al.*, 2010), *E. crassipes* (Gao-Lei Bo,2004), *C. fistula* (Nayan, 2011), *I. balsamina* (Wang *et al.*, 2009) and medicinal properties of *Q. Indica* and *H. rosa-sinensis* has been recorded by Sahu *et al*. (2012) and Ruban and Gajujakshmi (2012). The seed extracts of *C. viscosa* and *C.tora* also exhibited antibacterial (Ardha Jyoti and Suba Rao 2010 and Shukla *et al.*, 2013). Flayeh and Sylayman (2011) studied antifungal and antibacterial activity of *P. granatum.* The antimicrobial activity of various parts of *P. longifolia* var. *pendulae* (Faizi *et al.*, 2008), chemo-protective effect of *I. coccineae* (Latha and Paniker,2001), pharmacognostical activity of B. *racemosa* (Manohar *et al.*, 2011and Nirmal *et al.*,2011) already been recorded.

The anti bacterial property of leaf extract of *R. serpentina, P. granatum, A. lanata, E. hirta, P. longifolia, M. paradisica, H. rosa–sinensis, M. oleifera, flower extracts of A. basica, B recimosa, C. fistula, I. balsamina, I. coccinea, N. indicum,*

P. gajava,*Q. indica* and seed extracts of *C.tora* and *C. viscosa* is reported to be new in India.

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