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Evaluation of *Tecoma stans* and *Callistemon viminalis* Extracts against Potato Soft Rot Bacteria *In vitro*

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In the present study, the effect of leaves and branches extracts from *Callistemon viminalis* and *Tecoma stans* against the growth of some phytopathogenic potato soft rot bacteria namely; *Dickeya chrysanthemi* (DSM 4610), *Pectobacterium carotovorum* subsp. *carotovorum* (ipp038), *Pectobacterium wasabiae* (ipp041), *Pectobacterium atrosepticum* (1007) and *Dickeya dianthicola* (IPO 2114) were evaluated. *T. stans* leaves and branches extracts were masterly effective against the tested bacterial strains rather than the extracted ones from *C. viminalis* . Both of methanolic crude extract (MCE) and alkaloids (chloroform fraction) found in *T. stans* gave a good profiles of antibacterial activity. On the other hand, the most extracts of *C. viminalis* branches and other aqueous fraction extracts from *C. viminalis* and *T. stans* did not exhibit any activity against the growth of studied bacterial strains. Our pointed results could be considered *T. stans* extracts as bioagents against potato soft rot bacteria

Key words: Extracts, *Tecoma stans*; *Callistemon viminalis*; phytopathogenic; potato soft rot bacteria.

Potato (*Solanum tuberosum* L.) is one of the most important vegetables crops in Egypt for both local consumption and export. From all pathogens infecting potato seed production, bacteria are recognized as the most serious problem (Van der Wolf and De Boer, 2007). Most harmful and damaging bacterial diseases of seed potato production in Egypt are blackleg and soft rot caused by *Pectobacterium* spp. (El-Kazazz, 1984; Abdel-Alim, 1996; Ahmed, 2009; Behiry, 2009, 2013) and recently *Dickeya* species. The economic losses in seed potato production of tubers by bacterial soft rot during storage varied from 31.3% to 36.8% (Rasul *et al*., 1999).

Potato blackleg or soft rot can be caused by *P. atrosepticum*, *P. carotovorum* subsp. *carotovorum*, *P. carotovorum* subsp. *brasiliensis*, *P. wasabiae* and *Dickeya* spp. (Pérombelon, 2002; Duarte *et al*., 2004; Samson *et al*., 2005; Pitman *et al*., 2010). Disease symptoms caused by these different bacterial pathogens are indistinguishable. Resistance in commercial cultivars is largely absent and chemicals to cure tubers and plants during cultivation are not sufficient, moreover hygienic measures are insufficient to prevent seed infections

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(Van der Wolf and De Boer, 2007). Some plant extracts were documented as effective inhibitors of phytopathogenic bacteria (Leksomboon *et al*., 1998, 2000). Antimicrobial activities of several plant extracts against bacterial soft rot of potatoes were evaluated and a quite satisfactory result was obtained (Krebs and Jaggir, 1999; Bdliya *et al*., 2007).

The extracts of ethanol, methanol and water and some solvents fractionated from the methanol crude extract from different parts of *T. stans* had good antimicrobial activity against some pathogenic bacteria and antioxidant activity (Senthilkumar *et al*., 2010; Govindappa *et al*., 2011; Salem *et al*., 2013b). Chemically, the extracts of *T. stans* have been reported to have several bioactive compounds such as saponins, flavonoids, alkaloids, phenols, steroids, anthraquinones, tannins, terpenes, phytosterols, triterpenes, hydrocarbons, resins, volatile oil and glycosides (Binuti and Lajubutu, 1994; Raju *et al*., 2011; Salem *et al*., 2013a) which exhibited various biological activities such as antimicrobial, antifungal and antioxidant activities (Karou *et al*., 2006; Raju *et al*., 2011; Govindappa *et al*., 2011). Binuti and Lajubutu, (1994) observed that the extracts of stem bark were showed better antimicrobial activity than from leaves. Chrysoeriol, luteolin and hyperoside were isolated from the leaves (Ramesh *et al*., 1986).

The extracts dissolved from the inflorescence of *C. viminalis* in water and ethanol extracts have been reported strong antibacterial against *Chromobacterium violaceum* and *Agrobacterium tumefaciens* (Adonizio *et al*., 2006). The aqueous extracts of flowers and leaves have been shown an antibacterial activity (Srivastava *et al*., 2003). Other chemical compounds like *C*-methyl flavonoids, triterpenoids and phloroglucinol derivatives were reported in the genus of *Callistemon* (Chane-Ming *et al*., 1998; Wollenweber *et al*., 2000; Kim *et al*., 2009; Islam *et al*., 2010).

The main objective of this research was to evaluate the effect of some extracts from *Callistemon viminalis* and *Tecoma stans* leaves and branches against the growth of some phytopathogenic bacteria which causing sever soft rot diseases in potato and subsequently to establish it as a potential antibacterial agent.

MATERIALS AND METHODS

Plant material

Leaves and branches of *Callistemon viminalis* and *Tecoma stans* were collected during August, 2012 from Antoniadis Garden, Horticultural Research Institute, Alexandria, Egypt and recoded with voucher numbers at Egypt barcode of life project (www.egyptbol.org), Faculty of Agriculture, Alexandria University.

Preparation of extracts

Leaves and branches of *T. stans* and *C. viminalis* were air-dried at room temperature, ground to fine particles, and extracted in methanol (80%) to obtain the methanol crude extract (MCE). The MCE was concentrated under reduced pressure at 45°C with a rotary evaporator. Five grams from methanol extract was further fractionated by successive solvent extraction with ethyl acetate (EtOAc fraction), chloroform ($CHCl_3$ fraction) and then with *n*-butanol saturated with water (*n*-BuOH fraction). The remaining aqueous fraction was also used for activity testing (Aq. fraction) (Salem *et al*., 2013 a,b). Alkaloids (Cannell 1998) which observed in the chloroform ($CHCl_3$ fraction) were determined. Sample of about 1 g of MCE was dissolved in 50 mL of 99% methanol and treated with an equal volume of 1% aqueous HCl. The alkaloids fraction was precipitated by drop-wise addition of 10% NH_4OH. All the studied solvents were evaporated under reduced pressure at 40-60°C and weight of the dried mass was recorded.

Antibacterial activity

Antibacterial activity of the extracts with concentration of 2000 µg/mL was evaluated against the growth of the phytopathogenic potato soft rot bacteria presented in Table 1.

The agar disc diffusion method was employed for the determination of antimicrobial activities of the extracts (NCCLS, 1997). Briefly, the tested bacteria in a suspension of 0.1 mL of 10^8 CFU/mL were spread over the surfaces of the purred media in Petri dishes. Filter paper discs with 5 mm in diameter were loaded with 20 µL of the extract and placed over the inoculated dishes with the tested bacteria. The inhibition zones (IZs) diameters were recorded in millimeters. Negative control was prepared using respective solvent. Gentamicin (10 µg/disc) was served as a positive control for the tested bacteria. Minimum inhibitory concentrations (MICs) were determined by serial dilution of extracts (100, 250, 500, 1000 and 2000 µg/mL) in 96-well micro-plates (Eloff, 1998). All the extracts were dissolved in 10% dimethyl sulfoxide (DMSO, Sigma-Aldrich) and distilled water solution (1:1 *v/v*).

RESULTS AND DISCUSSION

Antibacterial activity of five different solvent extracts *i.e.*, MEC, EtOAc, $CHCl_3$, *n*-BuOH and Aq fractions at the concentration of 2000 µg/mL of leaves and branches from *T. stans* and *C. viminalis* is presented in Tables 2 and 3.

Data recorded in Table 2 and 3 showed that

The EtOAc and BuOH fractions from branches of *T. stans* gave good antibacterial activity against *Dickeya chrysanthemi* with IZ of 15 mm followed by MCE of *C. viminalis* leaves (14.5 mm IZ). While $CHCl_3$ fraction from leaves and branches of *T. stans* gave good activity against *D. chrysanthemi* with IZs values of 13 and 13.5 mm respectively. Also, the $CHCl_3$ fraction from *C. viminalis* leaves had good activity with IZ value of 12 mm.

Table 1. The bacterial strains used in the present study

No.	Strain No.	Bacterial strain	Geographical origin	Host	Accession No.	Source
1.	DSM 4610	*D.chrysanthemi*	Netherlands	Potato		Dr. Said Behiry Alexandria,Egypt
2.	IPO 2114	*D. dianthicola*	Type strain	Dianthus		
3.	ipp038	*P. carotovorum* subsp. *carotovorum*	Tehran, Iran	Potato	HQ424870.1	
4.	ipp041	*P. wasabiae*	Hamedan, Iran	Potato	HQ424871.1	
5.	1007	*P. atrosepticum*	Type strain	Potato		

The extracts from $CHCl_3$ fraction of branches from *T. stans* and MCE of *C. viminalis* leaves had good positive effect against *Pectobacterium carotovorum* subsp. *carotovorum* with IZ values of 12 mm followed by $CHCl_3$ and EtOAc fractions of leaves from *T. stans* with IZ values of 10.5 and 10 mm, respectively. The MCE from *C. viminalis* leaves showed the highest activity against the growth of *Pectobacterium wasabiae* with IZ value of 17 mm followed by BuOH fraction of *T. stans* branches (IZ value of 12 mm). Also, The MCE from *C. viminalis* leaves showed the highest activity against the growth of *Pectobacterium atrosepticum* with IZ value of 16 mm followed by BuOH fraction of *T. stans* branches (IZ value of 11.5 mm) and EtOAc fraction from *T. stans* leaves with IZ value of 11 mm.

The MCE from *C. viminalis* leaves showed the highest activity against the growth of *Dickeya dianthicola* with IZ value of 16 mm followed by BuOH fraction of *T. stans* branches (IZ value of 15 mm) and $CHCl_3$ fraction from *T. stans* branches with IZ value of 11.5 mm.

The tested phytopathogenic potato soft rot bacteria were showed susceptibility to *T. stans* leaves and branches extracts with different degrees rather than the extracts from *C. viminalis*. The obtained results are in agreement with (Govindappa *et al*., 2011) who showed that tannins, glycosides, triterpenes and steroids were the main groups found in the extracts of *T. stans* and could be responsible for the antibacterial activity. The MCE inhibited the growth of almost the tested bacterial strains. Alkaloids ($CHCl_3$ fraction) found in *T. stans* have been shown to possess good antibacterial activity (Erdemoglu *et al*., 2007; Maiza-Benabdesselam *et al*., 2007). The $CHCl_3$ fraction which contains the precipitated alkaloids was found to own a potential activity against the tested bacterial strains and the same results was found in the present study with potato soft rot bacteria. Phillipson and O'Neill, (1987) observed that action mechanism of alkaloids is attributed to their ability to intercalate with DNA.

Most of the extracts of *C. viminalis* branches did not show any activity against the growth of the studied bacterial strains. Also, the aqueous fraction extracts from *C. viminalis* and *T. stans* didn't show any activity. On the same bacterial strains, (Salem, 2013) showed that the bark extracts of *Delonix regia* and *Erythrina humeana* had a moderate activity against the growth of the studied bacterial strains. The EtOAc extract of *C. viminalis* leaves extract gave betulinic acid (Tshibangu *et al*., 2011). The antibacterial actively of extracts of *C. viminalis* could be related to the presence of several chemical groups like; glycosides, flavanoids, alkaloids, saponins, steroids, and tannins in the extract (Parekh *et al*., 2005; Kaur and Arora, 2009). Alkaloids, flavonoids and some phenols were presented in the polar extracts of *C. viminalis* and tannins, terpenes and quinines in non-polar extracts (Delahaye *et al*., 2009). According to the phytochemical analysis of methanol extract and its fractions from *T. stans* leaves and branches and leaves of *C. viminalis* which was reported in our previously studies (Salem *et al*., 2013 a,b), tannins, flavonoids, alkaloids, saponins and phenolics were the major chemical groups. Flavonoids have the ability to form complex with extracellular, soluble proteins and bacterial cell walls (Tsuchiya *et al*., 1996). Alkaloids and flavonoids have been found in the higher plants control the growth of microbial pathogen (Cannell 1998). Phenolic compounds can inhibit the enzyme by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more nonspecific interactions with the proteins (Balandrin *et al*., 1985; Mason and Wasserman, 1987). The previously results showed that the EtOAc fraction had the highest total phenolic compounds than other fractions and the

Table 2. Effect of extracts from *T. stans* observed against the growth of potato soft rot bacteria

Species	Part	Extract	IZs (mm)				
			DSM4610	Ipp0 38	Ipp0 41	1007	IPO 2114
TS	L	MCE	12.5 (500)	9 (100)	n.a -	10 (100)	9.5 (100)
		EtOAc	11.5 (100)	10 (250)	n.a -	11 (100)	10 (100)
		$CHCl_3$	13.5 (100)	10.5 (100)	n.a -	9 (100)	7.5 (1000)
		BuOH	11 (500)	9.5 (100)	8 (1000)	9.5 (1000)	10.5 (100)
		Aq.	n.a -	n.a -	n.a -	n.a -	n.a -
	B	MCE	12.5 (100)	9.5 (250)	n.a -	10 (100)	10 (100)
		EtOAc	15 (100)	9 (1000)	9 (1000)	10 (100)	10.5 (1000)
		$CHCl_3$	13 (100)	12 (100)	9 (100)	9.5 (100)	11.5 (1000)
		BuOH	15 (100)	n.a -	12 (1000)	11.5 (1000)	15 (100)
		Aq.	n.a -	n.a -	n.a -	n.a -	n.a -
	DMSO		n.a.	n.a.	n.a.	n.a.	n.a.
	Gentamicin*		34	24	25	30	35

*Positive control; discs of 10 µg Gentamicin.
DSM 4610, Dickeya chrysanthemi, ipp038; Pectobacterium carotovorum subsp. *carotovorum, ipp041; Pectobacterium wasabiae, 1007; Pectobacterium atrosepticum,* and *IPO 2114; Dickeya dianthicola*. TS, *Tecoma stans*; L, Leaves; B, Branches; IZs, Inhibition Zones (Values are expressed as mean of three replicates including disc diameter of 5 mm at 2000 µg/mL); MIC: Minimum inhibitory concentration values are given inter parenthesis as µg/mL. DMSO, Dimethylsulfoxide; n.a, not active; —, not determined; MCE- methanol crude extract; EtOAc-ethyl acetate fraction; $CHCl_3$-chloroform fraction; BuOH-*n*-butanol fraction; Aq-aqueous fraction.

high phenolic compounds play the main role and possess potent antimicrobial activities (Cowan, 1999; Adesegun *et al*., 2009). Hernández *et al*., (2005) reported that IZs values are not reflected to the antibacterial activity of a compound. For example, in the present study the MCE from *T. stans* leaves showed IZ value 10 mm against the growth of *Pectobacterium atrosepticum* and the MIC value was 100 µg/mL and the same MIC value was found by MCE of the leaves from *C. viminalis* but with IZ value of 16 mm. Fractionation of the weakly active MCE extract results in more active antibacterial partitions. For example, *Pectobacterium wasabiae* has showed resistance to MCE extract at 2000 µg/mL and the BuOH fraction from the leaves and branches of *T. stans* showed some activity with IZs values of 9 mm and 12 mm, respectively.

Table 3. Effect of extracts from *C. veminalis* observed against the growth of potato soft rot bacteria

Tree	Part	Extract	IZs (mm)				
			DSM4610	ipp038	ipp041	1007	IPO 2114
CV	L	MCE	14.5 (500)	12 (100)	17 (100)	16 (100)	16 (100)
		EtOAc	11 (100)	6 (100)	6 (100)	7 (100)	9 (100)
		$CHCl_3$	12 (100)	9 (100)	n.a —	8 (100)	7 (100)
		BuOH	10 (500)	8 (100)	6 (1000)	9 (1000)	8 (100)
		Aq.	n.a —	n.a —	n.a —	n.a —	n.a —
	B	MCE	10 (100)	8 (100)	n.a —	n.a —	n.a —
		EtOAc	n.a —	n.a —	n.a —	n.a —	n.a —
		$CHCl_3$	n.a —	n.a —	n.a —	n.a —	n.a —
		BuOH	n.a —	n.a —	n.a —	n.a —	n.a —
		Aq.	n.a —	n.a —	n.a —	n.a —	n.a —
	DMSO		n.a.	n.a.	n.a.	n.a.	n.a.
	Gentamicin*		34	24	25	30	35

For legend see Table 2. CV, *Callistemon viminalis*

CONCLUSION

In the present study, the extracts of leaves and branches from *Callistemon viminalis* and *Tecoma stans* were showed different degrees of activities against the growth of some phytopathogenic potato soft rot bacteria namely; *Dickeya chrysanthemi* (DSM4610), *Pectobacterium carotovorum* subsp. *carotovorum* (ipp038), *Pectobacterium wasabiae* (ipp041), *Pectobacterium atrosepticum* (1007) and *Dickeya dianthicola* (IPO 2114) were evaluated. The tested phytopathogenic potato soft rot bacteria were showed susceptibility to *T. stans* leaves and branches extracts with different degrees rather that the extracts from *C. viminalis.* The methanol crude extract inhibited the growth of almost the tested bacterial strains. Alkaloids ($CHCl_3$ fraction) found in *T. stans* have been shown to possess good antibacterial effect. Most of the extracts of *C. viminalis* branches did not show any activity against the growth of the tested bacterial strains.

Also, the aqueous fraction extracts from *C. viminalis* and *T. stans* didn't show any antibacterial activity.

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