Anti-bacterial activity of *Caesalpinia coriaria* (Jacq.) Willd. against plant pathogenic *Xanthomonas* pathovars: an eco-friendly approach

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Powdered leaf and pod material of *Caesalpinia coriaria* (Jacq.) Willd. was extracted with water and successively with different solvents viz., petroleum ether, benzene, chloroform, methanol and ethanol. Anti-bacterial activity assays of all the extracts against the important phytopathogenic *Xanthomonas* pathovars, known to cause diseases in tomato, french bean and cotton, were carried out by cup diffusion method. Aqueous pod extract showed significant activity. Among the five solvents extracts tested, methanol extract of both leaf and pod was most active against all the test bacteria, followed by ethanol extract. Comparison of the inhibitory activity of the extracts with the antibiotics bacterimycin 2000 and streptocycline revealed that methanol and ethanol extract of both leaf and pod and aqueous extract of pod were significantly higher than that of the antibiotics tested. Phytochemical analysis of leaf and pod materials revealed that antibacterial activity is due to the presence of phenolic and acidic fraction. Further separation of active fraction resulted in the loss of anti-bacterial activity, indicating a synergistic effect of the isolated active fraction. The results suggest that *C. coriaria* is a potential candidate plant for the management of phytopathogenic *Xanthomonas* which are known to cause diseases on cotton, french beans and tomato.

Keywords: anti-bacterial activity, *Caesalpinia coriaria*, *Xanthomonas* pathovars

Introduction

Pesticides are an essential input for preventing pre and post harvest crop losses (Mathur and Tannan, 1998; Saksena, 2001; Wheeler, 2002). Synthetic pesticides are commonly used to control phytopathogenic microorganisms (Agrios, 1997). Incessant and extensive use of these synthetic pesticides are posing serious problem to the life supporting systems due to their residual toxicity (Ferrer and Cabral, 1991; Gassner *et al*., 1997; Andrea *et al*., 2000;

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Harris *et al*., 2001; Campos *et al*., 2005). It is estimated that hardly 0.1% of the agro-chemicals used in crop protection reaches the target pest, leaving the remaining 99.9% to the environment to cause hazards to non target organisms including humans (Pimentel and Levitan, 1986). The large numbers of synthetic pesticides have been banned in the western world because of their undesirable attributes such as high and acute toxicity, long degradation periods, accumulation in the food chain and an extension of their power to destroy both useful and harmful pests (Barnard *et al*., 1997; Wodageneh and Wulp, 1997; Ortelli *et al*., 2005).

In-spite of use of all available means of plant protection, about 1/3 of the yearly harvest of the world is destroyed by pests and loss due to this is expected to be nearly $300 billion per year (Chandler, 2005). Many phytopathogenic bacteria have acquired resistance to synthetic pesticides (Sundin *et al*., 1994; Clarke *et al*., 1997; Williams and Heymann, 1998; White *et al*., 2002). Pathovars of *Xanthomonas* are known to cause diseases on several vegetable and cash crop and are reported to have developed resistance to kanamycin, ampicillin, penicillin and streptomycin (Weller and Saettler, 1980; Nafade and Verma, 1985; Verma *et al*., 1989; Bender *et al*., 1990; Rodriguez *et al*., 1997). This seriously hinders the management of diseases of crops and agricultural products (Dekker, 1987).

Considering the deleterious effects of synthetic pesticides on life supporting systems, there is an urgent need to search for alternative approaches for the management of plant pathogenic microorganisms (Hostettmann and Wolfender, 1997). Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (Balandrin *et al*., 1985; Mahajan and Das, 2003). Biopesticides has been suggested as an effective substitute for chemicals (Verma and Dubey, 1999; Kapoor, 2001). Reports are available on the use of several plant by-products, which posses antimicrobial properties, on several pathogenic bacteria and fungi (Dorman and Deans, 2000; Parameswari and Latha, 2001; Rath *et al*., 2001; Britto and Senthilkumar, 2001; Bylka *et al*., 2004; Shimpi and Bendre, 2005; Kilani, 2006), but reports are not available on the evaluation of inhibitory action of plants extract on phytopathogenic bacteria particularly in different pathovars of *Xanthomonas* which are known to cause many diseases in a wide variety of crops, causing considerable losses in yield and quality. This led the authors to screen *in vitro,* a large number of plants for antibacterial activity against important seed borne phytopathogenic X*anthomonas* pathovars, with the ultimate aim of developing plant based formulations for plant disease management (Satish *et al*., 1999; Mohana *et al*., 2006; Kiran and Raveesha, 2006; Raghavendra *et al*., 2006).

Caesalpinia coriaria (Jacq.) Willd. distributed in tropical and subtropical region belongs to the family *Caesalpinaceae* is used in traditional medicine. Pods are used in the treatment of bleeding piles. This plant is good for emollient properties useful in treating freckles and alleviates acute colic pain (Anon, 2000). Considering these, a detail investigation was conducted to test the efficacy of the different solvent extracts against important phytopathogenic bacteria and to identify the bioactive compound responsible for the antibacterial activity.

Materials and methods

Collection of plant materials

Fresh leaves and pods of *Caesalpinia coriaria* free from diseases were collected from Mysore (India), washed thoroughly 2-3 times with running tap water and once with sterile water, shade-dried, powdered and used for extraction. A voucher specimen of the plant is deposited in the herbarium of Department of Studies in Botany, University of Mysore, Mysore.

Preparation of the aqueous extracts

Fifty gm of shade dried, powder of leaves and pods of *C. coriaria* were macerated separately with 100 ml of sterile distilled water in a Waring blender (Waring International, new Hartford, CT, USA) for 10min. The macerate was first filtered through double layer muslin cloth and then centrifuged at 4000 g for 30 min. The supernatant was filtered through Whatman No. 1 filter paper and heat sterilized at 120ºC for 30 min. The extract was preserved aseptically in a brown bottle at 5ºC until further use. The extract was subjected to antibacterial activity assay.

Preparation of solvent extractions

Fifty gm of shade dried, powder of both leaf and pod of *C. coriaria* were filled separately in the thimble and extracted successively with 200 ml each of Petroleum ether, Benzene, Chloroform, Methanol and Ethanol using a Soxhlet extractor for 48 hours. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extract was weighed and preserved at 5ºC in airtight bottles until further use. One gm of each solvent residue was dissolved in 10 ml of methanol which served as the test extracts for antibacterial activity assay.

Phytochemical analysis of methanol extract

Methanol extract that showed highest antibacterial activity was subjected to phytochemical analysis (Anon, 1985; Harborne, 1998) and active fraction separation such as Fraction I (Phenolic compounds), Fraction II (Neutral compounds), Fraction III (Bases) and Fraction IV (Weaker acids) following the procedures of Roberts *et al*. (1981). The active fraction was further resolved by TLC and column chromatography using silica gel G and H (Merck) respectively with mobile phases Chloroform : Acetone (1:1.5). All the corresponding fractions and spots were again subjected to antibacterial activity assay at 50 µl concentration.

Plant pathogenic bacterial cultures

Authentic pure cultures of phytopathogenic *Xanthomonas axonopodis* pv *malvacearum* (*X. a*. pv. *m*) isolated from cotton (*Gossypium herbaceum* L.), *Xanthomonas axonopodis* pv *phaseoli* (*X. a*. pv. *p*) isolated from french bean (*Phaseolus vulgaris* L.) and *Xanthomonas campestris* pv *vasicatoria* (*X. c*. pv. *v*) isolated from tomato (*Lycopersicon esclentum* mill.) were obtained from DANIDA lab, University of Mysore, India.

Anti-bacterial activity assay

Antibacterial activity of aqueous extract, solvent extracts and isolated constituents was determined by cup diffusion method on nutrient agar medium (Anon, 1996). Cups were made in nutrient agar plate using sterile cork borer (5 mm) and inoculum containing 10^6 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 µl each of all aqueous, solvent extracts and isolated constituents were placed in the cups made in inoculated plates. The treatments also included 50 µl of sterilized distilled water and methanol separately which served as control. Antibiotics bacterimycin 2000 (Nitro propane hexadiol) (3 µg/ml) (Source: T. Stanes and Company Ltd., 23, Race-cource Road, Coimbatore-641018, India) and streptocycline (Streptomycin sulphate I.P. 90% Tetracycline Hydrochloride I.P. 10%) (1 µg/ml) (Source: Hindustan Antibiotics Ltd., PIMPRI, Pune-411018, India) at their respective recommended dosage were also treated for activity for comparative efficacy. The plates were incubated for 24 hours at 37ºC and zone of inhibition if any around the wells were measured in mm (millimeter). For each treatment six replicates were maintained. The data was subjected to statistical analysis using SPSS for windows software. The aqueous and methanol extracts of both leaf and pod showed highest

antibacterial activity, were further subjected to antibacterial activity assay at 10, 20, 30, 40, and 50 µl concentrations along with synthetic antibiotics bacterimycin and streptocycline for comparison.

Results

Anti-bacterial activity

Aqueous extracts

Anti-bacterial activity of aqueous leaf and pod extracts of *Caesalpinia coriaria* is presented in Table 1 and 2. Tukey HSD analysis of data revealed that, with increasing concentration of the aqueous extract, there was increase in antibacterial activity. Highly significant anti-bacterial activity of the aqueous extract at 50 µl was observed against all pathovars of *Xanthomonas*. Among the phytopathogenic *Xanthomonas* pathovars, *Xanthomonas axonopodis* pv. *malvacearum* was highly susceptible followed by *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas campestris* pv. *vesicatoria*. Pod extract recorded higher antibacterial activity than leaf extract.

Solvent extracts

The yield of extracts from leaf and pod were petroleum ether (1.8 & 1.1 gm), benzene (0.5 & 0.4 gm), chloroform (1.2 & 0.8 gm), methanol (26.8 & 31.0 gm) and ethanol (3.2 & 2.5 gm) respectively. Anti-bacterial activity of five different solvent extracts of both leaf and pod of *Caesalpinia coriaria* and synthetic antibiotics at 50µl concentration is presented in Table 1. Among the five solvents tested, methanol extract of both leaf and pod showed highly significant activity against all the test pathogens followed by ethanol and petroleum ether extract. Benzene and chloroform extracts of both leaf and pod did not show any activity against all *Xanthomonas* pathovars. The anti-bacterial activity of methanol extract of both leaf and pod of *Caesalpinia coriaria* at different concentrations is presented in (Table 2). Tukey HSD analysis of the data revealed that *Xanthomonas axonopodis* pv. *malvacearum* was highly susceptible among the *Xanthomonas* pathovars, where as *Xanthomonas campestris* pv. *vesicatoria* showed least inhibition.

Table 1. Zone of Inhibitory activity (in millimeter) of different extracts of *Caesalpinia coriaria* and antibiotics against some plant pathogenic pathovars of *Xanthomonas* at 50 µl concentration.

	Extracts		***X. a.* pv.*m***	***X. a.* pv.*p***	***X. c.* pv.*v***
1	Control aqueous	C	0.00	0.00	0.00
2	Control methanol	C	0.00	0.00	0.00
3	Aqueous extract	L	15.80±0.26	15.38±0.26	14.25±0.25
		P	21.13±0.29	19.75±036	17.50±0.29
4	Petroleum ether extract	L	12.88±0.29	10.75±0.36	12.38±0.26
		P	10.00±0.26	09.00±0.26	10.13±0.29
5	Benzene extract	L	0.00	0.00	0.00
		P	0.00	0.00	0.00
6	Chloroform extract	L	0.00	0.00	0.00
		P	0.00	0.00	0.00
7	Methanol extract	L	22.63±0.37	19.63±0.23	19.50±0.32
		P	19.50±0.18	19.38±0.32	17.50±0.32
8	Ethanol extract	L	18.50±0.32	16.75±0.25	16.13±0.29
		P	14.00±0.26	15.38±0.18	14.50±0.61
9	Methanol extract-Phenolic fraction	L	18.66±0.33	16.66±0.33	15.66±0.33
		P	16.33±0.33	14.33±0.33	12.33±0.33
10	Methanol extract-basic fraction	L	0.00	0.00	0.00
		P	0.00	0.00	0.00
11	Methanol extract-Neutral fraction	L	0.00	0.00	0.00
		P	0.00	0.00	0.00
12	Methanol extract-Acidic fraction	L	14.33±0.33	14.00±0.57	12.66±0.33
		P	12.06±0.33	12.66±0.33	10.33±0.33
13	Streptocycline	A	19.9±0.25	16.0±0.026	14.63±0.26
14	Bacterimycin 2000	A	10.00±0.43	11.38±0.026	11.25±0.25

Data given are mean of six replicates ± standard error, $p < 0.0001$
L- Leaf, P- Pod, C- Control, A-Antibiotic.
X. a. pv.*m* - *Xanthomonas axonopodis* pv *malvacearum*
X. a. pv.*p* - *Xanthomonas axonopodis* pv *phaseoli*
X. c. pv.*v* - *Xanthomonas campestris* pv *vasicatoria*

Table 2. Zone of Inhibitory activity (in millimeter) of aqueous and methanol extracts of *Caesalpinia coriaria* and antibiotics against some plant pathogenic pathovars of *Xanthomonas* at different concentrations.

Organisms	Extracts	Concentrations				
		10µl	20µl	30µl	40µl	50µl
X .a. pv. *m*	Aq(L)	7.75±0.25^{a}	9.00±0.26^{b}	10.88±0.29^{c}	13.88±0.29^{d}	15.00±0.26^{e}
	Aq(P)	9.50±0.18^{a}	14.00±0.26^{b}	18.00±0.26^{c}	19.75±0.25^{d}	21.13±0.29^{e}
	Met(L)	14.87±0.22^{a}	16.50±0.32^{b}	17.88±0.35^{c}	20.75±0.31^{d}	22.50±0.32^{e}
	Met(P)	10.25±0.25^{a}	13.63±0.37^{b}	14.63±0.26^{c}	16.13±0.29^{d}	19.00±0.26^{e}
	Strept(A)	09.75±0.25^{a}	12.88±0.29^{b}	15.87±0.22^{c}	17.62±0.26^{d}	19.95±0.25^{e}
	Bact(A)	00.00±0.00^{a}	07.00±0.26^{b}	08.88±0.35^{c}	10.00±0.26^{d}	10.00±0.43^{e}
X. a. pv *.p*	Aq(L)	00.00±0.00^{a}	8.25±0.25^{b}	10.13±0.22^{c}	12.00d±0.26^{d}	15.38±0.26^{e}
	Aq(P)	9.00±0.26^{a}	14.88±0.29^{b}	16.13±0.29^{c}	18.13±0.29^{d}	19.75±036^{e}
	Met(L)	12.25±0.25^{a}	13.38±0.37^{b}	16.13±0.29^{c}	17.50±0.23^{d}	19.25±0.25^{e}
	Met(P)	12.88±0.29^{a}	13.38±0.12^{b}	16.25±0.36^{c}	12.38±0.32^{d}	19.83±0.25^{e}
	Strept(A)	09.88±0.02^{a}	10.88±1.31^{b}	13.63±0.026^{c}	14.75±0.02^{d}	16.00±0.26^{e}
	Bact(A)	00.00±0.00^{a}	06.88±0.02^{b}	08.13±0.022^{c}	10.25±0.02^{d}	11.38±0.26^{e}
X. c. pv. *v*	Aq(L)	00.00±0.00^{a}	9.16±0.29^{b}	11.13±0.29^{c}	14.00±0.26^{d}	14.25±0.25^{e}
	Aq(P)	9.75±0.25^{a}	11.88±0.29^{b}	14.38±0.37^{c}	16.50±0.42^{d}	17.50±0.29^{e}
	Met(L)	12.75±0.31^{a}	14.75±0.25^{b}	16.38±0.37^{c}	12.75±0.31^{d}	19.38±0.26^{e}
	Met(P)	10.13±0.29^{a}	12.75±0.25^{b}	15.00±0.26^{c}	16.13±0.29^{d}	17.63±0.32^{e}
	Strept(A)	08.38±0.18^{a}	09.50±0.18^{b}	10.75±0.25^{c}	12.63±0.26^{d}	14.63±0.26^{e}
	Bact(A)	00.00±0.00^{a}	07.13±0.29^{b}	08.50±0.32^{c}	09.88±0.29^{d}	11.25±0.25^{e}

Mean of six replicate ± standard error, The means followed by the same letter(s) are not significantly different at P< 0.05 when subjected to Tukey HSD (row by row comparisons).
X. a. pv. *m* - *Xanthomonas axonopodis* pv *malvacearum*., *X. a*. pv. *p* - *Xanthomonas axonopodis* pv *phaseoli*., *X. c*. pv. *v* - *Xanthomonas campestris* pv *vasicatoria*.
Aq- Aqueous, Met-Methanol, Strep- Streptocycline, Bact- Bacterimycin 2000, L- Leaf, P- Pod, A-Antibiotic.

Anti-bacterial activity of the methanol and ethanol extracts (leaf and pod) and aqueous extract (pod) was highly significant when compared with that of synthetic antibiotics Streptocycline and Bacterimycin 2000.

Phytochemical analysis of methanol extract

The phytochemical analysis of methanol extracts of both leaf and pod revealed the presence of carbohydrates and glycosides, protein and amino acid, phenolic compounds, saponin, tannin, flavonoids, oils, gum and mucilage. Further phytochemical analysis (Roberts *et al.*, 1981) revealed that the antibacterial activity of methanol extract is due to the presence of phenolic and acidic compounds but the activity is less than that of crude methanol extract at 50 µl concentration (Table 1). In TLC separation phenolic fraction showed four

bands (R_f values 0.087, 0,333, 0.701, and 0.964) and acidic fraction showed five bands (R_f values 0.036, 0.079, 0.434, 0.565, and 0.876). Antibacterial activity was not observed in isolated compounds indicating the loss of antibacterial activity on further separation of the active fraction.

Discussion

The anti-bacterial activities of aqueous and solvent extracts were compared with standard Streptocycline and Bacterimycin 2000 and the results are reported in Table 1 and 2. The results show that the methanol extract of the plant parts showed more inhibitory effect than the other extracts. This tends to show that the active ingredients of the plant parts are better extracted with methanol than other solvents. The absence of antibacterial activity in the benzene and chloroform extracts indicates the insolubility of the active ingredients in these solvent. In general the activities against test bacterial culture used have shown good activity when compared with standard antibiotics. The phytochemical analysis of methanol extract revealed that the antibacterial activity is due to the presence of phenolic and acidic compounds and also observed that the activity is more in combination than separation. Further separation of the active fraction on TLC showed that the anti-bacterial activity was not observed in the isolated compounds. It is evident from the present investigations that the antibacterial activity in the methanol extracts of the leaf and pod but further separation of the methanol extract results in loss of antibacterial activity suggesting synergistic activity of the extract. There is a possibility of synergism between the compounds in a crude decoction than in isolated constituents (Daniel, 1999).

Field existences of antibiotic resistant phytopathogenic bacteria are increasing in recent years (Mandavia *et al*., 1999). WHO banned many agriculturally important pesticides due to wide range of toxicity against non-target organisms including humans which are known to cause pollution problem (Barnard *et al*., 1997). Some of the developing countries are still using these pesticides despite their harmful effects. Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganism, would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides (Verma and Dubey, 1999; Gottlieb *et al*., 2002). Many reports of antibacterial activity of plants extract against human pathogens and their pharmaceutical application are available (Cowan, 1999; Cragg *et al*., 1999; Newman *et al*., 2000; Gibbons, 2005), but not much has been done on the antibacterial activity of plants extract against plant pathogens (Satish *et al*., 1999). This is mainly due to lack of information on the screening/evaluation of

diverse plants for their antibacterial potential. Thus the present study reveals that *C. coriaria* is a potential candidate plant that could be successfully exploited for management of the diseases caused by different pathovars of *Xanthomonas* which are known to cause many diseases in wide variety of crops, causing considerable losses in yield and quality in an eco-friendly way. In the present investigations the antibacterial activity of *C. coriaria* against phytopathogenic bacteria has been demonstrated for the first time.

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