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Comparative study of some medicinal plants for their antibacterial properties against *Xanthomonas campestris* pv. *campestris,* the Black Rot pathogen of crucifers

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Aqueous extracts prepared from different parts of fifty five medicinal plant species were tested *in vitro* against *Xanthomonas campestris* pv. *campestris*, the black rot pathogen of several economic plants belonging to crucifer family. The antibacterial assay of the plant extracts was performed through poisoned food technique with three initial level of bacterial inoculum. Two experiments were set up, first to determine the bacteriostatic and bactericidal properties of plant extract (100% concentration) whereas, in second experiment, extracts at different concentrations (1% and 5%) were evaluated for their antibacterial properties under low and high density of *X.c.c.* Extracts were also evaluated to study their sensitivity towards high temperature to record any detrimental effect in antibacterial efficacy. Extracts of 33 medicinal plant species were found to posses bactericidal activity by completely inhibiting the colony development (0.0) after 48 h of incubation whereas, significant ($P \leq 0.5$) bacteriostatic properties were witnessed in 8 plant species. Extracts of *Acorus calamus, Gloriosa superba, Mucuna pruriens, Plumbago zeylanica* and *Psoralea corylifolia* totally arrested the colony development of X.c.c irrespective of density level, concentration of extract and exposure to higher temperature.

Key words: *Xanthomonas campestris* pv. *campestris*, Black rot of cabbage, medicinal plants, bactericidal and bacteriostatic properties

INTRODUCTION

Xanthomonas campestris pv. *campestris (X.c.c.)* is the causal agent of black rot in crucifer family (*Brassicaceae*) (Smith, 1897; Onsando, 1992) including broccoli, cabbage, cauliflower and radish. Black rot is one of the most important bacterial disease of crucifers worldwide (William, 1980) which causes considerable yield loss to the crop. Continuous cropping of the same species on the same piece of land creates favorable conditions for the establishment of this disease. Characteristic black rot symptoms result in blackened veins and V-shaped necrotic lesions at the foliar margin (Alvarez, 2000). *X.c.c* invades primarily through hydathodes, structures containing water pores located at leaf margins (Smith, 1897; Cook *et al.*, 1952). Under suitable weather conditions, the hydathodes exude copious quantities of fluid early in the morning, which collects around leaf margins as guttation drops. These drops become contaminated with epiphytic bacteria. Later, this guttation fluid may be drawn back into the leaf, carrying the suspended bacteria into the hydathode cavity and then into the vascular system. Periodic epidemics of black rot disease have occurred world-wide, especially in the developing regions of Africa and Asia, where high temperatures and humidity can aggravate the damage (Swings and Civerolo, 1993). However, an efficient, low pollutive control treatment as of yet, has not been developed (Swings and Civerolo, 1993). Though, antibiotics of Streptomycin group are routinely recommended for the management of this disease, their use over large acreage is not economically feasible. One major limitation of using chemical control agents is that the phytopathogenic

bacteria frequently develop a resistance to these compounds (Sigee, 1993).

In recent year much interest has been developed in the antimicrobial effects of medicinal plant extracts for plant disease control. Some plant extracts have been reported as effective inhibitors of phytopathogenic bacterial growth (Leksomboon *et al*, 1998; Leksomboon *et al*, 2000; Garden *et al*, 1978; Grainge and Alvarez, 1987). The forest of 'herbal state' Chhattisgarh, harbors a wealth of naturally grown medicinal plants which are used for the treatment of human diseases either traditionally by the local Baigas and Baidya or used commercially in the preparation of various Ayurvedic medicine. Many of these plant species were already reported to posses antibacterial properties against human pathogens (Ahmad *et al*, 1998; Lauk *et al*, 2003; Joshi, 1998; Tiwari *et al*, 2007). But these vast arrays of native medicinal flora have not been tested against plant pathogen especially *X. campestris* pv. *campestris*. Therefore, a comparative study has been undertaken to identify and evaluate the antibacterial property of medicinal plant species prevalent in the forest area of Chhattisgarh state.

MATERIALS AND METHODS

Xanthomonas campestris pv. *campestris* was isolated from naturally infected cabbage (*Brassica oleracea* L.) belonging to crucifer family and maintained on Pseudomonas Agar Base (PAB) (Hi media, 2003). Different parts *viz.* root, tuber, rhizome, whole plant, leaf, flower, fruit, bark and seed of fifty five medicinal plant species were collected from the forests as well as from the medicinal plant nursery established at College of Agriculture and Research Station (I.G.K.V.), Bilaspur, Chhattisgarh (Table 1). Plant materials were surface disinfected with 0.1% Mercuric Chloride solution and washed with three changes of sterilized water. Fresh plant materials were then chopped aseptically in a kitchen blender, dipped in required quantity of sterilized distilled water (1:1 w/v) and boiled on a water bath for 30-40 min to prepare crude stock solution (100%) (Tiwari *et al.*, 2007). Stock solutions were stored at 4°C until use.

Plant extracts were evaluated *in vitro* against *X.c.c* following poisoned food technique (Nene and Thapilyal, 2000). In the first set of experiment, 1 ml of stock solution (100%) in test tube was seeded with 0.1 ml bacterial suspension (inoculum I = 4.6 x 10^5 CFU/ml) of *X.c.c.* prepared from 48 h old culture. After 24 h and 48 h of incubation at 28±2°C, 0.1 ml suspension was seeded in the Petri dishes having PAB medium and after incubating the plates for 48 h at 28±2°C, the viable colonies were recorded. In the second set of experiment, antibacterial property of plant extracts was evaluated at different *X.c.c.* colony concentrations (inoculum II = 3.5 x 10^4 CFU/ml; inoculum III = 5.9 x 10^3 CFU/ ml). In the same set, plant extracts were also subjected to steam sterilization at 121°C for 15 min in an autoclave to determine the thermosensitivity of different plant extracts. 1 ml and 5 ml of stock solution were amended in 100 ml of sterilized semisolid (temperature 40-45°C) PAB medium to achieve a concentration of 1% and 5%. The medium was aseptically poured in Petri plates (90 mm) and allowed to solidify. The gelled plates were then seeded with 0.1 ml bacterial suspension at two levels prepared by serial dilution from 48 h old *X.c.c.* culture. Bacterial colonies were recoded after 48 h of incubation. All the experiments were conducted with three replications laid in completely randomized design. Data gathered were statistically analyzed using two factors.

RESULTS AND DISCUSSION

In the first experiment, extracts made from different parts of medicinal plant species showed a wide variability in terms of antibacterial activity against *X.c.c.* Out of fifty five extracts evaluated, thirty three extracts were found to posses bactericidal activity by completely inhibiting the colony development (0.0) even after 48 h of incubation. Species identified to have bactericidal properties are *Acorus calamus, Alstonia venenata, Andrographis peniculata, Asparagus racemosus, Bixa orellena, Cassia alata, Catharanthus roseus, Celastrus paniculatus, Centherum anthelminticum, Clerodendrum seratum, Coleus forskohlii, Cryptolepis buchananii, Curcuma zeodaria, Cutcuma aromatica, Cymbopogon winterianus, Equisitum diffusum, Gloriosa superba, Grewia hirsute, Gymnema sylvaestre, Helicetreae isora, Ipomoea mauritiana, Isomelus indica, Jatropha gosypifolia, Lavandula bipinnata, Mentha spictata, Millingtonia hortensis, Mucuna pruriens, Peucedanum nagpurense, Plumbago zeylanica, Psoralea corylifolia, Sagittaria sagittifolia, Sterospermum saveolans and Woodefordia flourbunda.* The remaining twenty two medicinal plant species were showed a wide spectrum of bacteriostatic properties against *X.c.c.* after 48 h of

Table 1 : Comparative efficacy of aqueous extracts of some medicinal plant species against *X. campestris* pv *campestries*. (Initial inoculum was 4.6×10^5 CFU/ml)

Scientific name	Vernacular Name	Parts used[s]	CFU[#] 100% extract 24 h*	CFU[#] 100% extract 48 h	Scientific name	Vernacular Name	Parts used[s]	CFU[#] 100% extract 24 h*	CFU[#] 100% extract 48 h
Abelmoschus moschatus	Latakasturi	S	15.0	20.0	*Gymnema sylvaestre*	Gudmar	L	0.0	0.0
Acorus calamus	Bach	Rz	0.0	0.0	*Hedychium coronarium*	Gulbakawali	F	18.3	28.3
Alstonia venenata	Korkot	L	0.0	0.0	*Helicetreae isora*	Marodphalli	L	0.0	0.0
Andrographis peniculata	Kalmegh	L	0.0	0.0	*Ipomoea mauritiana*	Bidarikand	T	0.0	0.0
Asparagus racemosus	Satavar	T	0.0	0.0	*Isomelus indica*	Isomelus	L	0.0	0.0
Baliospernum montanum	Chitawar	R	106.7	153.3	*Jatropha gosypifolia*	Bagranda	L	0.0	0.0
Bixa orellena	Sinduri	L	0.0	0.0	*Lavandula bipinnata*	Lavandula	Wp	0.0	0.0
Cassia alata	Hinglaj	R	0.0	0.0	*Mentha spictata*	Deshi pudina	L	0.0	0.0
Cassia occidentalis	Kasondi	S	833.3	966.7	*Millingtonia hortensis*	Aaskash neem	L	0.0	0.0
Catharanthus roseus	Sasdsuhagan	F	0.0	0.0	*Mimosa pudica*	Lajwanti	L	1666.7	1700
Celastrus paniculatus	Malkagni	L	0.0	0.0	*Mucuna pruriens*	Kenwach	S	0.0	0.0
Centherum anthelminticum	Vanjeera	S	0.0	0.0	*Nyctanthes arbor-tristis*	Harsingar	R	220.0	2300
Cissampelos pareira	Pathal	L	900	1100.0	*Peucedanum nagpurense*	Bhojraj	T	0.0	0.0
Cissus quandragularis	Hathjod	L	1266.7	1316.7	*Plumbago zeylanica*	Chitrak	L	0.0	0.0
Clematis smilacifolia	Clematis	F	2033.3	2066.7	*Psoralea corylifolia*	Babchi	S	0.0	0.0
Clerodendrum seratum	Bharang	Fr	0.0	0.0	*Pusidenitum glacum*	–	T	155	97.3
Coleus forskohlii	Pasandbhed	L	0.0	0.0	*Pygmaeopremna herbacea*	Patrangi	R	33.3	50.1
Costus speciosus	Keokand	T	2233.3	2500.2	*Sagittaria sagittifolia*	Sagittaria	L	0.0	0.0
Cryptolepis buchananii	Nagbel	T	0.0	0.0	*Sida acuta*	Balihari	S	2500.0	2600
Curcuma zeodaria	Jhool Bhaji	T	0.0	0.0	*Smilex zeylanica*	Ramdatun	Fr	15.0	20.0
Cutcuma aromatica	Jungli haldi	T	0.0	0.0	*Spherianthes indicus*	Gorakhmundi	F	1733.3	1833.3
Cymbopogon winterianus	Citronella	L	0.0	0.0	*Sterospermum saveolans*	Garud	B	0.0	0.0
Diplocydos palmatus	Bundela	Fr	425.0	1500.0	*Tribulus teristris*	Gokharu	S	56.7	70.2
Embelia tsjeriam	Vaividang	S	2350.0	2500.0	*Withania samnifera*	Ashwagandha	R	466.7	566.7
Equisitum diffusum	Hadjod	Wp	0.0	0.0	*Woodefordia flourbunda*	Dhavai	L	0.0	0.0
Gloriosa superba	Kalihari	T	0.0	0.0	*Zanthoxylum armatum*	Tejbal	L	38.0	40.0
Grewia hirsute	Gurdsakri	L	0.0	0.0	*Zerinium* sp.	–	L	56.7	70.0
Grewia tiliaefolis	Dahiman	L	12000	2100	Control (without extract)			2516.7	2620
					Steptocycline 100 ppm			0	0

[s] = (S = seed; Rz = rhizome; L = leaf; T = tuber; R = root; F = flower; Fr = fruit; Wp = whole plant);

* = Incubation time; # = Colony Forming Units

incubation (Table 1). Significant ($P \leq 0.5$) bacteriostatic properties in terms of reduction in colony count of *X.c.c.* were witnessed in extracts of *Abelmoschus moschatus* (20.0), *Smilex zeylanica* (20.0), *Hedychium coronarium* (28.3), *Zanthoxylum armatum* (40.0), *Pygmaeopremna herbacea* (50.1), *Zerinium* sp. (70.0), *Tribulus teristris* (70.2) and *Pusidenitum glacum* (97.3). However, medicinal plant species *i.e. Sida acuta* (2600.0) followed by *Embelia tsjeriam* (2500.2) which showed the least bacteriostatic activity compared with control (2620.0) after 48 h of incubation. Previously, Mukherjee and Biswas (1981) tested crude extracts of 25 selected plants and some were found significantly effective against *Xanthomonas* sp. Raghavendra *et al*, (2006) found *in vitro* that the activity of aqueous extract of. *Samanea saman* (Jacq.) Merr. was satisfactory against *Xanthomonas* pathovars.

Table 2 : Colony forming units of *X. campestris* pv *campestris* recorded in response to thermal treated/not treated aqueous extracts of some medicinal plant species,

	Thermal Treatment				Not Treated			
	1%	5%	1%	5%	1%	5%	1%	5%
Scientific name	$3,5 \times 10^{4\%}$		5.9×10^{3}		$3,5 \times 10^{4\%}$		5.9×10^{3}	
A. moschatus	151.4	89.5	0	0	75.6	49.1	12.4	0
A. calamus	0	0	0	0	0	0	0	0
A. venenata	600	300	0	0	250	20	0	0
A. peniculata	133.3	120	67.5	34	0	0	0	0
A. racemosus	2000	1500	500	400	1800	1700	450	350
B. montanum	553.1	345.6	153.0	97.2	110.1	91	87.5	45.9
B. orellena	166.6	136.6	10	0	0	0	0	0
C. alata	70	55.9	40.1	23.7	16.0	11.1	0	0
C. occidentalis	1206.6	800	567	345.8	402.2	266.7	189	115.3
C. roseus	1566.6	1266.6	105	10	906.6	700	40	5
C. paniculatus	900	400	5	0	800	100	5	0
C. anthelminticum	800	250	51.6	0	633.3	166.6	31.6	0
C. pareira	1566.6	1200	60	48.3	1200	800	23.3	0
C. quandragularis	2026.6	1066.6	595.3	345.1	1340.3	712.1	294	194.2
C. smilacifolia	2000	1500	666.7	500	2000	1400	588.2	411.8
C. seratum	200	81.6	45	5	0	0	0	0
C. forskohlii	2000	1563	101.6	45	1500	900	40	10
C. speciosus	1583.3	1233.3	25	0	1400	1000	83.3	20
C. buchananii	145.7	76.9	134	45.2	35.3	17.2	12	0
C. zeodaria	700	100	100	0	0	0	0	0
C. aromatica	1100	900	13	5	800	600	0	0
C. winterianus	120.5	65.2	46.9	23.5	0	0	0	0
D. plamatus	870.3	666.7	33.3	26.8	435.2	333.3	16.7	13.4
E. tsjeriam	1500	1000	88.3	43.3	1200	733.3	43.3	0
E. diffusum	275.3	88.8	28.6	9.2	3	0	0	0
G. superba	0	0	0	0	0	0	0	0
G. hirsute	150.6	75.1	89	55.1	75.3	37.6	44.5	27.6
G. tiliaefolia	2500	1866.6	600	400	2100	1633.3	600	200
G. sylvaestre	1200	750	90	30	0	0	0	0
H. coronarium	83.7	41.7	49.4	30.6	64.4	32.1	38	23.5
H. isora	66.8	27.4	39.3	16.1	55.7	22.8	0	0
I. mauritiana	33.4	21.5	13.7	6.9	0	0	0	0
I. indica	1200	966.6	342.9	276.2	133.3	107.4	38.1	30.7
J. gosypifolia	2000	1600	900	433.3	1666.6	1200	700	316.6
L. bipinnata	113.6	46.6	66.8	27.4	56.8	23.3	0	0
M. spictata	1300	866.6	11.6	0	0	0	0	0
M. hortensis	966.7	733.3	5	0	0	0	0	0
M. pudica	2233.3	1173.3	2200	1133.3	345	145.9	78.3	5
M. pruriens	0	0	0	0	0	0	0	0
N. arbor-tristis	1500	1200	500	300	1500	0	0	0
P. nagpurense	1666.6	1400	537.6	451.6	1600	1200	516.1	387.1
P. zeylanica	0	0	0	0	0	0	0	0
P. corylifolia	0	0	0	0	0	0	0	0
P. glacum	1200	900	35	15	1200	700	58.3	16.6
P. herbacea	112	68.2	24	17.3	56	34.1	12	0
S. sagittifolia	51	32.1	15	10.5	10.5	0	0	0

[Continued]

S. acuta	3080	2506.6	1232	1002.6	1540	1253.3	616	501.3
S. zeylanica	137.6	81.4	56	34	68.8	20.7	0	0
S. indicus	2000	1030.3	1365.6	313.6	952.4	490.6	650.3	149.4
S. saveolans	27.2	15	10.5	0	0	0	0	0
T. teristris	750	500	45	18.2	394.7	263.2	23.7	9.6
W. somnifera	1800	1466.6	514.3	396.4	1600	1200	457.1	324.3
W. flourbunda	46.4	29.2	15.6	9.5	13.1	0	0	0
Z. armatum	206.6	100	85	53.3	93.0	45.5	38.6	24.2
Zerinium sp.	1500	1000	90	20	1300	733.3	50	0
Control	2516.7	2620						
Streptocyline	0.0	0.0						

% = Initial inoculum

CD at 5% Thermal treatment/not treated – 1378.7

CD at 5% Concentration – 1378.7

CD at 5% Species = 228.8

CD at 5% Dilution – 1378.7

Coefficient of Variation – 45.5%

Data from Table 2 revealed that out of fifty five medicinal plants extracts *Acorus calamus, Gloriosa superba, Mucuna pruriens, Plumbago zeylanica* and *Psoralea corylifolia* completely inhibited (0.0) the growth of *X.c.c.* irrespective extract concentrations, inoculum level and exposure to high temperature condition. The antibacterial properties of *Andrographis peniculata, Bixa orellena, Clerodendrum seratum, Curcuma zeodaria, Cymbopogon winterianus, Gloriosa superba, Gymnema sylvaestre, Ipomoea mauritiana, Mentha spictata, Millingtonia hortensis, Mucuna pruriens,* and *Sterospermum saveolans* extracts affected by high temperature condition irrespective of extract concentration (Table 2). Antibacterial property of most of the extracts was affected when exposed to high temperature condition. Although most of the extracts were identified to posses antibacterial

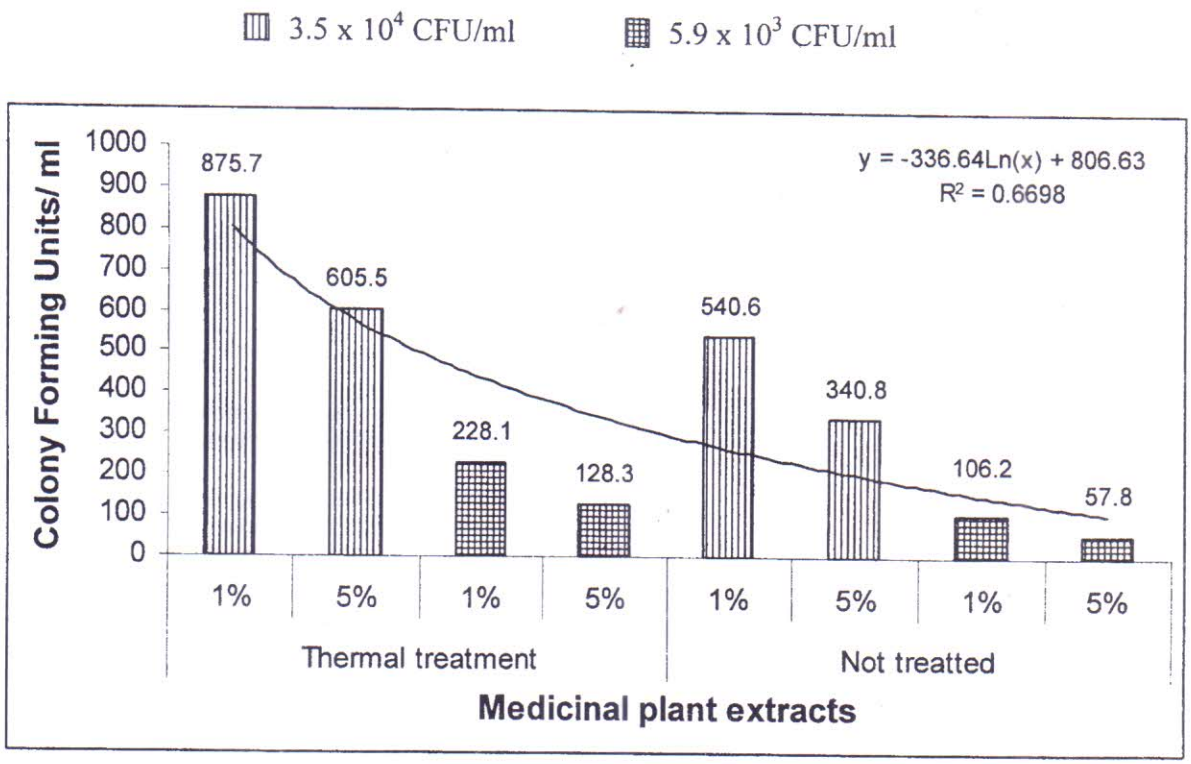

Fig. 1 : Mean CFU recorded in sterilized and unsterilised plant extract treatments at two concentrations (1% and 5%) upon tw3o initial inoculum levels. Trend line in the graph showing decrease colony count at higher extract concentration as well as in unsterilised extract than sterilized extracts.

properties, the activity was decreased with increase in the density of *X.c.c.* inoculum as less number of extracts were found to inhibit complete growth of *X.c.c.* at higher inoculum density (3.5 x 10^4 CFU/ml). It was also indicated from Table 2 that antibacterial properties of the extracts were affected when subjected to higher temperature condition. Moreover, the mean colony count of *X.c.c.* was considerably higher after application of sterilized plant extract at 1% (875.7) and 5% (605.5) than unsterilised extract at 1% (540.6) and 5% (340.8) concentration when the inoculum level was 3.5 x 10^4 CFU/ml. Similar trend was also recorded in the lower inoculum level (5.9 x 10^3 CFU/ml). Thus a deteriorating trend line in terms of effectivity of unsterilized to sterilized extract was plotted (Fig. 1).

The present *in vitro* study preliminary indicates the potentiality of some medicinal plant extracts in controlling the growth of *Xanthomonas campestris* pv. *campestris* which cause diseases of several important vegetables. Detail study on these identified medicinal plant species possessing high antibacterial activity will be useful in further research on the identification and characterization of the inhibitory fractions, their synthesis and deployment for management of plant diseases *in vivo*. Moreover, the unexplored medicinal plant species showing antibacterial properties can also be tested against clinical pathogens. In addition it can be suggested that medicinal plant should be extracted in aseptic condition and not to be sterilized with moist heat which may considerably deteriorate its antibacterial properties.

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