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Anti-bacterial evaluation of botanical formulation for the management of bacterial blight of pomegranate caused by *Xanthomonasaxonopodis* Pv. *punicae*

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Abstract
Inhibitory activity of solvent extraction of botanicals *Viz*., *Allium sativum*, *Garciniaindica*and *Prosopisjuliflora*, were evaluated against *Xanthomonasaxonopodis* pv. *Punicae* under *in-vitro* condition. Botanicals which are extracted from soxhletapparatus using different solvents. Among the botanicals tested *Prosopisjuliflora* with ethanol extract was found to be most effective with the highest average zone of inhibition of 31.33 mm. Followed by Methanol extract of *Prosopisjuliflora* (30.67 mm), Methanol extract of *Garciniaindica* (29.67 mm), methanol extracts of *Allium sativum* (24.67 mm). Shelf life of effective solvent botanical formulations stored at 4°C, 25°C, 30°C, 35°C and 40°C under *in vitro* conditions. Results reaveled that solvent extraction of *Prosopisjuli flora* recorded more efficiency, followed by solvent extraction of *Allium sativum* and *Garciniaindica* against *Xanthomonasaxonopodis* pv. *punicae*.

Keywords: Inhibitory activity, solvent extraction, botanicals, shelf life and formulation

Introduction
Bacterial blight of pomegranate caused by *Xanthomonasaxonopodis* pv. *Punicae* (Hingorani and Singh. 1959) [2], Vauterin, *et al.* (1995) [11] is known to occur in different pomegranate growing areas of the country *viz.*, Maharashtra, Karnataka and Andhra Pradesh. Pomegranate, the boon commercial fruit crop to the farmer, turned as a big bane after the severe incidence of bacterial blight. 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) [1]. Green plants are found to be an effective reservoir for the bioactive molecules and can provide valuable sources for discovery of natural pesticides. Use of botanicals is now emerging as one of the important means in protection of crop produce and the environment from pesticidal pollution, which is a global problem. Therefore, there has been a growing interest in research concerning the alternatives of chemical pesticides and antimicrobial active compounds, including the plant extracts and essential oils of aromatic plants (Pradhanang, *et al.* 2003) [8]. Importance of formulations and extraction with solvents to explore the maximum efficacy of botanicals. Hence it has been screened different solvents for the extraction of botanicals in present study.

Material methods
Agar well diffusion method
The efficacy of the botanicals was confirmed by Agar well diffusion method (Holder and Boyse. 1994) [3]. The culture of *X. axonopodis* pv. *punicae* was added to nutrient medium and poured in to the Petri plate and allowed to solidify. The agar plates were punched aseptically with 6-mm sterilized cork borer to prepare wells. The wells were filled with 100 µl of botanical extracts. All plates were incubated (30°C) for 48h. After incubation, the diameters of any clear zones around the wells were measured in milli meter.

Soxhlet apparatus method (Gargade method. 2013)
The three botanicals *viz*., *Allium sativum* (Bhima purple), *Garciniaindica* and *Prosopisjuliflora* were extracted with different solvents *viz*., Acetone, Benzene, Chloroform, Ethanol, Hexane

and Methanol and subjected for condensation through soxhlet apparatus.

Soxhlet extraction is a continuous process of botanical extraction with solvents. The powdered plant material is packed in thimble, on which solvents were poured at 1:10(w/v). And the botanicals were filtered to the lower part of thimble. The thimble along with filtrate was fixed to soxhlet apparatus.

Adjusted the temperature of apparatus to 65°C. The condensed solvents were analysed for their efficiency against *Xap* under *in vitro* through agar well method and record the inhibition zone in mm. The final condensed extracts of effective solvents (ethanol and methanol) were stored at different temperatures (25°C, 30°C, 35°C and 40°C) sample was drawn at 15 and 30 days for the evaluation of the efficiency under *in vitro*.

Results and Discussion

The solvents *viz.*, acetone, benzene, chloroform, ethanol, hexane, methanol and petroleum ether extracts of *Allium sativum* (Bhima purple), *Garciniaindica* and *Prosopisjuliflora* botanical formulations were evaluated *in vitro* against the growth of *X. axonopodis.* pv. *Punicae*re presented in Table 1. The results revealed that ethanol extract of *P. juliflora* showed highest inhibition zone of 31.33 mm at 1:5 w/v concentration. Followed by methanol extract of *P. juliflora* (30.67 mm), acetone extract of *P. juliflora* (27.33 mm), petroleum etherextract of *P. juliflora* (24.67 mm), benzene extract of*P. juliflora* (23.33 mm) and chloroform extract of *P. juliflora* (20.67 mm) at 1:5 w/v concentration.

However when the solvents were extracted with *Garcinia indica*, the methanol extract of *G. indica* showed highest inhibition zone of 29.67 mm at 1:5 w/v concentration. Followed by ethanol extract of *G. indica* (27.33 mm), acetone extract of *G. indica* (21.33 mm), petroleum etherextract of*G. indica* (18.67 mm), benzene extract of *G. indica* (16.67 mm) and chloroform extract of *G. indica* (15.33 mm) at 1:5 w/v concentration.

Results also showed when the solvents extracted with *Allium sativum* (Bhima purple), the methanol extract of *A. sativum* (Bhima purple) showed highest inhibition zone of 24.67 mm at 1:5 w/v concentration. Followed by ethanol extract of *A. Sativum* (Bhima purple) (22.67 mm), acetone extract of *A. Sativum* (Bhima purple) (17.33 mm), benzene extract of *A. Sativum* (Bhima purple) (14.67 mm), petroleum etherextract of*A. Sativum* (Bhima purple) (13.33 mm), and chloroform extract of*A. Sativum* (Bhima purple) (14.33 mm) at 1:5 w/v concentration. Results also revealed no inhibition of bacterial growth was seen when the solvents tested alone.

The results of data in Table 2, showed the efficacy of botanical formulations were evaluated *in vitro* against the growth of *X. axonopodis.* pv. *punicae*. Results showed that ethanol extract of *P. juliflora* showed highest inhibition zone with 31.33 mm at 1:5 w/v concentration at first day of the experiment. Followed by methanol extract of *G. indica*(29.33 mm),methanol extract of *P. juliflora* (28.67 mm), ethanol extract of *G. indica* (26.67 mm), methanol extract of *A. sativum* (Bhima purple) (24.67 mm) and ethanol extract of *A. sativum* (Bhima purple) (22.67 mm)at 1:5 w/v concentration.

After 15th day ethanol extract of *P. juliflora* with inhibition zone 30.33, 27.33, 26.67, 23.33 and 20.00 mm at 4°C, 25°C, 30°C, 35°C and 40°C respectively. Followed by methanol extract of *P. juliflora* (28.00, 25.33, 23.33, 21.33 and 17.33 mm), methanol extract of *G. indica*(28.33, 27.33, 26.67, 21.33 and 17.33 mm), ethanol extract of *G. indica*(26.00, 24.67, 22.67, 18.67 and 15.33 mm), methanol extract of *A. sativum* (Bhima purple) (24.00, 22.67, 20.33, 16.67 and 12.33 mm) and ethanol extract of *A. sativum* (Bhima purple) (22.33, 18.67, 18.33, 12.67 and 8.00 mm) at 4°C, 25°C, 30°C, 35°C and 40°C respectively at 1:5 w/v concentration.

After 30th day ethanol extract of *P. juliflora* with inhibition zone 28.00, 24.67, 23.33, 20.67 and 16.67 mm at 4°C, 25°C, 30°C, 35°C and 40°C respectively. Followed by methanol extract of *P. juliflora* (26.67, 22.67, 20.67, 17.33 and 14.67 mm), methanol extract of *G. indica*(28.00, 24.67, 24.67, 18.67 and 15.33 mm), ethanol extract of *G. indica*(25.67, 22.67, 20.67, 15.33 and 12.67 mm), methanol extract of *A. sativum* (Bhima purple) (24.00, 16.33, 15.67, 12.00 and 7.33 mm) and ethanol extract of *A. sativum* (Bhima purple) (22.00, 14.00, 13.67, 8.67 and 5.33 mm) at 4°C, 25°C, 30°C, 35°C and 40°C respectively at 1:5 w/v concentration. Results also revealed that efficacy of the botanical formulations decreases with increase in temperature and days.

The results in accordance with work of Pathmanathan, *et al.* (2010) who extracted the botanicals with different solvents *viz.*, acetone, benzene, dichloromethane, hexane, ethyle acetate, ethanol and methanol from rotary evaporator. Among the solvent used, ethanol and methanol resulted more yield compared to other solvents and they also reported bulb of *Allium sativum*, leaf of *Eucalyptus citriodora* and *Ocimum sanctum* extracted with ethanol and methanol solvents gave highest zone of inhibition against *Bacillus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*and *Escherichia coli.*

Shachi singh (2012) [9] reported among different solvents chosen ethanol was found to be best solvent generating highest yield, this was because both polar and nonpolar compounds get extracted in ethanol and also have more antibacterial activity.

Parekha, *et al.* (2006) recorded cold aqueous extract of bulb of *A. sativum* demonstrated that the growth of *Staphylococcus aureus* was inhibited by agar well diffusion.

Singh, *et al.* (2001) [10] reported that *Adenocalymmaalliaceum* extracts retained their activity against *Alternariatenuissima* even after 35 days at 10°C.

Onyeagba, *et al.* (2004) [4] reported that the absence of inhibitory effect due to the inactivation of active principles that may be thermo labile means loss of characteristic properties by action of heat. It has been also reported that the allicin is the biologically active alkaloid of *Allium sativum* is not stable at higher temperature.

Conclusion

From the present investigation it is concluded that treatment T17 (Ethanol extract of *Prosopisjuliflora*) was found to be the best treatmentagainst *Xanthomonasaxonopodis* pv. *punicae* and in shelf life evaluation of botanicals extracted with solvents, ethanol and methanol extraction of *Garciniaindica* recorded highest shelf life period.

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Table 1: Efficiency of solvent extracted botanical formulations from soxhlet apparatus against *Xanthomonasaxonopodis* pv. *punicae*

Treatments	Treatment details	Zone of inhibition (diameter in mm)
T_1	Acetone extracts of *A.s*	17.33 (4.22)
T_2	Benzene extracts of *A.s*	14.67 (3.89)
T_3	Chloroform extracts of *A.s*	14.33 (3.78)
T_4	Ethanol extracts of *A.s*	22.67 (4.81)
T_5	Methanol extracts of *A.s*	24.67 (4.96)
T_6	Petroleum ether extracts of *A.s*	13.33 (3.71)
T_7	Acetone extracts of *G.i*	21.33 (4.67)
T_8	Benzene extracts of *G.i*	16.67 (4.14)
T_9	Chloroform extracts of *G.i*	15.33 (3.97)
T_{10}	Ethanol extracts of *G.i*	27.33 (5.27)
T_{11}	Methanol extracts of *G.i*	29.67 (5.44)
T_{12}	Petroleum ether extracts of *G.i*	18.67 (4.37)
T_{13}	Acetone extracts of *P.j*	27.33 (5.27)
T_{14}	Benzene extracts of *P.j*	23.33 (4.88)
T_{15}	Chloroform extracts of *P.j*	20.67 (4.59)
T_{16}	Methanol extracts of *P.j*	30.67 (5.58)
T_{17}	Ethanol extracts of *P.j*	31.33 (5.64)
T_{18}	Petroleum ether extracts of *P.j*	24.67 (5.01)
T_{19}	Acetone	0.00 (0.70)
T_{20}	Benzene	0.00 (0.70)
T_{21}	Chloroform	0.00 (0.70)
T_{22}	Ethanol	0.00 (0.70)
T_{23}	Methanol	0.00 (0.70)
T_{24}	Petroleum ether	0.00 (0.70)
T_{25}	Control	0.00 (0.70)
	SEm±	0.06
	CD (0.01)	0.22

*The results in the parentheses are square root transformed values

Note * : *A.s* : *Allium sativum*(Bhima purple), *G.i* : *Garcinia indica, P.j* : *Prosopis juliflora.*

Table 2: Shelf life of effective solvent botanical formulations extracted from soxhlet apparatus against *Xanthomonasaxonopodis* pv. *Punicae* under *in vitro* conditions

Duration	Treatment details	Zone of inhibition (diameter in mm)					
		4°C	25°C	30°C	35°C	40°C	Average
1st day	Ethanol extracts of *A.s*	22.67 (4.76)	22.67 (4.76)	22.67 (4.76)	22.67 (4.76)	22.67 (4.76)	22.67
	Methanol extracts of *A.s*	24.67 (24.96)	24.67 (24.96)	24.67 (24.96)	24.67 (24.96)	24.67 (24.96)	24.67
	Ethanol extracts of *G.i*	26.67 (5.16)	26.67 (5.16)	26.67 (5.16)	26.67 (5.16)	26.67 (5.16)	26.67
	Methanol extracts of *G.i*	29.33 (5.41)	29.33 (5.41)	29.33 (5.41)	29.33 (5.41)	29.33 (5.41)	29.33
	Ethanol extracts of *P.j*	31.33 (5.59)	31.33 (5.59)	31.33 (5.59)	31.33 (5.59)	31.33 (5.59)	31.33
	Methanol extracts of *P.j*	28.67 (5.35)	28.67 (5.35)	28.67 (5.35)	28.67 (5.35)	28.67 (5.35)	28.67
15th day	Ethanol extracts of *A.s*	22.33 (4.72)	18.67 (4.32)	18.33 (4.28)	12.67 (3.55)	8.00 (2.91)	16.00
	Methanol extracts of *A.s*	24.00 (4.89)	22.67 (4.76)	20.33 (4.50)	16.67 (4.08)	12.33 (3.51)	19.20
	Ethanol extracts of *G.i*	26.00 (5.09)	24.67 (5.01)	22.67 (4.81)	18.67 (4.37)	15.33 (3.97)	21.46
	Methanol extracts of *G.i*	28.33 (5.32)	27.33 (5.27)	26.67 (5.21)	21.33 (4.67)	17.33 (4.22)	24.19
	Methanol extracts of *P.j*	28.00 (5.29)	25.33 (5.08)	23.33 (4.88)	21.33 (4.67)	17.33 (4.22)	23.13
	Ethanol extracts of *P.j*	30.33 (5.50)	27.33 (5.27)	26.67 (5.21)	23.33 (4.88)	20.00 (4.52)	25.53
30th day	Ethanol extracts of *A.s*	22.00 (4.69)	14.00 (3.74)	13.67 (3.69)	8.67 (3.02)	5.33 (2.91)	12.73
	Methanol extracts of *A.s*	24.00 (4.89)	16.33 (4.04)	15.67 (3.95)	12.00 (3.52)	7.33 (2.70)	15.06
	Ethanol extracts of *G.i*	25.67 (5.06)	22.67 (4.81)	20.67 (4.59)	15.33 (3.97)	12.67 (3.62)	19.40
	Methanol extracts of *G.i*	28.00 (5.29)	24.67 (5.01)	24.67 (5.01)	18.67 (4.37)	15.33 (3.97)	22.26
	Methanol extracts of *P.j*	26.67 (5.41)	22.67 (4.81)	20.67 (4.59)	17.33 (4.22)	14.67 (3.89)	20.40
	Ethanol extracts of *P.j*	28.00 (5.29)	24.67 (5.01)	23.33 (4.88)	20.67 (4.59)	16.67 (4.14)	22.66
	Control	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00
	SEm±	0.08	0.07	0.07	0.07	0.09	
	CD (0.01)	0.29	0.24	0.27	0.31	0.33	

*The results in the parentheses are square root transformed values

Note * : *A.s* : *Allium sativum*(Bhima purple), *G.i* : *Garcinia indica, P.j* : *Prosopis juliflora.*

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