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Evaluation of *piper betle* l. Leaf extracts for biocontrol of important phytopathogenic bacteria

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Plant diseases are the major bottlenecks in agriculture, generally overcome by the use of pesticides. But the extensive use of pesticides has led to a wide spectrum of environment and human health hazards. The better alternative is "Biological control" which is an eco-friendly strategy, especially with botanicals proving to be better alternatives in disease management. The present study was to search a new botanical for the control of some important plant pathogenic bacteria under *in-vitro*. Solvent extracts of leaves of *Piper betle* viz., petroleum ether, chloroform, ethyl acetate and methanol extract subjected to antibacterial activity through cup and disc diffusion assay showed inhibitory activity against the tested bacteria.. Petroleum ether extract recorded lower inhibition zone ranging from 14-20 mm, while higher inhibition ranging from 26-40 mm was recorded in methanol extract. Chloroform and ethyl acetate extracts recorded moderate inhibition ranging from 15-25 mm. Minimum inhibitory concentration (MIC) of methanol and chloroform extracts ranged from 0.019-0.131 mg/ml and 0.029-0.131 mg/ml, respectively. Phytochemical analysis of methanol and ethyl acetate extracts revealed the presence of flavanoids, tannins, steroids and glycosides; chloroform extract revealed the presence of alkaloids and glycosides. Further work is in progress to isolate the active compounds. It is proved that different extracts of *P. betle* showed significant activities against a broad array of plant pathogenic bacteria can be a new source of botanical pesticides plant disease management.

Key words: *Piper betle*; antibacterial; phytopathogenic bacteria; flavanoids; alkaloids

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Introduction

Modern agriculture and industrial farming have delivered tremendous gain in productivity and efficiency. However, plant diseases are one of the major bottlenecks in agricultural production, which result in losses up to 40% of global food production (Svary *et al*., 2012).

To overcome this pesticides have become an important tool as a protective agent for boosting food production. There are over 500 compounds registered as pesticides or metabolites of pesticides (Rekha *et al*., 2006). Applications of such pesticides are being used during production, post harvest treatment, storage and transportation (FAO/WHO, 2004).

Pesticides have been linked to a wide spectrum of human health hazards ranging from short impact such as headache, nausea to chronic impacts like cancer, reproductive disorder and endocrine disruption. The amount of applied pesticides reaching the target organism is about 0.1% while the remaining bulk contaminates the environment (Carriger *et al*., 2006). Some amount of harmful pesticides residues remain in the harvested fruits, vegetables and crops causing permanent danger to environment, quality of food and consumer creating health hazards (Solecki *et al*., 2005; Rekha *et al*., 2006) have stated that endosulfan residues of organochlorine in wheat were found in all market samples of India where pesticides residues were higher than MRL'S. The adverse effect of pesticides on soil microbial diversity and activities has been described by several workers (Littlefield-Wyer *et al*., 2008). Extensive use of pesticides has led to the development of copper resistant strains (Cupples and Elmhierst, 1999).

In 1950's , soon after the introduction of antibiotics in human medicine the potential of these wonder drugs to work on plant diseases was explored and only streptomycin and tetracycline had significant usage. But, again due to indiscriminate use of these antibiotics, the bacteria gained resistance to these antibiotics which destabilized plant disease control. Pathavors of *Xanthomonas* are known to cause diseases on several vegetable and cash crops are reported to have developed resistance to Kanamycin and Ampicilin (Rodriguez *et al*., 1997).The consistent use of sreptomycin against *Xanthomonas campestris* pv.. *vesicatoria* has resulted in wide spread resistant populations (Minsavage *et al*., 1990). Recovering from the euphoria of green revolution India is battling from residual effects of extensively used chemical fertilizers, pesticides and antibiotics.

Biological control is now an eco-friendly strategy to reduce the dependence of high risk chemicals, fertilizers and antibiotics for disease management. As a part of their defense system many plants produce secondary metabolites which have antimicrobial properties. These natural products known

as botanical pesticides or herbal medicines have long been used in the control of micro-organisms causing plant and human diseases.

The antimicrobial properties of botanicals have encouraged the authors to screen for the antibacterial property of a locally available plant *Piper betle* against selected plant pathogenic bacteria. *Piper betle* Linn is a perennial dioecious, semi woody climber, nodes swollen, papillose when young glabrous at maturity. Leaves alternate, simple and yellowish green to bright green in color, berries are rarely produced, co crescent into a fleshy spadix (Wealth of India, 1980). The leaves have a strong pungent aromatic flavor and are widely used as masticators in Asia. It is popularly known as "Pan" or "veelayadhele" in Kannada or "betle vine" or betle pepper in English. *Piper betle* is cultivated in Srilanka, India, Malaya Peninsula, Phillippine Island and East Africa (Jayawera, 1982).

In traditional medicine of India and China *P. betle* is used in to treat bronchitis, asthma, catarrh, cough, leprosy and dyspepsia and diphtheria. It has digestive stimulant, carminative and aphrodiacac properties. Various pharmacological activities of *Piper betle* such as antifungal (Tewari and Nayak, 2003), gastro protective (Majumder *et al*., 2003), wound healing (Santhanam and Nagarajan, 1990), hepato protective activity (Saravanan *et al*., 2002), antioxidant (Santhakumari 2003; Pin et al. 2010), anti inflammatory (Pin et al. 2010), anti-malarial activity (Al-Adhroey *et al*., 2011) , antiallergic (Wirotesangthong *et al*., 2008), antidiabetic activity and antifertility on male rats (Rathnasooriya and Premakumara,1997) have been reported.

The antibacterial activity against human pathogenic bacteria has been reported by several researchers (Ghanwate and Thakare, 2012; Rajeshbabu *et al*.,2011; Chakraborty and Bharkha Shah, 2011), but antibacterial activity against plant pathogenic bacteria causing several important diseases in crops, fruits and vegetables has not been studied so far. The literature revealed that not much work has been done regarding *P. betle* leaf extracts as botanical pesticides to control plant pathogens. In the present investigation, antibacterial activity of aqueous and various solvent extracts against some common phyto-pathogenic bacteria has been evaluated.

Materials and methods

Plant material

Apparently healthy leaves of *P. betle* were collected in 2010 from Mansagangotri campus, Mysore District, Karnataka and used for the preparation of aqueous and different solvent extracts. Material was identified with the help of the taxonomists in the Department of studies in Botany,

University of Mysore and a voucher specimen of the plant is deposited in the Herbarium, Department of Studies in Botany, University of Mysore, Mysore.

Test pathogens

The bacteria *Xanthomonas viz, Xanthomonas axonopodis pv malvacearum* (X. a. m) known to cause angular leaf spot of cotton, *X.axonopodis p.v. vesicatoria* (x. c. v) causal organism of bacterial spot of tomato *Xanthomonas oryzae* causing bacterial blight of paddy were obtained from Department stock, Department Botany, University of Mysore, Mysore ,India. *Agrobacterium tumefaciens* causing crown gall disease of stone fruits (MTCC 431), *Xanthomonas campestris p.v. campestris* causing black rot of cabbage (MTCC 2286), *Erwinia carotovorum* sub sp *carotovora* causing soft rot disease of potato (MTCC 1428) were obtained from MTCC, Chandigarh, India. Standard type cultures of *Pseudomonas solanacearum* (NCIM 5103) causing bacterial wilt in solonaceous members and *Pseudomonas syringae* (NCIM 5102) causing bacterial canker and dieback of fruits were obtained from NCL, Pune, India.All the test bacteria were sub-cultured on nutrient agar and frequently sub-cultured. These bacteria served as test pathogens for the assay.

Preparation of extract

Aqueous extract

Samples (50 g) of thoroughly washed fresh leaves *P. betle* were macerated with 50 ml sterile distilled water in a warning blender (Warning International, New Hartford, CT, USA) for 10 min. The macerate was first filtered through double layered cloth and then centrifuged at 4000 rpm for 10 min. The supernatant was filtered through Whatman No. 1 filter paper and sterilized at 120 °C for 10 min. The extract was cooled at room temperature and lyophilized .The concentrated extract and preserved at 5 °C in an airtight bottle until further use.

Solvent extract

Thoroughly washed mature leaves were shade dried and powdered with the help of warning blender. 50 g of the powder was filled in the thimble and extracted successively with petroleum ether, chloroform, ethyl acetate and methanol using a soxhlet extractor for 48 h. The extracts were concentrated using rotary flash evaporator and preserved at 5 °C in an airtight bottle until further use. All the extracts were tested for their antibacterial activity.

Antibacterial activity assay

Antibacterial activity of aqueous extract and solvent extracts was determined by agar cup diffusion (Prez *et al*., 1990) and disc diffusion method(NCCLS) Cups are made in nutrient agar plate containing 20 ml of agar media using sterile cork borer (7mm) and inoculum containing 10^6 CFU/ml of bacteria were spread on the solid media with a sterile swab moistened with the bacterial suspension. The dried aqueous and solvent extracts were reconstituted in sterile water and methanol to a concentration of 100 mg/ml.

Aqueous extract and solvent extracts of 100 µl were placed in the cups made in the inoculated plates. Also, 100 µl of sterilized distilled water and methanol were placed in the cups separately which served as negative control. Antibiotic streptocycline (streptomycin sulfate IP and 90% tetracycline hydrochloride IP 10%; 1mg/ml) and R-Bacternol (1mg/ml) at their respective dosage were also tested which served as positive control.

For disc diffusion method 100 µL of extracts were loaded to sterile discs of 6mm, also 100 µL of sterile distilled water and methanol were loaded to sterile discs and placed on the solidified nutrient agar which served as negative control. Antibiotic Streptocycline (1mg/mL) and R-Bacternol (1mg/mL) at their respective dosage (100µg /disc) were also tested which served as positive control. The plates were incubated for 24 h at 37° C and zones of inhibition if any around the discs were measured. For each treatment three replicates were maintained and repeated twice.

Minimum Iinhibitory Concentration (MIC)

MIC was determined in 96 well sterile flat bottom micro titer plates based on micro dilution assay which is an automated turbidometric and colorimetric method as described by (Das, 2010). Inoculum of the test bacteria was prepared from 24 h cultured bacteria and a suspension was made in sterile/saline water and adjusted to 0.5M Mc Farland standard solution turbidity.

The crude extracts of methanol, ethyl acetate and chloroform were diluted to the concentration of 100 mg/ml which served as stock solution. The 96 well plates were prepared by dispensing 200 µL of broth and 100 µL of the extract to the first well. A two-fold serial dilution was made and final concentrations were 5-0.019 mg/mL. The inoculum suspension of 10 µL of each bacterial strain was added to each well.

The wells containing nutrient broth with inoculum and solvent served as negative control. The plates were incubated at 37 °C for 24 h and the turbidity was measured at 620 nm using micro plate reader (LT4000, LABTECH

Instruments, UK). The lowest concentration that inhibited visible growth was recorded as the MIC based on the readings.

The MIC was also detected by adding 10 µL/well of TTC (2, 3, 5-triphenyl tetrazolium chloride, Sigma) dissolved in water (TTC 2 mg/mL) and incubated under appropriate cultivation conditions for 30 min in the dark (Qaiyami, 2007). Viable organism reduced the dye to pink color compound. The lowest concentration at which the color change occurred was taken as the MIC value. All MIC tests were repeated in triplicates.

Statistical analyses

Statistical calculations were carried out using one way analysis of variance (ANOVA) and the significances of the differences between means are calculated using Tukeys multiple range test under the significance level of $P < 0.05$.

Phytochemical analysis

Phytochemical analysis of petroleum ether, chloroform, ethyl acetate, methanol and water extract was carried out for the detection of active secondary metabolites or different constituents such as tannins, alkaloids, flavanoids, terpenoids, steroids, carbohydrates, proteins and saponins. The dried extracts were reconstituted in methanol and 1 mL of each extract was subjected to standard phytochemical analysis according to the procedure described by Harborne (1998).

Results

The results of antibacterial activity of aqueous and solvent extracts of *P. betle* by cup diffusion method are presented in Table-1. Petroleum ether, chloroform, ethyl acetate and methanol extract subjected to antibacterial activity showed inhibition against the tested bacteria, but the zone of inhibition varied. Petroleum ether extract recorded lower inhibition zone ranging between 14-20 mm, while higher inhibition ranging between 26-40 mm was recorded in methanol extract. Chloroform and ethyl acetate recorded a moderate inhibition ranging from 19-25 and 15-20 mm, respectively. Aqueous extract did not showed activity against any of the bacteria tested.

Table 1. Antibacterial activity of different extracts of *Piper betle* and antibiotics on phyto pathogenic bacteria by agar well diffusion method (in mm)

Test Bacteria	Solventcontrol	Petroleum. ether	Chloroform	Ethyl acetate	Methanol	Streptocycine	R-Bacternol
X.c.pv. *campestris* (*X.c*.pv. *c)*	0.00	21.5±0.28^{a}	20.25±0.25^{de}	18.5±0.64^{ab}	28.5±0.85^{de}	27.5±0.95^{c}	12.5±0.50^{d}
*X. a.*pv. *malvacearum* (*X..a.*pv. *m)*	0.00	14.5±0.28^{d}	19.5±0.28^{e}	19.5±0.28^{a}	35.5±1.08^{abc}	22.25±0.25^{d}	0.0
X. Oryzae (X. o)	0.00	19.5±0.28^{b}	22.5±0.28^{bc}	16.75±0.25^{bc}	33.5±1.8^{bc}	27±0.25^{c}	16.0±0.40^{ab}
X.a.pv.*vesicatoria (X.a.* pv. *v)*	0.00	21.5±0.28^{a}	21.5±0.28^{cd}	18.75±0.47^{ab}	38.5±0.40^{a}	23.5±0.28^{d}	14.25±0.25^{bcd}
Agrobacterium tumefaciens (A.t)	0.00	15.25±0.25^{d}	19.7±0.25^{e}	15.75±0.25^{c}	38.0±0.50^{ab}	23.0±0.40^{d}	13.25±0.25^{cd}
Erwinia carotovora (E. car)	0.00	15.75±0.25^{d}	22.75±0.47^{bc}	18.75±0.47^{ab}	25.5±0.86^{e}	31.0±0.57^{ab}	16.25±0.75^{a}
Pseudomonas solanacearum (P. sol)	0.00	17.25±0.47^{c}	25.25±0.25^{a}	19.75±0.85^{a}	31.5±0.64^{cd}	32.0±1.08^{a}	0.0
Pseudomonas syringae (P. sy)	0.00	15.5±0.28^{d}	23.50±0.64^{b}	20.0±0.40^{a}	36.5±0.84^{ab}	28.75±0.47^{bc}	15.0±0.40^{abc}

Values are mean of four independent replicates. Figures followed by different letters in columns differ significantly when subjected to Tukey ($P<0.05$).

The results of antibacterial activity by disc diffusion method (Table-2) also recorded good inhibition zone as in cup diffusion method, but the zone of inhibition was slightly lesser against all the bacteria. The maximum zone was recorded in methanol extract ranging between 23-28 mm.

Table 2. Antibacterial activity of different extracts of *Piper betle* and antibiotics on phyto pathogenic bacteria by Disc diffusion method

Test Bacteria	Solvent control	Pet. ether	Chloroform	Ethyl acetate	Methanol	Streptocycline	R-Bacternol
X.c.pv. *campestris(X.c*.pv. *c)*	0.00	15.25±0.25^{a}	15.5±0.28^{de}	14.25±0.47^{cd}	22.5±0.25^{de}	27.5±0.95^{c}	12.5±0.50^{d}
X..a.pv. *malvacearum* (*X..a*.pv. *m)*	0.00	10.75±0.25^{c}	13.75±0.25^{f}	16.5±0.28^{bc}	24.0±0.25^{bc}	22.25±0.25^{d}	0.00
X. Oryzae(X. o)	0.00	15.25±0.47^{a}	18.75±0.25^{ab}	13.75±0.62^{d}	23.0±0.40^{cd}	27±0.25^{c}	16.±0.40^{ab}
X.a.pv.*vesicatoria(X.a..*pv. *v)*	0.00	15.5±0.28^{a}	16.75±0.25^{cd}	15.0±0.40^{cd}	27.50±0.50^{a}	23.5±0.28^{d}	14.25±0.25^{bcd}
Agrobacterium tumefaciens (A.t)	0.00	13.25±0.25^{b}	14.0±0.70^{ef}	13.0±0.40^{d}	27.25±0.47^{a}	23.0±0.40^{d}	13.25±0.25^{cd}
Erwinia carotovora(E.car)	0.00	11.75±0.25^{bc}	17.25±0.25^{bc}	14.75±0.62^{cd}	20.50±0.28^{e}	31.0±0.57^{ab}	16.25±0.75^{a}
Pseudomonas solanacearum (P. sol)	0.00	15.25±0.25^{a}	20.0±0.40^{a}	17.25±0.64^{ab}	21.25±0.47^{de}	32.0±1.08^{a}	0.00
Pseudomonas syringae (P. sy)	0.00	11.75±0.47^{bc}	19.5±0.28^{a}	19.25±0.47^{a}	25.75±0.25^{ab}	28.75±0.47^{bc}	15.0±0.40^{abc}

Values are mean of four independent replicates. Figures followed by different letters in columns differ significantly when subjected to Tukey ($P<0.05$)

Among the bacteria tested *X. axonopodis* pv. *vesicatoria* was highly susceptible to all the extracts with a maximum inhibition zone of 21.5 mm in petroleum ether extract and 38.5 mm in methanol extract. *E. carotovora* was less susceptible to petroleum ether and methanol extracts and moderately to chloroform and ethyl acetate extracts. The potential of antibacterial activity of different solvent extracts of *P. betle* indicates that methanol extract is the most potent followed by chloroform, ethyl acetate and petroleum ether extracts.

Petroleum ether extract showed maximum activity against *X. axonopodis* pv. *vesicatoria* (21.5 mm) and *X. campestris* pv. c*ampestris* (21.5 mm) while

least activity was recorded against *X. axonopodis* pv. *malvacearum*. Ethyl acetate extract recorded highest activity against *P. syringae* (20 mm) and least activity against *A. tumefaciens* (15 mm). Chloroform extract showed remarkable activity against *P. solanacearum* (25 mm) and least activity against *X. axonopodis* pv. *malvacearum* (19 mm). Methanol extract recorded highest activity against *X. axonopodis* pv. *vesicatoria (*38.5 mm) and least activity against *E. carotovora* (25.5 mm).

The standard antibiotics streptocycline and R-Bacternol were also subjected to antibacterial activity. Streptocycline recorded a maximum inhibition zone of 32 mm. R-Bacternol recorded least activity against all the tested bacteria with a maximum inhibition zone of 16 mm but did not showed activity against *X. axonopodis* pv. *malvacearum* and *P. solanacearum* at the tested concentration.

The zone of inhibition recorded by methanol extract was more than the antibiotic streptocycline against all the tested bacteria. This showed that methanol extract is highly potent with a broad spectrum antibacterial activity.

Table 3. MIC of Methanol, chloroform and Ethyl acetate extract of *Piper betle* against plant pathogenic bacteria(mg/ml)

Extracts(mg/ml)	**Bacteria**							
	X.c.pv. *c*	*X.a*.pv.*m*	*X. o*	*X.a*.pv. *v*	*A. t*	*E. car*	*P. sy*	*P. sol*
Methanol	0.078	0.078	0.104	0.040	0.019	0.108	0.40	0.131
Ethyl acetate	0.26	0.208	0.26	0.091	0.116	0.145	0.091	0.104
Chloroform	0.091	0.065	0.13	0.078	0.029	0.081	0.078	0.078

X.a.pv.*m –Xanthomona saxonopodis* pv.*malvacearum, X.a*.pv.*v -X.axonopodis* pv.. *vesicatoria,* X.o-*Xanthomonas oryzae,* A.t-*Agrobacterium tumefaciens ,X.c*.pv.*c- Xanthomonas campestris* pv. *campestris, E.car –Erwinia carotovora, P.sol-Pseudomonas solanacearum,P.sy-Pseudomonas syringae.*

The minimum inhibitory concentration of different solvent extracts is presented in Table-3.The extracts which showed significant activity were considered for MIC determination. *A. tumefaciens* recorded the least MIC value of 0.019 mg in methanol extract. *X. axonopodis* pv. *vesicatoria* and *P. syringae* recorded the least MIC value of 0.091 mg in ethyl acetate extract. *A. tumefaciens* recorded the least MIC value of 0.029 mg in chloroform extract.

Phytochemical analyses of all the extracts are presented in Table-4. Tannins, flavonoids, glycosides and proteins were present in aqueous, methanol and petroleum ether extracts. Steroids are present in methanol and petroleum ether extracts. Alkaloids and glycosides are present in petroleum ether and chloroform extracts. Terpenoids are present only in petroleum ether extract and saponins in aqueous extract.

Table 4. Phytochemical analyses of aqueous and solvent extracts of *Piper betle*

Phytochemical compounds	Extracts				
	Aqueous	Petroleum ether	Chloroform	Ethyl acetate	Methanol
Alkaloids	-	+	+	-	-
Flavanoids	+	+	-	+	+
Terpenoids	-	-	-	-	-
Tannins	+	+	-	+	+
Steroids	-	+	-	-	+
Glycosides	+	+	+	+	+
Carbohydrates	-	-	-	+	+
Proteins	+	+	+	+	+
Saponins	+	-	-	-	-

+ = Present; - = Absent

Discussion

Biological control is an ecologically sound environment friendly strategy to reduce the dependence of high risk chemicals and antibiotics for disease management. The advantages of plant extracts over synthetic chemicals as increased interest of possible applications of plant derived secondary metabolites to pest and pathogen control.

Considering these facts in the present investigation, aqueous and different solvent extracts of a perennial plant *Piper betle* were subjected to antibacterial activity against important plant pathogenic bacteria causing very important diseases to vegetables, fruits and crops.

All the solvent extracts of *P. betle* showed activity against the test pathogens with variation in the inhibition zone. Among the tested solvent extracts, petroleum ether extract was the least active extract while methanol was the most effective extract showing a maximum zone of inhibition ranging up to 40 mm. The activity of the extracts was compared with standard antibiotics streptocycline and R-Bacternol (1mg/ml). The inhibition zone recorded by R-Bacternol was very less when compared to the extracts at the tested concentration. Streptocycline was another antibiotic tested against the phyto-pathogenic bacteria where the inhibition zone ranged from 23-32 mm.

The activity recorded by streptocycline was lesser than the methanol extract of *P. betle*. The antibiotic streptocycline in pure form showing lesser inhibition zone than the crude methanol extract speaks the potency of methanol extract in controlling the phyto-pathogenic bacteria. The MIC of the extracts at a very low concentration indicates the potency of different *piper betle* extracts. The increase in antibacterial spectrum of extract and decreased risk of pathogen resistance are observed in crude extracts, which contains a mixture of many

active compounds (Yazdani *et al*., 2011). The literature review shows that the methanol extract of *P. betle* recorded an inhibition of 82.25% radial growth of the anthranose fungus, colleotrichum capsici (Jonny *et al*., 2011). The aqueous extracts of *P.betle* as shown a maximum inhibition of 4.50 mm against *X.axanopoids*.pv..citri while solvent extracts have not been tested for phytopathogenic bacteria (Manonmani *et al*., 2009). Various extracts of *piper betle* have recorded good inhibition against various human pathogens (Ghanwate and Thakare, 2012; Rajeshbabu *et al*.,2011; Chakraborty and Bharkha Shah, 2011). Aqueous extract of this plant has shown good activity against dental plaque forming bacteria (Fathilah *et al*., 2009). Several other workers have shown that all the extracts have good antimicrobial potential of which methanol is highly potent followed by ethanol, ethyl acetate, chloroform and petroleum ether against many tested human pathogens (Shukla *et al*.,2009; Fawad *et al*. 2012; Chakraborty and Bharkha shah, 2011).The literature shows that antimicrobial activity is more in organic solvent extracts than in aqueous extract which has been evident in our investigations.

In the present study, plant powder are sequentially extracted with different solvents in increasing polarity order since polarity of the solvents seem to play an important role in the extraction of natural products which influences the antibacterial activity of the extract (Parekh and Chanda, 2007) and partial separation of active components may be an advantage to reduce the antagonistic effects of chemical constituents because of the compound present in crude mixture may interfere with the others (Furneis *et al*., 1978).

Phytochemical analysis revealed the presence of phenols, tannins and steroids in most of the extracts which indicates that *P. betle* extracts are phenol rich compounds. The literature shows that *piper betle* leaves are rich in a wide variety of secondary metabolites such as phenolic compounds (chavicol,hydroxyl chavicol),volatile oils (safrole,eugenol,isoeugenol,eugenol methyl ester), fatty acids (stearic and palmitic acid) and hydroxyl fatty acids (palmatic and myristic), which *in vitro* illustrate the antibacterial activity (Shukla *et al*., 2009).

Nalina and Rahim (2007) have reported that steroids present in the *P. betle* extracts were involved in surface interaction with the bacterial cell wall and membrane leading to alterations in the primary structure of cell wall and membrane ultimately leading to pore formation and degradation of the bacterial components. The phenolic compounds and tannins present in the plant extracts operates either by metal ion depletion or by direct action on the microbial membrane(Mc Donald *et al*.,1996), also tannins are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens (Kamba *et al*., 2010).

The literature survey reports various pharmacological activities of *P. betle* extracts. But *P. betle* extracts have not been exploited for controlling plant pathogens so far. The factors such as habitation, season of collection, maturity of plants etc. affect the concentration of their bioactive chemical constituents and antibacterial activity. So, in the present investigation many plant pathogenic bacteria causing diseases on vegetable, fruits and crops were selected initially for a new alternate control measures. The good control of many of these bacteria by various extracts (Tables 1 and 2) indicated the potentiality of the plant *P. betle* which could be exploited for management of phyto pathogenic bacteria at large.

Conclusion

Exploitation of naturally available chemicals from plants which retards the reproduction of undesirable microorganisms 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. It is proved in the present investigation that different extracts of *P. betle* showed significant activity against a broad array of plant pathogenic bacteria and forms new sources of botanical pesticides for biological control.

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