Annals of Plant Sciences
ISSN: 2287-688X
Research Article

Activity test of crude extracts of invasive plants *Ageratina adenophora* and *Ipomoea carnea* ssp. *fistulosa* against human pathogenic bacteria

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Abstract: The aqueous (distilled water) and alcoholic (methanol) crude extract from the leaves of invasive alien plant species *Ageratina adenophora* and *Ipomoea carnea* ssp. *fistulosa* were evaluated against six human pathogenic bacteria, three Gram negative: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniaea* (ATCC 15380) and *Proteus mirabilis* (ATCC 49132) and three Gram positive: *Enterococcus faecalis* (ATCC 29212) *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 25923) using disc diffusion method. Different concentrations of plant extracts (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml and 250 mg/ml) were applied and diameter of zone of inhibition (ZOI) of bacterial growth were recorded. Both plant extracts showed antibacterial activity against Gram positive and Gram-negative bacteria. Methanolic extract of plant extracts exhibited good activity against tested bacteria when compared to aqueous extract. Among tested plants *I. carnea* ssp. *fistula* was more active than *A. adenophora*. The zone of inhibition of bacterial growth increased with increasing concentrations. The demonstration of activity against all these organisms had shown that both alien invasive species; *Ageratina adenophora* and *Ipomoea carnea* ssp. *fistulosa. can* be used to produce raw materials/substances for further development of diverse antibiotics with broad spectrum of activity.

Key words: Pathogenic bacteria; Antibacterial; Invasive plants; Crude plant extract.

Introduction

Antibiotics are one of the most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. In recent times, there have been increases in antibiotic resistant strains of clinically important pathogens, which have led to the emergence of new bacterial strains that are multi-resistant (Aibinu *et al.*, 2003, 2004). The non-availability and high cost of new generation antibiotics with limited effective span have resulted in increase in morbidity and mortality (Williams 2000). Therefore, there is a need to look for substances from other sources with proven antimicrobial activity.

Recent trends show that the discovery rate of active novel chemical entities is declining (Lam 2007). Natural products of higher plants may give a new source of antimicrobial agents (Runyoro 2006, Shahidi 2004). The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of world (Reddy *et al.*, 2001; Ramasamy and Charles, 2009; Sahu and Devkota, 2016). Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc., which have been found *in vitro* to have antimicrobial properties (Dahanukar *et al.*, 2000, Cowan 1999; Shinde and Mulay, 2015). In an effort to expand the spectrum of antibacterial agents from natural resources, invasive alien species *Ageratina adenophora (Asteraceae) and Ipomoea carnea* (Convolvulaceae) has been selected.

In Nepal, Invasive Alien Species (IAS) is of a major problem and causing a negative impact on the environment, especially on biodiversity of the country, either socially or economically. Management of such alien invasive plant is necessary for conservation of biodiversity and native species. Antibacterial study of invasive alien plants helps to find the potentialities of

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effect of these plants on bacterial growth. Further phytochemical study also helps to find the chemical constituents that may be useful for pharmaceutical formulation. Invasive plants have important ecological and economic impacts world-wide and increasing attention is now being paid to eradication and management efforts (Pimental *et al.,* 2000, Pysek and Richardson 2010). So the result of present study may have great significance in the management of such invasive alien weed.

Materials and Methods

The leaf sample of *Ageratina adenophora* and *Ipomoea carnea* ssp. *fistulosa* was collected from Kirtipur and river side of Jhojhi Kataiya village respectively. The leaves were washed thoroughly 2–3 times with running tap water, chopped into small pieces and then air dried on the newspaper at room temperature till they become completely dry and then powdered with the help of grinder. Powder was stored in air tight zipper bag.

Preparation of extracts

25g of dried powdered leaf samples of *Ageratina adenophora* and *Ipomoea carnea* ssp. *fistulosa* were soaked separately in 250 ml of methanol (95%) and distilled water for 72 h. Then the mixture was stirred at 24 h interval using a sterile glass rod (Alagesaboopathi, 2011). The plant samples squeezed and then filtered with the help of 3 layered cotton cloth. Water content of distilled water filtrate was evaporated on heating mantle using water bath till the solution reduced to semisolid form (Bhattarai and Shrestha 2009). The methanolic filtrate was concentrated down with rotary vaccum evaporator under negative pressure and extracts were then transferred into sterile labeled bottles and they were made into semisolid form by evaporation to water bath at 50°C. Obtained crude extracts were weighted and made the bottles air tight and stored in a refrigerator at temperature 4°C for prior to use (Mahida and Mohan 2007).

Plant extract concentration

Different concentrations of the extracts (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, and 250 mg/ml) were made in both solvents i.e. methanol and distilled water separately. Methanol and distilled water solvents were used as negative control and antibiotics (Ampicillin and Amoxicillin at 10 mg/ml) were introduced as positive control (Oyedeji *et al.* 2010).

Collection of microorganisms

Three Gram negative bacteria: *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniaea* (ATCC 15380) and *Proteus mirabilis* (ATCC 49132) and three Gram positive bacteria: *Enterococcus faecalis* (ATCC 29212) *Bacillus subtilis* (ATCC 6633) and *Staphylococcus aureus* (ATCC 25923) were selected for the antibacterial study. The bacterial strains used for the test were obtained from Department of Microbiology, Teaching hospital, Maharajgunj, Nepal. They were taken on slants and later cultured in test tube having nutrient broth.

Antibacterial Test

The disc diffusion method (Peach and Tracey 1950, Balouiri *et al.,* 2016) was used for the antibacterial study with slight modifications. The paper disc (5mm diameter) was made by punching the filter paper Sartorious stedium 292. The test discs were prepared by dipping and saturating sterilized punched filter paper in both positive and negative control and different concentrations of the plant extracts (50 mg/ml, 100 mg/ml, 150 mg/ml, 200 mg/ml, and 250 mg/ml). For antibacterial test, Nutrient Agar media was applied (HI media method recommended). The melted Nutrient Agar poured almost 10–10 ml in each petriplates (which were already sterilized, divided into six chambers, labeled with the date, code name of the bacteria and the concentrations code) and was left to solidify for 15–20 minutes. The inoculums of bacteria were transferred into petriplates containing solidified media using sterile cotton swab. The sterile cotton swab was dipped into well mixed distilled water test culture and was spread on the media by moving the swab in Z –shape. One swab was used for single bacterium. Seven replicates were used for each bacterium. The culture plates were allowed to dry for 5–10 minutes. Then in each petriplate in each chamber different concentrated and controls disc were put with the help of sterile forceps. The plates were then incubated at 37°C

for 24 h. Microbial growth was determined by measuring the diameter of zone of inhibition (ZOI).

Preliminary Phytochemical Screening

Preliminary qualitative phytochemical screening was carried out on aqueous and methanol extract applying the standard protocols described by (Harborne 1973, Sofowara 1993, Trease & Evans 1989). Change in color was noted for the result.

Data analysis

The antibacterial assay was done in seven replicates and standard deviation (S.D.) from the mean was calculated. The effect of extracts on bacterium was evaluated by a one-way analysis of variance (ANOVA) using SPSS version 16. Differences of letters at $p<0.05$ were determined by the TUKEY test.

Results and Discussion

In present study the antibacterial activities of two invasive alien species viz. *Ageratina adenophora* and *Ipomoea carnea* ssp. *fistulosa* of Nepal was investigated against six human pathogenic bacteria. The antibacterial capacity of crude extracts of plants at different concentrations was measured by Zone of inhibition (ZOI). There was significant differences in mean value of ZOI of bacterial growth in different concentrations of plant leaf extract (Tables 1 & 2).

During the study it was found that the methanolic and distilled water extract of both plants showed different activities against an array of Gram +ve and Gram –ve bacteria. *Ipomoea carnea* ssp. *fistulosa* was observed more active by showing ZOI against selected bacteria by inhibiting all of them in distilled water extract at 250 mg/ml (Table 1) and at all concentrations in methanolic extract (Table 2). This is may be due to the weed extract-based synthesis of silver nanoparticles is very efficient against selected human pathogens (Daniel *et al*., 2012, Adsul 2012) also found that the tested bacteria *Proteus vulgaris* was inhibited by acetone crude extract from leaves of *I. carnea* ssp. *fistulosa* but it was not able to inhibit *E. coli, B. cereus*, and *S. aureus*. These differences in result may be due to different habitat and different solvents used for the crude extract of plant material.

Methanolic extract of *Ageratina adenophora* produced zone of inhibition against all bacterial growth at five concentrations (Table 2) while in distilled water extract it was able to show zone of inhibition with *K. pneumoniae, E. faecalis*, *E. coli*, *B. subtilis* and *S. aureus* (Table 1). The plant was found to be ineffective against *Proteus mirabilis* at all concentrations. Baral and Maharjan (2011) also found the similar result. According to their result, the methanolic extract of *Ageratina adenophora* was effective against *Bacillus subtilis, Staphylococcus aureus, K. pneumoniae,* and *E. coli* at different concentrations. But it was not able to inhibit *P. mirabilis* and extract of distilled water only inhibited *B. subtilis*. Similarly, Barajas *et al*., (2013) found that essential oil of *A. Jahnii* and *A. pichinchensis* showed the zone of inhibition against *S. aureus* and *E. faecallis* but they were not able to inhibit the growth of *E. coli, K. pneumoniae, P. aeruginosa*. Bhattarai and Shrestha (2009) reported that organic solvent ethanol extract of *A. adenophora* showed antibacterial effect towards *Proteus* spp., *S.* spp., *B. subtilis, S. aureus*, *P.mirabilis*, and water solvent extract showed antibacterial effect towards *E. coli, S.aureus*, *S.* spp, *P.* spp. and *B. subtilis*. These results are somewhat dissimilar with this present study which may be due to the difference in plant part used, employed bacteria, used solvent, phytochemical differences between species and habitat of plant (Bhalodia and Shukla 2011).

Methanolic extract of *Ipomoea carnea* ssp. *fistulosa* at 50 mg/ml –250 mg/ml concentration had shown the highest ZOI; 12 mm– 14 mm diameter and 11 mm–14 mm diameter on *Enterococcus faecalis* and *Escherichia coli* respectively (Table 2). At 50–250 mg/ml concentrations the ZOI was 11 mm–13 mm diam. recorded on *Bacillus subtilis.* In distilled water extract, the highest ZOI ;10 mm–12 mm diameter and 10 mm–11 mm diameter on *Staphylococcus aureus* and *Enterococcus faecalis* respectively at 200 mg/ml – 250 mg/ml concentrations was recorded (Table 1). The

inhibitory effect of the tested extracts might be due to natural bioactive materials present in these extracts (Rai and Carpinella, 2006). Similarly, methanolic extract of *Ageratina adenophora* had shown the highest ZOI (11 mm–14 mm diameter) on *Proteus mirabilis* at 50 mg/ml – 250 mg/ml, (10 mm–13 mm diameter) on *Bacillus subtilis* at 50 mg/ml–250 mg/ml concentrations respectively. At 50 mg/ml –250 mg/ml concentration ZOI was shown (10 mm–12 mm diameter) on *Staphylococcus aureus.* At 150 mg/ml – 250 mg/ml concentrations the ZOI was shown (10–12 mm diameter) on *K. pneumoniae* and *E. coli* (Table 2). In distilled water extract *Ageratina adenophora* had shown the highest ZOI (8 mm diameter) at 250 mg/ml concentration on *K. pneumoniae* and *E. faecallis* (Table 1).

Both plants did not show effect against some tested bacteria at all concentrations. Distilled water extract of *I. carnea* ssp. *fistulosa* didn't show effect on *E. coli* at 50 mg/ml–200 mg/ml concentrations and on *B. subtilis* and *P. mirabilis* at 50 mg/ml (Table 1). DW extract of *A. adenophora* did not show effect on *P. mirabilis* at 50 mg/ml–250 mg/ml concentrations (Table 1), *B. subtilis* at 50 mg/ml–200 mg/ml concentrations, *K. pneumoniae* at 50 mg/ml–150 mg/ml concentrations and *E. coli* at 50 mg/ml-100 mg/ml (Table 1) concentration. With no antibacterial activities of extract of some plants against some selected bacteria may be due to the low concentration of extracts (Sudharameshwari and Radhika 2007, Bhalodia and Shukla 2011) and can thus only be proven by using large doses (Farnsworth 1993).

Three Gram negative and three-gram positive bacteria were tested with two invasive alien species plant extract in the present study. In comparison, generally Gram-positive bacteria use to find more susceptible than Gram negative bacteria (Parekh *et al.,* 2005, Akila *et al.,* 2016). But in this study mixed type of result was found. Among Gram positive bacteria *S. aureus* showed the highest zone of inhibition (12 mm diameter) followed by *E. faecalis* (11 mm diameter) at 250 mg/ml concentrations in DW extract of *I. carnea* ssp. *fistulosa* (Table 1). In *A. adenophora* DW extract, *E. faecalis* and *K. pneumoniae* showed equal ZOI (8 mm) at 250 mg/ml (Table 1). In methanolic extract of *I. carnea* ssp. *fistulosa* showed the highest ZOI (14 mm) against *E. coli* and *E. faecalis* at higher concentration at 250 mg/ml and *A. adenophora* showed the highest ZOI (14 mm) diameter at 250 mg/ml in methanol extract against *P. mirabilis* (Table 2). Almost similar type of result was found by several researchers (Biswas *et al.,* 2002). In their study they found that the neem leaves and seeds showed antibacterial activity against a wide spectrum of Gram- positive and Gram-negative microorganisms.

It is revealed from the Tables 1 and 2 that crude extract of two selected IAS has potential antibacterial activity against at least two bacteria from 50 mg/ml to 250 mg/ml concentrations in both distilled water and methanol extract. Methanol extract was found more active than aqueous extracts. The methanol extract of both plants inhibited the selected bacterial growth at all concentrations but distilled water extract was active only at higher concentrations and DW extract of *A. adenophora* was found inactive against *Proteus mirabilis* at all concentrations. This greater effectiveness of methanol extract compared to aqueous extract may be due to differences in constituents and amount of extraction of phytochemicals, which are toxic to targeted pathogens, present in leaf parts of tested invasive alien plants (Bhalodia and Shukla 2011). The leaf extracts of selected plants contain alkaloids, saponins and tannins (Table 3). Alkaloids and tannins have antibacterial and antihelminthic properties (Hedberg *et al.,* 1983), The methanol extract had antimicrobial activity against selected organisms; this may be due to the ability of the methanol to extract some of the active properties like phenolic compounds, saponin, bryophyllin and other secondary metabolites which are reported to be antimicrobial from the tested plants (Okwu and Josiah 2006). In aqueous solution, there may be lack of solubility of activity constituents or may be due to loss of some active compounds during extraction process of the plant sample (Sampathkumar *et al.,* 2008).

In the result, with the increase in concentration of plant extracts, the zone of inhibition of bacteria was also increased. Similar type of result was found by previous researcher (Lalfakzuala 2014). In their study methanolic extract of the leaves of four common weed were investigated on *Bacillus subtilis* and *Bacillus pumilis* at different concentration. Minimum ZOI was recorded at 25 mg/ml and maximum at 100 mg/ml. Comparison of the extract with commercial antibiotics Ampicillin and Amoxicillin had shown that the antibiotics were more effective in inhibiting the growth of the organisms than the extract (Tables 1 & 2). This may be due to the presence of stronger bioactive principles in the antibiotics and their molecular size which permit their penetration into the cells of the organisms (Oladunmoye 2006).

Table 1: Zone of Inhibition (ZOI) mm in distilled water leaf extract of *tested plant*

Bacterial strain	Zone of inhibition (ZOI) mm in DW leaf extract of selected plant spp										
		Concentrations (mg/ml)					Control				
	Plant spp	50	100	150	200	250	Negative DW	Positive Ampi.	Positive Amox.	P	F
K.p.	*A. adenophora*	0±0 a	0±0 a	0±0 a	7±7 b	8±0 .1 b	0±0 a	37±4 d	29±7 c	.000	155.65
	I.carnea	6±.4 b	6±.1 b	6±.3 b	7±.6 b	7± .8 b	0±0 a	37±4 d	29±7 c	.000	125.39
E.f.	*A. adenophora*	6±3 b	6±3 b	6±.2 b	7±.3 b	8±.3 b	0±0 a	34±.4 d	26±4 c	.000	359.58
	I.carnea	6±.3 b	7±.8 bc	8±1 bc	10±.8 cd	11 ±1 d	0±0 a	34±.4 f	26±4 e	.000	282.99
E.coli	*A. adenophora*	0±0 a	0±0 a	6±0 b	6±0 b	6±0 b	0±0 a	33±4 c	33±4 c	.000	304.51
	I.carnea	0±0 a	0±0 a	0±0 a	0±0 a	6±2 b	0±0 a	33±4 c	33±4 c	.000	293.65
B.s.	*A. adenophora*	0±0 a	0±0 a	0±0 a	0±0 a	6±.2 a	0±0 a	28±7 b	24±7 b	.000	87.50
	I.carnea	0±0 a	6±2 b	7±.1 b	9±.6 b	9±.6 b	0±0 a	28±7 c	24±7 c	.000	65.39
P. m.	*A. adenophora*	0±0 a	0±0 a	0±0 a	0±0 a	0±3 a	0±0 a	27±1 c	21±1 b	.000	540.37
	I.carnea	0±0 a	6±.5 b	7±.2 bc	8±.7 bc	10±1 c	0±0 a	27±1 e	21±1 d	.000	348.32
S.a.	*A. adenophora*	6±.3 a	6±.2 a	7±.6 a	7±.8 a	7±.8 a	0±0 a	26±7 b	21±11 b	.000	32.72
	I.carnea	6±.7 ab	8±1 ab	9±1 b	10±1 b	12±1 b	0±0 a	26±7 c	21±11 c	.000	27.95

The values were expressed as Mean ±S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. Tukey HSD multiple comparison test was done to compare the different letters at P< 0.05

Abbreviations: *K.p.=Klebsiella pneumoniae; E.f.=Enterococcus faecalis; E.coli=Escherichia coli; B.s.=Bacillus subtilis; P.m.=Proteus mirabilis; S.a.=Staphylococcus aureus*; Ampi=Ampicillin; Amoxi=Amoxicillin, values are mean ± SD of seven replicates

Table 2: Zone of Inhibition (ZOI) mm in methanol leaf extract of tested plants

Bacterial strain	Zone of inhibition (ZOI) mm in methanol leaf extract of selected plant spp										
		Concentrations (mg/ml)					Control				
	Plant spp	50	100	150	200	250	Negative Meth.	Positive Ampi.	Positive Amox.	P	F
K.p.	*A. adenophora*	9±1 b	10±.5 b	10±1 b	11±1 b	12±1 b	0±0a	37±4 d	29±7 c	.000	97.15
	I.carnea	9±2 b	9±2 b	9±2 b	11±1 b	11±1 b	0±0a	37±4 d	29±7 c	.000	88.00
E.f.	*A. adenophora*	8±1 b	8±.5 b	9±1 b	10±1 b	11±1 b	0±0a	34±.4d	26±4 c	.000	250.6
	I.carnea	12±1 b	12±.8 b	12±1 b	13±1 b	14±.8 b	0±0a	34±.4d	26±4 c	.000	223.31
E.coli	*A. adenophora*	8±1 b	9±.5 b	10±1 b	11±1 b	12±1 b	0±0a	33±4 c	33±4 c	.000	179.2
	I.carnea	11±2 b	13±1 b	13±1 b	13±1 b	14±1 b	0±0a	33±4 c	33±4 c	.000	135.72
B.s.	*A. adenophora*	10±.8b	11±.6b	12±.5b	12±.5 b	13±.5 b	0±0a	28±7 c	24±7 c	.000	45.57
	I.carnea	11±.8b	12±1 b	12±1 b	13±.8 b	13±1 b	0±0a	28±7 c	24±7 c	.000	43.06
P. m.	*A. adenophora*	11±1 b	11±1 b	12±.7b	13±.9 b	14±.9 b	0±0a	27±1 d	21±1 c	.000	220.09
	I.carnea	7±.9 b	10±2bc	11±2bc	11±1 c	12±1 c	0±0a	27±1 e	21±1 d	.000	161.19
S.a.	*A. adenophora*	10±.4b	11±.6b	12±.5b	12±.6 b	12±.6 b	0±0a	26±7 c	21±11 c	.000	24.72
	I.carnea	8±1 ab	9±1 b	9±1 b	10±1 b	11±1 b	0±0a	26±7 c	21±11 c	.000	26.81

The values were expressed as Mean ± S. D. and statistically analysis using One Way ANOVA for obtaining F and P value. Tukey HSD multiple comparison test was done to compare the different letters at P< 0.05

Abbreviations: A. *K.p. =Klebsiella pneumoniae; E.f. =Enterococcus faecalis; E.coli=Escherichia coli; B.s. =Bacillus subtilis; P.m. =Proteus mirabilis; S.a.=Staphylococcus aureus*; Ampi=Ampicillin; Amoxi=Amoxicillin, values are mean ± SD of seven

Table 3. Preliminary phytochemical screening of leaf extract of *A. adenophora* and *I. carnea* ssp. *fistulosa.* Responses to various tests were denoted by +, ++ and +++ signs indicating weak, moderate and strong reactions respectively while - for no reaction

Plant spp	Phytochemical constituents									
	Distilled water					Methanol				
	Tannin	Saponins	Terpenoids	Alkaloids	Flavonoids	Tannin	Saponins	Terpenoids	Alkaloids	Flavonoids
Ageratina adenophora	+++	++	+++	+++	-	+++	+++	-	+++	+++
Ipomoea carnea ssp. *fistulosa*	+	+++	+	++	-	+	+++	-	+++	+++

Conclusion

The result of this study had shown that both *Ageratina adenophora* and *Ipomoea carnea* ssp. *fistulosa* extracts had varied antibacterial activities against the tested organisms. The demonstration of activity against all these organisms had shown that both plants can be used to produce raw materials/substances for further development of diverse antibiotics with broad spectrum of activity. These findings can form the basis of further studies to isolate compounds, to find new therapeutic principles. Invasive alien species are considered as one of the greatest threat to natural ecosystem of the earth. Exploitation of these rapidly growing species can be done on making the different pharmaceutical products in one hand while proper management on the other side.

Acknowledgement

This work was supported by the University Grants Commission, Kathmandu, Nepal, under Faculty Research Grants.

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Cite this article as:

Ritu K. Das and Anjana Devkota. Activity test of crude extracts of invasive plants *Ageratina adenophora* and *Ipomoea carnea* ssp. fistulosa against human pathogenic bacteria. *Annals of Plant Sciences* 9.1 (2020) pp. 3699-3706.

http://dx.doi.org/10.21746/aps.2020.9.1.4

Source of support: University Grants Commission, Kathmandu, Nepal.
Conflict of interest: Nil.