Biosciences, Biotechnology Research Asia Vol. **7**(1), 413-417 (2010)

Antibacterial activities of *Ipomoea carnea* stem

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(Received: March 12, 2010; Accepted: April 21, 2010)

ABSTARCT

Natural products are used as traditional medicines from ancient times. They are having a great importance in *Ayurveda*. One of the medicinal plant species is *Ipomoea carnea*,which belongs to convolvulaceae family and fistulosa sub-family. Many Ipomoea species having antimicrobial activities were reported in literature. The present study explored the antibacterial activities of hexane, chloroform, ethyl acetate, acetone and methanol extracts of stem of *I. carnea* against, gram negative bacteria *Escherichia coli(*ATCC10536), *Klebseilla pneumoniae* (ATCC33495*),Proteus mirabilis* (ATCC12453) and *Pseudomonas aeruginosa (*ATCC10662*)* and gram positive becteria *Bacillus subtilis* (ATCC11774), *Staphylococcus aureus* (ATCC1026) and *Bacillus cereus* (ATCC10876). Air shade dried powdered material was extracted using solvents of increasing polarity from non-polar (n-haxane) to polar (methanol) solvents. These extracts were tested against above seven bacterial strains in concentration 40 ìL/ml. The antibacterial activity was determined by using disc diffusion method. Streptomycin was used as a standard. Acetone and methanol extracts of *I. carnea* stem showed significant activity. Therefore, these extracts were selected for further investigation to determine its therapeutic potential.

Key words: *Ipomoea carnea,* extract, antibacterial activity, dics diffusion method, Steptomycin.

INTRODUCTION

In present days antibiotic resistance has become a global concern[1]. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens[2]. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and mechanism of action for new and re-emerging infectious diseases[3]. Therefore researchers are increasingly turning their attention to folk medicines, looking for new leads to develop better drugs against microbial infections and screening of several medicinal plants for their potential antimicrobial activities[4,5]. *I. carnea*, a wild herb, largely available in all states of India is a native of South America. It is a green manure crop and also used as a folk medicine[7]. Ash of *I. carnea* leaves is used in a skin disease in some rural areas of Chhattisgarh, India[6]. A preliminary pharmacological study on the glycosides from the leaves of *I. carnea* is reported[7]. There are reports on synergistic effect of insecticides with plant extracts of *against* malarial Vector *Anopheles stephensi* [8]. Antimicrobial activity of, metal complexes prepared from the leave proteins are reported[9]. Many Ipomoea species having antimicrobial activities are reported in literature[1,10]. However, there is no information available about the antimicrobial activity of *I. carnea* stem against some selected bacteria. The present study explores the antibacterial activity of hexane, chloroform, ethyl acetate, acetone and methanol extract of *I. carnea* stem against gram negative bacteria *Escherichia coli(*ATCC10536), *Klebseilla pneumoniae* (ATCC33495*), Proteus mirabilis* (ATCC12453) and *Pseudomonas aeruginosa (*ATCC10662*)* and gram positive bacteria *Bacillus subtilis* (ATCC11774), *Staphylococcus aureus* (ATCC1026) and *Bacillus cereus* (ATCC10876). Streptomycin was used as the standard.

MATERIAL AND METHODS

Collection and identification of plant materials

The plant material was collected from the river sides of Pune, Maharastra, India. The plant was authenticated at Botanical Survey of India, Pune. Its authentication no is ELICAI.,BSI/WC/Tech/ 2009/96.

Preparation of plant extract

Air shade dried and powdered stem material of *I. carnea* (50mg) was extracted with solvents (250ml) n-hexane(1), chloroform(2),ethyl acetate(3), acetone(4) and methanol(5) by refluxing for 18 hours. Solvent were recovered under reduced pressure to obtain the crude extracts. Solution of different concentration were prepared using acetone as solvent. Broad fractionation of acetone extract(4) was carried out to get five major fractions as 10% ethyl acetate-hexane(A), chloroform(B), ethyl acetate(C), acetone(D) and methanol(E). Similarly, methanol extract was fractionated as chloroform($A^{/}$), 10% methanol-chloroform($B^{/}$),50% methanol-chloroform($C^{/}$)and methanol($D^{/}$).

Bacterial Strains

On the basis of pathogenic importance, seven pathogenic bacterial strains, gram negative bacteria *Escherichia coli(*ATCC10536), *Klebseilla pneumoniae* (ATCC33495*), Proteus mirabilis* (ATCC12453) and *Pseudomonas aeruginosa (*ATCC10662*)* and gram positive bacteria *Bacillus subtilis* (ATCC11774), *Staphylococcus aureus* (ATCC1026) and *Bacillus cereus* (ATCC10876) were selected . All bacterial strains were maintained at 4°C on nutrient agar (Hi-Media) slants and cultured at 37°c using same agar medium.

Antibacterial activity assay

The paper disc diffusion method was used to determine the antibacterial activity. Sample of each extract, (100 mg) was dissolved in respective solvent (1ml). Steriled filter paper discs (5mm) were impregnated with 40 µL of these solvent extracts. Adequate amount of Muller –Hinton Agar was dispensed into sterile plates and allowed to solidify under aseptic conditions. The count of the bacterial strains was adjusted to yield 1×10^{7} to 1×10^{8} ml^{-1}. The test organisms (0.1ml) were incubated with a sterile spreader on the surface of the solid medium in plates. The Agar plates inoculated with test organism were incubated for one hour before placing the extract impregnated paper discs on the plates. The sterile discs impregnated with different extracts were placed on Agar plates. The plates were incubated at 37ºC for 24 hrs. Acetone was used as a solvent for dissolving the extracts and was used as the control in the assay. The antibacterial activity results were calculated as a mean of 3 replicates. Streptomycin was used as standard. Antibacterial activity was assessed based on the measurement of the diameter (in mm) of the clear zones of growth of inhibition.

RESULTS AND DISCUSSION

The extractive yield of different extracts of *I. carnea* stem is reported in Table 1. The extractive yield varied for different solvents used. The maximum yield was in methanol (20%) and minimum was in hexane (5%).

The antibacterial activities of hexane, chloroform, ethyl acetate, acetone and methanol extracts were assayed in vitro by Agar disc diffusion method against seven different bacterial strains. The results are shown in Table 2.

Agar disc diffusion assay of activities of the extracts showed a variable clear zone for different bacteria. In all tested extracts, no clear zones were created in *Bacillus subtilis* and *Staphylococcus aureus.* Hexane extract showed activity only against two tested bacterial strains, namely, *Klebseilla pneumoniae* and *E. Coli* .It was inactive against gram (+) ve bacteria. Chloroform and ethyl acetate extracts showed almost equal

Table 1: Extractive yields in different solvents

Solvent	Yield (%)
Hexane	5
Chloroform	10
Ethyl acetate	7
Acetone	15
Methanol	20

activities against both gram (+) ve and gram (-) ve bacteria. Methanol extract showed activity against both gram (+) ve and gram (-) ve bacteria and was found more active than the chloroform and ethyl acetate extracts. The highest antimicrobial activity was shown by the acetone extract against *Bacillus cereus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. It showed activity against both gram (+) ve and gram (-) ve bacteria. The most susceptible bacteria was the *Escherichia coli*, because all extracts showed higher activity against *Escherichia coli* than other strains. The most resistant bacteria were *Bacillus subtilis* and *Staphylococcus aureus*.

Non-polar hexane and semi-polar chloroform extracts were more active against *Klebseilla pneumoniae* than polar ethyl acetate, acetone and methanol extracts. Methanol and acetone extracts showed more activities than other extracts against *Bacillus cereus*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Escherichia coli*. These extracts were further fractioned and activity was tested against bacterial strains.

Activities of acetone and methanol extract fractions

Table-3 presents the results of antibacterial activities of the acetone extract fractions: 20% ethyl acetate-hexane(A), chloroform(B), ethyl acetate(C), acetone(D) and methanol(E).

All the fractions of both acetone and methanol extracts showed the highest activities against *Escherichia Coli*. Fraction D and D/ of both the extracts showed the highest activity against *Escherichia coli*. The effect of concentration of these two fractions on antibacterial activity was studied further. MIC study of these two fractions against *Escherichia coli* was carried out and the results are listed in Table-5.

For both fraction D and D/ , the MIC was found 25 µg/mL. and activity was increased with increasing concentration.

From the results, it can be concluded that Gram (-) ve bacteria are more susceptible towards the plant (stem) extract than the Gram (+) ve bacteria. Among the five extracts , acetone extract followed by methanol extract showed better

Table 2: Activities of different extracts

Extract	Zone of inhibition(mm)*						
	Klebseila pneumonia	**Bacillus subtilis**	***Staphylococcus aureus***	***Bacillus cereus***	***Proteus mirabilis***	***Pseudomonas aeruginosa***	***Escherichia coli***
Hexane	8	-**	-	-	-	-	9
Chloroform	8	-	-	8	7	7	9
Ethyl acetate	7	-	-	7	7	7	9
Acetone	7	-	-	9	9	9	10
Methanol	7	-	-	8	8	8	10
Streptomycin	21	39	37	19	16	23	29

*Zone of inhibition including the diameter of filter paper disc (5mm)

** No activity

antibacterial activity. The compounds responsible for these antibacterial activities have not been isolated. However, further work is going on to determine the different bioactive compounds from the plant and their full spectrum of efficacy. Phytochemical analysis of the plant revealed the presence of phenolic compounds, terpenoids, alkaloids, flavonoids and steroids.Some of which have antioxidant and antibacterial activities . The antibacterial properties of the plant may be due to the individual or combined effect of the molecules present in it. Compositional variation of plant active biomolecules in different extracts is responsible for exhibition of different antibacterial activity[2].

Table 3: Activities of different fractions of acetone extract

Fraction	*Bacillus cereus*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Escherichia coli*
A	7	7	7	9
B	8	8	8	10
C	7	7	8	9
D	8	9	9	13
E	9	9	7	11

Table 4: Activities of different methanol extract fractions

Fraction	*Bacillus cereus*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Escherichia coli*
A/	8	8	7	9
B/	8	8	8	11
C/	9	9	8	11
D/	9	8	8	12

Table 5: MIC of the D and D/ fractions

Fraction	Concentration (µg/ml)	Zone of Inhibition (mm)*
D	400	13
	200	11
	100	9
	50	8
	25	7
	10	-
D/	400	12
	200	11
	100	10
	50	9
	25	8
	10	-

*Zone of inhibition including the diameter of filter paper disc (5mm)

Based on these results it can be conclude that the plant (stem) extracts of *I. carnea* have great potential as antibacterial compounds, especially antibacterial agent against infectious diseases caused by the bacteria *Escherichia coli*.The present study of in vitro antibacterial evaluation of these extracts form a primary platform for further phytochemical and pharmacological studies on this largely available plant. These promissory extracts opened the possibility of finding new clinically effective antibacterial compounds and establish *I. carnea* as a natural source of bioactive compounds.

ACKNOWLEDGMENTS

Authors are thankful to the Head, Department of Chemistry, S.P.College and the principal, S.P.College, Pune, India, for providing necessary laboratory facilities for the work.

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