

Antioxidant and Antibacterial Activity of Stem Aqueous Extract of *Ceropegia juncea* Roxb. (Asclepiadaceae)

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Ceropegia juncea Roxb. (Asclepiadaceae) is a medicinal plant whose fleshy stem extract was tested and the same possessed antioxidant properties including radical scavenging activity. The antibacterial activity was tested by agar diffusion method against *Bacillus subtilis*, *Citrobacter koseri*, *Enterobacter aerogens*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The minimum inhibitory (MIC) and the minimum bacterial concentration (MBC) values varied for the six bacterial genus tested.

Keywords: Antibacterial activity, Antioxidant activity, *Ceropegia juncea*, DDPH, MIC, MBC.

Herbal remedies from medicinal plants have been used traditionally in many parts of the world where access to formal healthcare is limited (Adewole and Caxton-Martins, 2006). Herbal remedies may have recognizable therapeutic effects (Bailey and Day, 1989); or can also have toxic side-effects (Keen *et al.*, 1994). The use of medicinal plants provides an indication of beliefs about illness and its treatment that may conflict with beliefs of workers in the formal healthcare system (Morgan and Watkins, 1988).

Ceropegia juncea Roxb. (Asclepiadaceae) is an important medicinal herb, which is used as a source of 'Soma' a plant drug of the Ayurvedic medicine with a wide variety of uses.

The fleshy stem and root tubers used as a raw material for traditional and folk medicines for the treatments of stomach and gastric disorders (Nikam and Savant, 2009). *C. bulbosa* and *C. tuberosa* root tubers contain an alkaloid called ceropegin (Nadkarni (1976), consumed after cooking (Mabberley (1997). The root tubers also contain starch, sugars, albuminoids, fats, gum, crude fiber and valuable constituents in many traditional Indian Ayurvedic drug preparations that are active against many diseases especially diarrhea and dysentery. The ceropegin is an analgesic and tranquilizer which acts against ulcers and inflammation (Adibatti *et al.*, 1991). In view of this, the present study was designed to evaluate the antioxidant and antibacterial activity of *Ceropegia juncea* fleshy stem.

MATERIAL AND METHODS

Collection and identification of plant material

Fresh stem of *Ceropegia juncea* Roxb. family Asclepiadaceae was collected from a naturalized population in Nartharmalai (Pudukottai District), Tamil Nadu, India (Fig 1, a,b & c). For periodic harvest of explants, stocks were

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maintained in the herbal garden at Department of Botany, National College, Tiruchirappalli, Tamil Nadu. The taxonomic identities of these plants were confirmed by Flora of the Presidency of Madras (Gamble, 1925). Fresh stem material was washed under running tap water, air dried in shade, homogenized to fine powder and stored in sterile air-tight bottles for the experimental work.

Identification of chemical compounds in *C. juncea* stem by GC-MS method

The identification of bioactive compounds of *C. juncea* stem was carried out by GC / MS method as described by (Adams (2001) and Christos Liolios et al (2007).

Bacterial cultures

The bacterial cultures tested in this work *Bacillus subtilis* NCBT 008, *Citrobacter koseri* NCBT 042, *Enterobacter aerogens* NCBT 012, *Escherichia coli* NCBT 001, *Klebsiella pneumonia* NCBT 018, *Pseudomonas aeruginosa* NCBT 054 were maintained in immobilized condition in Nutrient Gelatin medium containing Gelatin 15g, Nutrient broth 100 ml with pH 7.2 (Harrigan and McCance, 1969) in Microbiology Lab, Department of Botany, National College, Tiruchirappalli.

Preparation of stem extract

100 gms of dried stem powder was extracted using 300 ml of sterile distilled hot water and 90% methanol (v/v). The immersed stem powder was kept in a shaker (60 rpm) for a week and filtered through Whatman No.1 filter paper. The extract was concentrated using simple distillation and lyophilisation method and stored in sterile vials at 4° C for further work.

Total phenolic content assay

Total phenolic content assay was conducted using Folin Ciocalten reagent method (Sato *et al.*, 1996). An aliquot of 0.5 ml (100 mg/ml in 80% methanol) of the extract was mixed with 0.5 ml of Folin-Ciocalten reagent and 0.05 ml of 10% Na₂CO₃ and absorbance was measured at 735 nm after 1 hr of incubation at room-temperature. Gallic acid was used as the standard for the calibration curve and the total phenolic contents were expressed as mg gallic acid equivalents per gram of tested extract.

Measurement of α,α -Diphenyl- β -picrylhydrazyl radical scavenging activity

Quantitative measurement of radical scavenging properties was carried out in a universal

bottle. The reaction mixture contained 50 ml of test samples (0.1 mg/ml) or 80% MeOH as a blank and 5 ml of 0.04% (w/v) solution of α,α -Diphenyl- β -picryl- hydrazyl in methanol (Oktay *et al.*, 2003). Two different known antioxidants such as Vitamin E (0.1 mg/ml) and butylated hydroxytoluene (BHT, Sigma) (0.1 mg/ml) were used for comparison as positive control. The colorimetric test for free radicals relies on the reaction of a specific antioxidant (AH) with α,α -Diphenyl- β -picrylhydrazyl (α,α -Diphenyl- β -picrylhydrazyl-H+A) and the method was adopted from Ohnishi *et al.* (1994). In the radical form α,α -Diphenyl- β -picrylhydrazyl resulted in a maximum absorption at 517 nm but upon reduction by an antioxidant, the pale-yellow non-radical form was produced and hence the absorption at 517 nm disappeared.

Decolourization was measured at 517 nm after incubation for 30 min. Measurements were taken in triplicate, α,α -Diphenyl- β -picrylhydrazyl scavenging effect was calculated using the equation.

α,α -Diphenyl- β -picrylhydrazyl

$$\text{Scavenging Effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100$$

A₀ was the absorbance of the control and A₁ was the absorbance in the presence of sample (Oktay *et al.*, 2003). The actual decrease in absorption induced by the test compounds was compared with the positive controls. Extract concentration providing 50% inhibition (IC₅₀) was calculated from the graph of percentage of inhibition against extract concentration. Tests were carried out in triplicate.

Measurement of reducing power

The reducing power of the stem extracts of *Ceropegia juncea* and butylated hydroxytoluene were determined according to the method of Yen and Chen (1995). The extracts (1-10 mg/ml) and butylated hydroxytoluene (1-6 mg/ml) were mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide and then incubated at 50° C for 20 min. An equal volume of 1% trichloroacetic acid was added to the mixture to stop the reaction. The mixture was centrifuged at 4500 rpm for 10 min. The supernatant was mixed with distilled water and 0.1% FeCl₃ at a ratio of

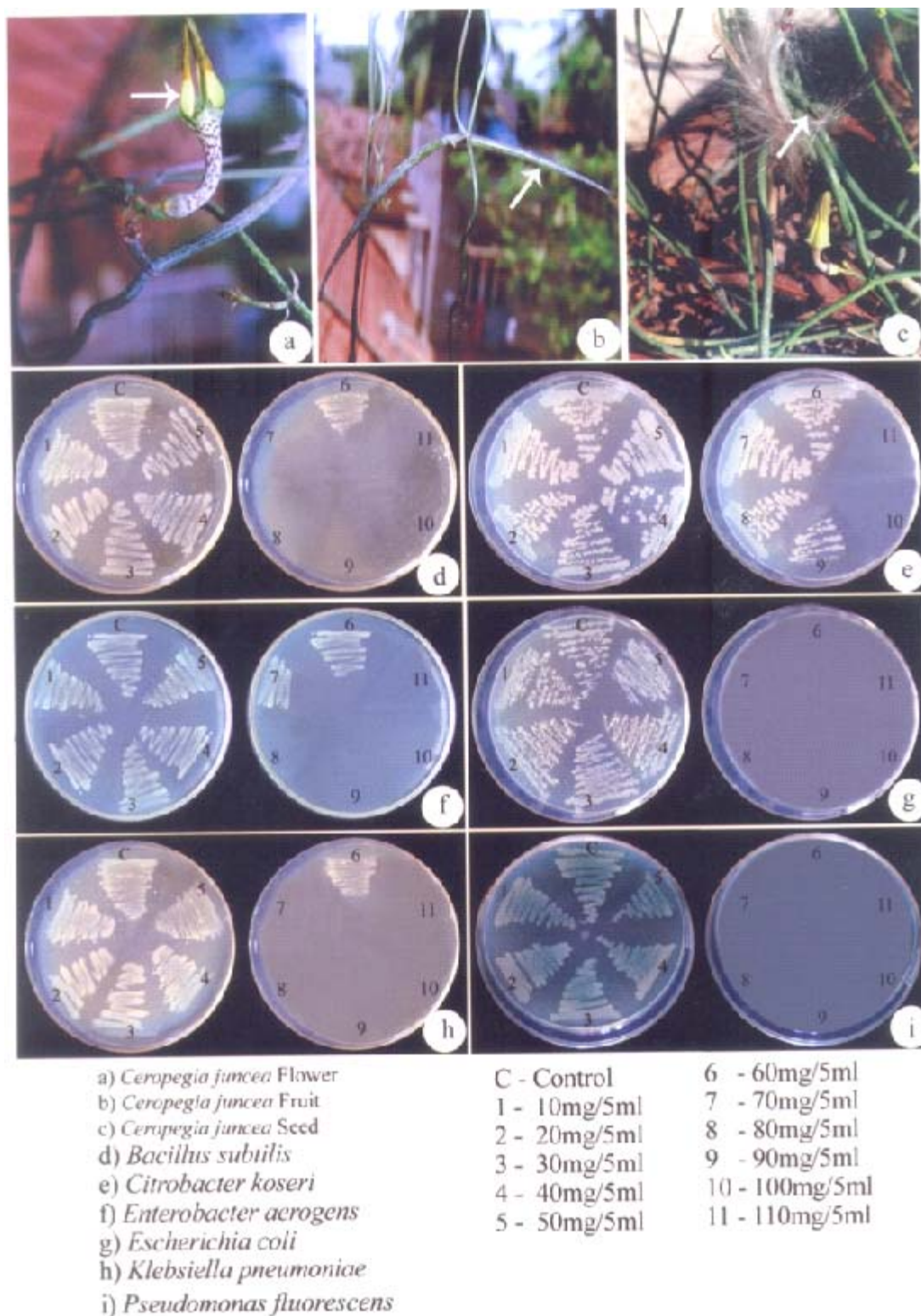


Fig 1. Antibacterial activity of *ceropogia juncea* Roxb.(asclepiadacea) fleshy stem

1:1:2 (v/v/v) and then absorbance was measured at 700 nm.

RESULTS AND DISCUSSION

Bioactive compounds

The chemical compounds isolated from *C. juncea* stem is presented in (Table 1), which revealed Betulin and Lupeol, the two major compounds might have been responsible for antioxidant and antibacterial activities.

Antioxidant studies - DPPH and reducing power

The scavenging effect of the stem methanol extracts on DPPH radical exhibited strong radical scavenging activity. It might be due to their hydrogen-donating ability (Oyaizu, 1986; Hyang-Sook *et al.*, 2000) and is generally associated with the presence of reductants (Pin-Der and Duth, 1998). The total phenolic content of the crude extract of the leaf (100 mg/ml in 80% methanol) of *C. juncea* was 12.0 mg (mg gallic acid equivalent/g).

Table 1. Identification of chemical compounds in *C. juncea* stem by [GC-MS study]

No	RT	Name of the compound	Molecular	MW	Peak Area %
1.	7.34	2,-Formyl-9-[a-d-ribofuranosyl] hypoxanthine.	C ₁₁ H ₁₂ N ₄ O ₆	296	3.65
2.	7.96	Phenol,2,6-bis(1,1-dimethylethyl)-4-methyl-,methylcarbamate	C ₁₇ H ₂₇ NO ₂	277	0.25
3.	9.00	N-Ethyl-N-methyl-4-nitrosobenzennamine	C ₉ H ₁₂ N ₂ O	164	3.18
4.	11.16	2,Piperidinone,N-[4-bromo-n-butyl]-	C ₉ H ₁₆ BrNO	233	0.15
5.	11.64	3,7,11,15,- Tetramethyl-2-hexadecan-1-ol	C ₂₀ H ₄₀ O	296	1.44
6.	11.90	Phytol	C ₂₀ H ₄₀ O	296	0.32
7.	13.08	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	C ₂₀ H ₃₀ O ₄	334	0.53
8.	13.46	Pentadecanoic acid,2,6,10,14-tetramethyl-methyl ester	C ₂₀ H ₄₀ O	312	0.11
9.	14.97	Phytol	C ₂₀ H ₄₀ O	296	1.27
10.	15.49	Pyrrolo[1,2-a] pyrimidine-4(6H)-one, 7,8-dihydro-3-acetyl-2-amino-	C ₉ H ₁₁ N ₃ O ₂	193	2.10
11.	30.64	Androstane-11,17-dione,3-[(trimethylsilyl)oxy]-17-[o-(phenylmethyl)oxime],(3a,5a)	C ₂₉ H ₄₃ NO ₃ Si	481	4.14
12.	31.14	1- Monolonoleoylglycerol trimethylsilyl ether	C ₂₇ H ₅₄ O ₄ Si ₂	498	2.87
13.	32.40	2,2-Dimethyl -6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol	C ₁₄ H ₂₄ O ₄	256	4.90
14.	33.67	Lupeol	C ₃₀ H ₅₀ O	426	30.38
15.	34.40	Betulin	C ₃₀ H ₅₀ O ₂	442	44.69

Table 2. Scavenging effect percentage of crude stem extract of *Ceropegia juncea* and known antioxidant BHT and Vitamin-E at 0.1 mg/ml respectively

Sample	Scavenging effect %
BHT	8.5
Vitamin-E	4.0
<i>C. juncea</i> stem extract	8.0

Scavenging effect on α,α-Diphenyl-β-picrylhydrazyl radical

The leaf extract was screened for DPPH radical scavenging activity according to the method Ohnishi *et al.* (1994). *C. juncea* stem extract exhibited stronger radical scavenging activity than butylated hydroxytoluene and vitamin E (Table 2).

The concentration of the crude extract decreased α,α-Diphenyl-β-picrylhydrazyl to 50% of the initial radical concentration (IC₅₀) in 30 min.

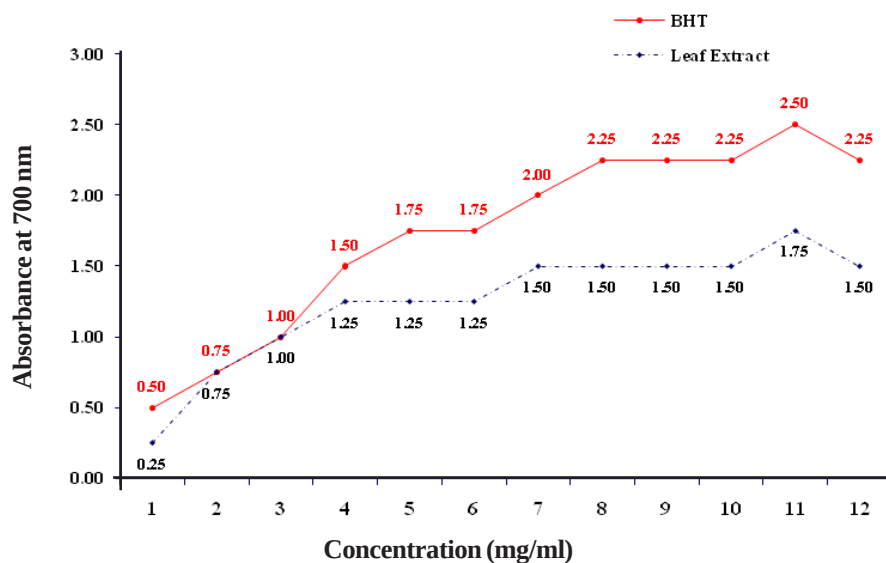


Fig. 3. Reducing power of the crude stem extract of *C. juncea* and known antioxidants butylated hydroxytoluene (BHT). Increase in the absorbance at 700 nm indicates the reducing power

This parameter is widely used to measure antioxidant power. A low IC_{50} is indicative of the presence of strong antioxidant compounds such as flavones, catechins and xanthan (Shimada *et al.*, 1992; Schinella *et al.*, 2000). Strong radical scavenging activity of stem extract of *C. juncea* might be due to their hydrogen donating ability as stated by Oyaizu (1986), Hyang-Sook *et al.* (2000) and is generally associated with the presence of reductants (Pin-Der and Duth, 1998). At proper concentration, the crude extracts may act as electron donors and react with free radicals to convert them to more stable products and terminate radical chain reaction (Duh and Yen, 1997). The results demonstrated that the stem crude extracts of *C. juncea* have potent free radical scavenging activity. Reducing power of *C. juncea* stem extract

The presence of reductants (antioxidants) in the stem extract would result in the reduction of the Fe^{3+} ferricyanide complex to its ferrous form. The formation of Perl's Prussian blue measured at 700 nm can be used to monitor the amount of Fe^{2+} complex. The dose-response curve for the reducing power of the stem extract is shown in Fig.3. Butylated hydroxytoluene (BHT) was used as positive control and the reducing powers of stem extract also increased with the increase in the concentration. At a dose of 10.0 mg/ml, the stem extract showed reducing power value 1.75 when

compared with the BHT control 2.5 respectively. These results revealed that the stem extract acts as electron donors and could react with free radicals, converting them to more stable products and terminating the radical chain reaction (Yen and Chen, 1995; Yamaguchi *et al.*, 1998).

The above results suggest that the *C. juncea* stem extract can eliminate human health problems in which free radicals play a major role (Karbownik and Lewinski, 2003; Lugasi *et al.*, 1999). There is a continuing effort in the food industry to seek additional plant sources that are safer and better antioxidants *C. juncea* stem extracts can serve as a suitable natural source of antioxidant.

Antibacterial Activity

The minimal inhibitory concentration (MIC) value and the minimum bacterial concentration (MBC) value varied for the six bacteria tested for this study. *Bacillus subtilis* showed MIC 60 mg/ 5ml and MBC 70mg/ 5ml, *Citrobacter koseri* showed MIC 90mg /5 ml and MBC 100mg/ 5ml; *Enterobacter aerogens* showed MIC 70 mg/ 5ml and MBC 80mg/ 5ml; *Escherichia coli* showed minimum values of MIC 50 mg/5 ml and MBC 60 mg/5 ml; *Klebsiella pneumoniae* showed MIC 60 mg/5 ml and MBC 70 mg/5 ml whereas *Pseudomonas aeruginosa* showed maximum value. MIC 50 mg/5 ml and MBC 60 mg/5 ml. (Fig 1,d,e,f,g,h & i).

Traditionally plant leaf extracts are used to treat microbial infections including antibacterial antitumor, anti-cancer and cytotoxic studies (Haddock, 1994; Oberlies *et al.*, 1995; Mor, 2000; Chang, 2001; Padma *et al.*, 2001; Liaw, 2002; Chang *et al.*, 2003; Pathak *et al.*, 2003; Yuan *et al.*, 2003; Kojima, 2004).

C. juncea is rich in many phytochemical compounds like Betulin, Lupeol, 2,2-Dimethyl-6-methylene-1-[3,5-dihydroxy-1-pentenyl]cyclohexan-1-perhydrol, 1-Monoloneoleoylglycerol trimethylsilyl ether, Androstane-11,17-dione,3-[(trimethylsilyloxy)-17-[o-(phenylmethyl)oxime],(3a,5a), Pyrrolo[1,2-a]pyrimidine-4(6H)-one,7,8-dihydro-3-acetyl-2-amino-, Phytol, Pentadecanoic acid,2,6,10,14-tetramethyl-methyl ester, 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester, Phytol, 3,7,11,15,-Tetramethyl-2-hexadecan-1-ol, 2,Piperidinone,N-[4-bromo-n-butyl]-, N-Ethyl-N-methyl-4-nitrosobenzennamine, Phenol,2,6-bis(1,1-dimethylethyl)-4-methyl-,methylcarbamate, 2,-Formyl-9-[a-d-ribofuranosyl]hypoxanthine. Many phytochemicals like Flavonoidal and other Polyphenolic compounds have antioxidant effect (Sharma Paras *et al.*, 2011) and active against multi-drug resistant (MDR) cancer cells by ATP-blocking properties (Oberlies *et al.*, 1995; Pathak *et al.*, 2003; Tormo *et al.*, 2003; Kojima, 2004) Betulin and Lupeol the major bioactive compounds of *C. juncea* stem extract possess antimicrobial properties and active against the tested bacteria.

In conclusion, *C. juncea* stem extract can serve as a safe natural plant antioxidant to the food industry. In addition it can also be a potential antibacterial source and the bioactive compounds will find a place in the formulation of herbal medicine to treat bacterial mediated infections.

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