Int J Pharm Bio Sci 2013 Oct; 4(4): (B) 187 - 191

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ANTIMICROBIAL ACTIVITY OF *IN VITRO* AND *IN VIVO* PLANT EXTRACTS OF *CEROPEGIA PUSILLA*. WIGHT AND ARN AN ENDEMIC MEDICINAL PLANT

K.KALIMUTHU* R.PRABAKARAN AND C. BRINDHA**

Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-641018, India
*** PSG College of Arts and Science Coimbatore.*

ABSTRACT

The present study aimed at to evaluate the antimicrobial activity of methanol extracts of the endemic medicinal plant, *Ceropegia pusilla* against five different bacterial strains namely, *Shigella sonnei* MTCC 2957, *Klebsiella pneumoniae* MTCC 109, *Escherichia coli* MTCC 118, *Staphylococcus aureus* ATCC 43300 and *Bacillus* sp., and four different fungal strains namely, *Aspergillus* sp., *Mucor* sp., *Penicillium* sp., and *Candida albicans* ATCC 60192, by agar well diffusion method. The methanol extract from the *in vitro* plant showed higher inhibitory effect towards the bacterium, *Klebsiella* sp., moderate inhibitory effect against the fungal strains *Penicillium* sp., and *Candida albicans*. There is no inhibitory effect towards the other bacterial and fungal strains whereas the extract from *in vivo* plant showed a higher inhibitory effect towards the bacterium *Klebsiella* sp., and fungal strains *Mucor* sp., *Penicillium* sp., and *Candida albicans*., moderate inhibitory effect against *Staphylococcus* sp., and no inhibitory effect against the other bacterial and fungal strains. On the basis of this experimental result, it can be concluded that methanol extract of *in vitro* and *in vivo* plants of *Ceropegia pusilla* could be considered for further isolation and evaluation as therapeutic antimicrobial.

KEYWORDS: ***Ceropegia pusilla*, *in vitro* and *in vivo* plants, methanol extract, Antimicrobial activity.**

K.KALIMUTHU
Plant Tissue Culture Division, PG and Research Department of Botany, Government Arts College (Autonomous), Coimbatore-641018, India
Email : k_kalimuthu@rediffmail.com

**Corresponding author*

INTRODUCTION

Infectious diseases represent an important cause of morbidity and mortality among the general population, particularly in developing countries. Bacterial species presents the genetic ability to acquire and transmit resistance against currently available antibacterials. These antimicrobial resistant bacteria are the cause of numerous clinical problems worldwide and the development and increase of resistance among pathogens causing nosocomial and community-acquired infections and are associated with the widespread utilization of antibiotics [1-4]. Hence it is very important to develop new antimicrobial drugs especially from natural products. Medicinal plants act as a rich source of anti-microbial agents, and used as a potent source of the powerful drugs. The raw drug obtained from various parts of different plant species has different medicinal properties [5]. Considering the vast potentiality of plants as sources for antimicrobial drugs several authors have investigated the antimicrobial activity of many medicinal plants [6-9]. In India, high species richness in plants enhances the scope of the medicinal plant research. Western Ghats is very rich in its medicinal wealth. The forests and other vegetations of this region is a treasure house of about 700 medicinal plants and out of which some are used for traditional and folk huge medicinal practices. Many are exploited commercially for their active enzymes and their commercial value. *Ceropegia*, is an old world tropical genus, includes about 200 species and distributed in tropical and sub-tropical Asia, Africa, Australia, Malaysia, and in the canary of Pacific Islands [10]. In India, 50 species are present [11], out of which 28 are endemic to peninsular India [12] and among them *Ceropegia pusilla* is an endemic and endangered plant, grows widely in the hilly tracts of South India [13]. The tubers are edible and contain an alkaloid called ceropegin [14], used in Ayurvedic drug preparation that are active against many diseases especially diarrhea, dysentery and syphilis. The root tuber also contains starch and used as a nutritive tonic and blood purifier [15-16]. This plant is also used as an antidote for snake bite [17]. Hence the present study reveals the mysteries of the antimicrobial activity of the medicinal plant *Ceropegia pusilla* (*in vitro* and *in vivo*) which has a possible application in the pharmacological industries to produce new drugs of higher potential against the pathogens dominating the human life.

MATERIALS AND METHODS

(i)Plant materials

Plant of *Ceropegia pusilla* was collected from Ooty, Nilgiris District, Tamilnadu (India) and authenticated by Botanical Survey of India (BSI/ SRC/ 5/23/2012-13/ tech, 1268).

(ii)Crude methanol extract preparation

The *in vitro* and *in vivo* fresh plants were carefully washed with tap water, rinsed with distilled water and air dried for one hour. Then it was cut into small pieces, dried in room temperature for two weeks, grounded into powder with the help of hand mill and stored in room temperature. The whole plant powder was macerated in methanol 95% (v/v) in 1:3 proportions at room temperature, undergoing mechanical shaking for 4hours followed by filtration. The extract obtained was concentrated in a rotary evaporator at 40°C and the residue was extracted twice again analogously, thereby obtaining the crude methanol extract.

(iii)Test microorganisms

The microorganisms used in this study includes *Staphylococcus aureus* ATCC 43300, *Shigella sonnei* MTCC 2957, *Klebsiella pneumoniae* MTCC 109, *Escherichia coli* MTCC 118 and *Bacillus* sp., and fungal strains *Aspergillus* sp., *Candida albicans* ATCC 60192 *Mucor* sp., and *Penicillium* sp., were obtained from the Department of Microbiology, Hindustan College of Arts and Science, Coimbatore. The bacterial and fungal strains were cultured on respective selective media and stored at 20°±2°C.

(iv)Preparation of inoculums

Exactly 18 hour broth culture of the test bacteria isolates was suspended into sterile nutrient broth and fungal strains in sterile distilled water. 1.0 × 10^6 CFU/ml.

(v)Antimicrobial assay – Well diffusion method

The modified agar well diffusion method was employed to determine the antimicrobial activities [18]. About 0.2 ml of the standardized 24 hour old broth culture of the test organisms were spread onto sterile Muller Hinton Agar plates. These were then allowed to set. Methanol extracts (30 µg/ml) of in vitro and in vivo plants were prepared. With the aid of a sterile cork borer, wells of about 6 mm in diameter were bored on the plates. About 0.5 ml of the extract was dispensed into the wells and then allowed to stand for about 15 minutes for pre diffusion of the extracts to occur. The plates were then incubated at 37^0C for 24 hours. At the end of the incubation period, inhibition zones formed on the agar plates were observed for various bacterial and fungal strains and are tabulated.

RESULTS AND DISCUSSION

The antimicrobial activity of methanol extract of the whole plant of *C. pusilla* was assayed against five species of bacteria including *Shigella sonnei*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Bacillus* sp., and four species of fungi including *Aspergillus* sp., *Mucor* sp., *Penicillium* sp., and *Candida albicans* by agar well diffusion method. Results of antimicrobial activity assays are presented in Table 1&2 and Figures 1&2.

Table 1

Antibacterial activity of methanol extract of C.pusilla whole plant.

Methanol extract of	*Escherichia Coli*	*Klebsiella* sp.,	*Shigella* sp.,	*Staphylococcus* sp.,	*Bacillus* sp.,
In vitro plant	R	S	R	R	R
In vivo plant	R	S	R	I	R

Table 2

Antifungal activity of methanol extract of C.pusilla whole plant.

Methanol extract of	*Aspergillus* sp.,	*Mucor* sp.,	*Penicillium* sp.,	*Candida albicans*
In vitro plant	R	R	I	I
In vivo plant	R	S	S	S

Key note: R-Highly Resistant I-Intermediate (moderately sensitive) S-Sensitive

Figure 1

Antibacterial patterns of methanol extract of C.pusilla whole plant.

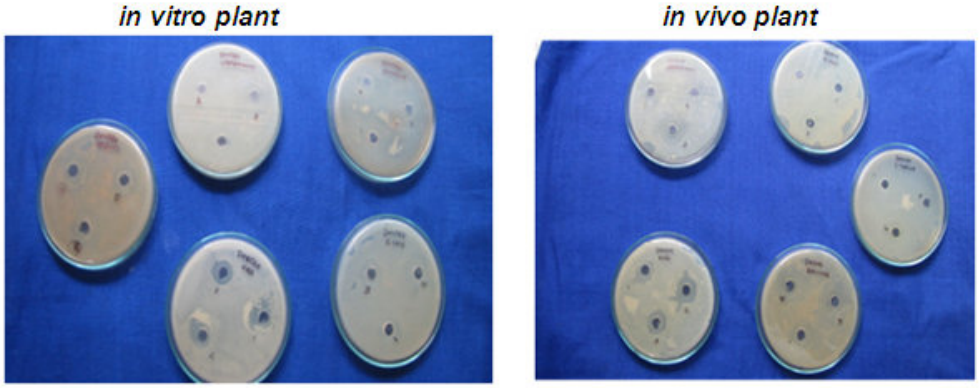
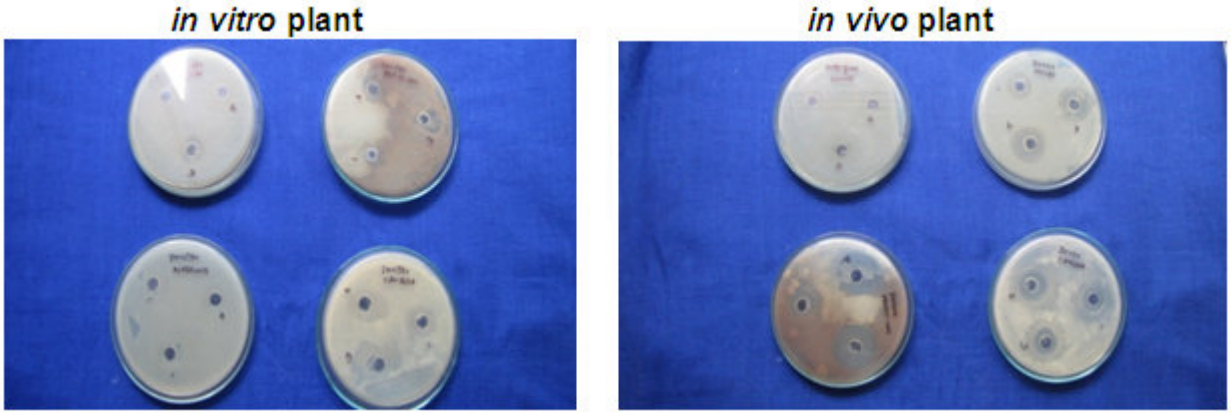


Figure 2
Antifungal patterns of methanol extract of C.pusilla whole plant.



The methanol extract of the in vitro whole plant showed effective antibacterial activity against *Klebsiella* sp., and moderately inhibit the growth of the fungi *Penicillium* sp., and *Candida albicans*. The *in vitro* plant extract did not show any antimicrobial activity against the other test microorganisms. The methanol extract of the *in vivo* whole plant also showed good antibacterial activity towards *Klebsiella* sp., fungus *Mucor* sp., *Penicillium* sp., and *Candida albicans*. The extract also moderately inhibited the growth of the bacterium of *Staphylococcus* sp. All other test microorganisms showed resistance against the methanol extract of *in vivo* plant. Plants that have antimicrobial compounds present in them have tremendous potential to be used for therapeutic purposes without any undesirable effects that are usually observed while using synthetic compound [19]**.** The results obtained in the present study indicate the presence of antimicrobial compounds in the crude extracts of this plant that can inhibit the growth of some micro-organisms which show a correlation between the reported uses of these local plant species against different microbial pathogens. This provides justification for the use of this plant in folk medicine to treat various infectious diseases. Its antimicrobial activities can be further enhanced if the active components are purified and proper dosage for administration is determined. Therefore, further studies will be needed on the methanol extract of both *in vitro* and *in vivo* plants of Ceropegia pusilla for the isolation of respective pure compounds, to confirm its potentiality towards medicinal uses with respect to heal infectious diseases

.

REFERENCES

1. Harvey, J. and A. Gilmour. Characterization of recurrent and sporadic Listeria monocytogenes isolates from raw milk and nondairy foods by pulsed-field gel electrophoresis, monocin typing, plasmid profiling, and cadmium antibiotic resistance determination. App. Env. Microbiol. 67:840-847. (2001).
2. Ruiz-Bolivar, Z., R. A. Poutou-Piñ ales and A. K. Carrascal-Camacho. Resistencia antimicrobiana y a desinfectantes de Listeria spp. NOVA – Pub. Científica Ciencias Bioméd. 6(10):101-236. (2008).
3. Sakoulas, G. and R. C. Moellering. Increasing Antibiotic Resistance among Methicillin-Resistant *Staphylococcus aureus* Strains. Clin. Infec. Dis. 46(5):360-367,(2008).
4. Howden, B. P., J. K. Davies, P. D. R. Johnson, T. P. Stinear and M. L. Grayson. Reduced Vancomycin Susceptibility in *Staphylococcus aureus*, including Vancomycin-Intermediate and

Heterogeneous Vancomycin-Intermediate strains: Resistance mechanisms, laboratory detection, and clinical mplications. Clin. Microbiol. Rev. 23(1):99.(2010).

5. Srivastava, J., J. Lambert and N. Vietmeyer, Medicinal plants: An expanding role in development. World Bank Technical Paper. No. 320. (1996).
6. Srinivasan, D., S. Nathan, T. Suresh and O. Perumalsamy. Antimicrobial activity of certain Indian medicinal plants used in folkloric medicine. J. Ethnopharm. 74:217-220.(2001).
7. Kumarasamy, Y., P. Cox, M. Jaspars, L. Nahar and S. Sarker. Screening seeds of Scottish plants for antibacterial activity. J. Ethnopharm. 83:73-77. (2002).
8. Masika, P. J. and A. J. Afolayan.. Antimicrobial activity of some plants used for the treatment of livestock disease in the Eastern Cape, South Africa. J. Ethnopharm. 83:129–134. (2002)
9. Hamill, F. A., S. Apio, N. K. Mubiru, R. Bukenya- Ziraba, M. Mosango, O. W. Maganyi and D. D. Soejarto. Traditional herbal drugs of Southern Uganda, II: literature analysis and antimicrobial assays. J. Ethnopharm. 84:57- 78. (2003).
10. Bruyns PV Three new succulent species of Apocyanaceae (Asclepiadoideae) from Southern Africa. Kew Bull. 58:427-435. (2003).
11. Surveswaran S, Kamble MY, Yadav SR, Sun M .Molecular phylogeny of *Ceropegia* (Asclepiadaceae, Apocyanaceae) from Indian Western Ghats. Plant Syst. Evol. 281:51-63. (2009).
12. Ahmedulla M, and NayarMP Endemic plants of the Indian region peninsular India. Bot. Survey India, The Kolkata 1. (1986)
13. Botanical Survey of India, Studies on rare and endangered species. http://www.envfor.nic.in/bsi/research.html. (2002)
14. Jain SK, Defillips RA ,Asclepiadaceae. In: Medicinal plants of India. Algonac, India 1:89-94. (1991).
15. Prakash JW, Raja RDA, Anderson NA, Williams C, Regini GS, Bensar K, Rajeev R, Kiruba S, Jeeva S, Das SSM . Ethnomedicinal plants used by Kanitribes of Agasthiyarmalai biosphere reserve southern Western Ghats. Indian J. TraditKnowl. 7:2008; 410 -413. (2008).
16. Mabberley DJ , the Plant Book. Cambridge University Press, Cambridge, pp.: 96-98. (1997)
17. Duraisamy Suresh and Subramaniam Paulsamy ,Phenological observation and population dynamics of six uncommon medicinal plants in the grasslands of Nilgiris, Western Ghats, India Maejo Int. J. Sci. Technol.4(02), 185-192. (2010)
18. Collins, C. H., Lynes, P. M., Grange, J. M. Microbiological Methods (7^{th} edition). Butter wont-Heinemann Ltd, Britain pp. 175-190. (1995)
19. Fridous AJ, Islam SNLM and Faruque ABM, Antimicrobial activity of the leaves of *Adhatoda vasica*, *Calatropis gigantium*, *Nerium odoratum* and Ocimum sanctum. Bangladesh J. Bot. 227, (1990).