Ethnobotanical Leaflets 12: 1067-72. 2008.

In Vitro Evaluation of Antibacterial Activity Using Crude Extracts of *Catharanthus roseus* L. (G.) Don.

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Issued 17 November 2008

ABSTRACT

Catharanthus roseus popularly known as Madagascar periwinkle is a potential source for anti-leukemic alkaloids. The present study aims to evaluate the possibility for the presence of novel bio-active compounds against pathogenic bacteria, as most of the pathogens develop drug resistance against commonly used antibiotics. To determine antibacterial activity, crude extracts from different parts of *C. roseus* were tested against bacterial strains of clinical significance. Extraction of bio-active principles in appropriate solvent was followed by evaluation of antibacterial activity by disc diffusion assay against selected bacterial stains. Data depicts that the pattern of inhibition largely depends upon extraction procedure, plant part, physiological and morphological state of plant, extraction solvent and microorganism tested. Of different plant materials tested, extracts prepared from leaves showed significantly higher efficacy. Extracts prepared using organic solvents exhibited higher antibacterial activity when compared to their corresponding aqueous extracts. However, no activity was observed in the aqueous extracts. Among the extracts that were significantly active, extract obtained using ethanol exhibited maximum activity against bacterial strains tested. Gram (-) stains were more sensitive when compared to Gram (+) bacteria. The study implicates that bio-active compound(s) of *C. roseus* could potentially be exploited as antibacterial agents.

KEYWORDS: Disc diffusion assay; antibacterial activity; *C. roseus;* bio active compounds.

INTRODUCTION

Emerging and reemerging infectious diseases and spread of deadly drug-resistant strains pose a challenge to public health care services. In particular, emergence of resistance to antibiotics has hampered the pace by which newer antibiotics are being introduced into the public domain (Russell, 2002). This drives the discovery of novel antimicrobial therapeutic agents from the medicinal herbs (Gootz, 1990). Alternatively, global attention has been shifted towards hunting novel bio-molecules of plant origin for the development of new drugs. Since, the phytochemicals are more specific, biodegradable and are suppose to have fewer side effects. Phytochemicals offer unique platform for structural diversity and biological functionality which is indispensable for drug discovery

(Nisbet, 1997; Verpoorte, 1998).

Despite ever increasing advancement in the field of medicine and molecular diagnosis it is estimated that 80% of the world population is still dependant on the plant derived pharmaceuticals. WHO report depicts that plant based products or its derivatives accounts for nearly 28% of drugs available in the market (Newman et al., 2003). Natural products as such and their derivatives have historically been exploited as a valuable source of novel therapeutic agents (Koehn and Carter, 2005). Further, a large proportion of plant based compounds are used as lead molecules in drug discovery to produce synthetic molecular analogs that have similar skeletons yet intricate structures. This implicates that phytochemicals play a critical role in diversity-oriented synthesis (DOS) of natural product-like pharma-compounds (Marcaurelle and Johannes, 2008).

Human beings have exploited the plants for curing ailments since antiquity. Traditional systems of medicine like Ayurveda, Unani, Homeopathy and Siddha solely rely on phyto-pharmaceuticals that are obtained from selected medicinal plants (herbs) based on traditional knowledge gained over a period of time and expertise by the traditional healers. Plants continue to be major resources for therapeutic compounds. Ethnobotanical and ubiquitous plants serve as a rich resource of natural drugs for research and development (Kong et al., 2003). Medicinal plant-based drugs owe the advantage of being simple, effective and exhibit broad spectrum activity (Chin et al., 2006). Medicinal plant products when compared to their synthetic counterparts minimize the adverse side effects. Indigenous systems of medicine that use plant-based drugs could provide both concepts of therapy as well as therapeutic agents to complement modern medicine in management of diseases. The global interest in therapeutic potential of phytochemicals during the last few decades is therefore quite obvious.

Traditionally, *C. roseus* has been used in folk medicine to treat diabetes and high blood pressure. As antidiabetic remedy, it was believed to promote insulin production and increase utilization of sugars from food. Its diuretic action, alleviate high blood pressure. However, in modern medicine alkaloids and chemotherapeutic agents form *C. roseus* are known for their anticancer pain-relieving and properties.

C. roseus (Madagascar periwinkle) belongs to the family Apocynaceae. It is short-lived perennial plant with dark green and glossy leaves. Pharmacological studies have revealed that *C. roseus* contains more than 70 different types of alkaloids (indole alklaloids) and chemotherapeutic agents that are effective in treating various types of cancers – breast cancer, lung cancer, uterine cancer, melanomas, and Hodgkin's and non-Hodgkin's lymphoma (Verpoorte, 1998). The anticancer drugs vincristine and vinblastine are obtained from alkaloids of *C. roseus*. Besides anti-cancer activity, alkaloids from this plant are known for their antihypertensive and antispasmodic properties (Verpoorte et al., 2002). In vitro studies have shown that the plant large number of alkaloids upon elicitation (El Sayed et al., 2004). The enormity of work conducted on this medicinal plant is so large that since 1950s about 2000 publications and about 295 patents dealing with this plant and its products have appeared thus far. Ironically, amidst such enormous data, only handful of publications deals with the antimicrobial potential of this plant (Govindaraji, 2007). Considering the medicinal value that this plant has, we in the present study evaluated the antibacterial potential in crude extracts of leaves, stem, root and flower against selected clinical bacterial strains.

MATERIALS AND METHODS

Plant Material

C. roseus was collected from the fields in Madurai, India, and taken to the laboratory. The Flora of Presidency of Madras (Gamble, 1993) and The Flora of Tamil Nadu Carnatic (Matthew, 1983) were used for identification and authentication of the plants. Plant materials (leaves, stem, root and flower) were washed separately under running tap water, followed by rinse using sterilized distilled water. Excess of water was removed from the plant material using filter paper before they were used for extraction.

Extract Preparation

Aqueous extract

Ten g of plant material was macerated in pestle and mortar with 100 ml distilled water at room temperature and then filtered using muslin cloth. Filtrate obtained was subsequently passed through Whattman's No. 1 Filter paper under aseptic conditions and the filtrate was collected in fresh sterilized glass tubes and used within 24h for evaluation of antibacterial activity.

Solvent Extraction

Ten g of each plant part was mixed with 100 ml organic solvent (ethanol or methanol). The mixture thus obtained was filtered through muslin cloth and subsequently passed through Whattman's No. 1 Filter paper. The filtrate was concentrated by evaporation of solvent at room temperature. Extracts were stored at 4°C until further use.

Bacterial Strains

A total of eight bacterial strains including both Gram-negative and Gram-positive bacteria *Escherichia coli, Pseudomonas aeruginosa, Serratia marcescens, Salmonella typii, Staphylococcus aureus, Streptococcus pyrogens, Bacillus cereus and Bacillus subtilis* (Table 1) were selected to assess susceptibility patterns against the phytochemical extracts. The bacterial cultures were maintained in nutrient agar slants at 37°C. Each of the microorganisms was freshly cultured prior to susceptibility testing by transferring them into a separate test tube containing nutrient broth and incubated overnight at 37°C.

Antibacterial Susceptibility Assay

Extracts obtained by various plant parts were evaluated for antibacterial activities by disc diffusion assay (DDA). All extracts were filter sterilized before they were used in the experiments. Petri dish containing 20 ml of Nutrient Agar (NA) was inoculated with approximately 100 µl of seed culture and allowed to solidify. Sterile disc were loaded with 100 µl of extract and incubated at 37°C overnight along with positive and negative controls. The experiment was performed in triplicate. The antibacterial activity of each extract was recorded based on the inhibition of bacterial growth by the extract at the end of incubation period.

Sterilized distilled water and other solvents used in preparation of extracts were used as negative control. Tetracycline was used as positive control to determine the efficacy of extracts against selected bacteria.

RESULTS AND DISCUSSION

Results of the present study indicate that antibacterial activity of the extracts varied significantly depending upon the plant part used viz., leaf, stem, root, and flower. Further, data obtained demonstrates that the antibacterial activity of plant parts depends largely upon the extraction procedure, type of solvent used for extraction, and the bacterial strains tested. Data indicate that extracts prepared from leaves exhibited better antibacterial activities than those extracts prepared from other parts of the plant. Almost all parts of the plant showed significant antibacterial

activity (Table 2-4). The leaf extracts exhibited maximum inhibition, followed by root, stem extracts. However, floral extract were comparatively inactive towards the microbial strains tested. Phytochemical extracts were found to be inhibitory than their respective aqueous extracts. Ethanol was found to be a more suitable solvent for the maximum extraction of active metabolites. Gram-positive bacteria were found more susceptible as compared to Gram-negative species. Antibacterial activities were dose-dependent. However, the efficacies of plant extracts were less than the standard.

Herbal medicines are a valuable and readily available resource for primary health care and complementary health care systems. Unfortunately, many species of plants containing substances of medicinal value have yet to be discovered; though large numbers of plants are constantly being screened for their antimicrobial effects. It has been suggested that phytochemical extracts from plants holds promise to be used in allopathic medicine as they are potential sources of antiviral, antitumoral and antimicrobial agents (Nair et al., 2005). The present study reveals the antibacterial potential of crude extracts of different parts of *C. roseus*. Almost all parts of the plant exhibited significant antibacterial activity. Nevertheless, leaf extracts demonstrated maximum antibacterial activity. In a similar study, the leaf extracts of *C. roseus* was found to have significant antibacterial activity against *Xanthomonas campestris* (Satish et al., 1999). Furthermore, Gram-positive bacteria were found to have more susceptibility as compared to Gram-negative bacterial species. It has been shown in various studies that polarity of antibacterial compounds is crucial for their activity (Goyal et al., 2008). Therefore it is obvious that extracts prepared using organic solvents were more active against bacterial species. Similar observations have been reported by Thongson et al., (2004). In a study with *C. roseus* it has been pointed out that the pattern of inhibition largely depends upon extraction procedure, plant part, physiological and morphological state of plant, extraction solvent and microorganism tested. It has been demonstrated that extracts prepared using dried plant material is much more effective than the fresh plant materials (Goyal et al., 2008).

This implicates that if a lead molecule is identified from such studies, plant tissue culture techniques can be harnessed for the production of plant secondary metabolites (Verpoorte et al., 2002). Therefore, a study with large number of clinical pathogens with phytochemicals is expected to provide a hint to fish-out an effective lead molecule.

REFERENCES

1. Chin Y, Balunas MJ, Chai HB and Kinghorn AD (2006) Drug discovery from natural sources. *AAPS J*, 8:E239-53.
2. El-Sayed M, Choi YH, Frédérich M, Roytrakul S and Verpoorte R (2004) Alkaloid accumulation in *Catharanthus roseus* cell suspension cultures fed with stemmadenine. *Biotechnol Lett*, 26:793–798.
3. Gamble JS (1993). Flora of the Presidency of Madras. Vol I-III. Bishen Singh Mahendra Pal Singh. Dehra Dun-India.
4. Gootz TD (1990) Discovery and development of new antimicrobial agents. *Clin Microbiol Rev* 3:13-31.
5. Govindaraji V (2007) PGR mediated in vitro metabolic engineering of alkaloid production in somatic explants of *Catharanthus roseus* (L.) G. Don. M. Phil. Dissertation, PRIDE, PU, Salem.
6. Goyal P, Khanna A, Chauhan A, Chauhan G and Kaushik P (2008) In vitro evaluation of crude extracts of *Catharanthus roseus* for potential antibacterial activity. *Int J Green Pharm* 2:176-81.
7. Koehn FE and Carter GT (2005) The evolving role of natural products in drug discovery. *Nat Rev Drug*

Discov 4:206-20.

8. Kong JM, Goh NK, Chia LS and Chia TF (2003) Recent Advances in traditional plant drugs and orchids. *Acta Pharmacol Sin* 24:7-21.
9. Marcaurelle LA and Johannes CW (2008) Application of natural product-inspired diversity-oriented synthesis to drug discovery. *Prog Drug Res*. 66(187):89-216.
10. Mathew KW (1985). The Flora of Tamil Nadu Carnatic, The Rapinant Herbarium, St. Josephs College, Tiruchirapalli, India.
11. Nair R, Kalariya T and Chanda S (2005) Antibacterial Activity of Some Selected Indian Medicinal Flora. *Turk J Biol* 29:41-47.
12. Newman DJ, Cragg GM and Snader KM (2003) Natural products as sources of new drugs over the period 1981-2002. *J Nat Prod* 6:1022-37.
13. Nisbet LJ and Moore M (1997) Will natural products remain an important source of drug research for the future? *Curr Opin Biotechnol* 8:708-12.
14. Russell AD (2002) Antibiotic and biocide resistance in bacteria: Introduction. J *Appl Microbiol Symp Suppl* 92:1S-3S.
15. Satish S, Raveesha KA and Janardhana GR (1999) Antibacterial activity of plant extracts on phytopathogenic *Xanthomonas campestris* pathovars. *Lett Appl Microbiol* 28:145-147.
16. Thongson C, Davidson PM, Mahakarnchanakul W and Weiss J (2004) Antimicrobial activity of ultrasound-assisted solvent-extracted spices. *Lett Appl Microbiol* 39:401-6.
17. Verpoorte R (1998) Exploration of nature's chemodiversity: the role of secondary metabolites as lead for drug development. *Drug Dev Today* 3:232–238.
18. Verpoorte R, Contin A and Memelink J (2002) Biotechnology for the production of plant secondary metabolites. *Phytochem Rev* 1:13–25.

Table 1. Bacterial strains used in the present study.

S. No	Bacterial strain	Gram (+/-)
1	*Escherichia coli*	-
2	*Pseudomonas aeruginosa*	-
3	*Serratia marcescens*	-
4	*Salmonella typii*	-
5	*Staphylococcus aureus*	+
6	*Streptococcus pyrogens*	+
7	*Bacillus cereus*	+
8	*Bacillus subtilis*	+

Table 2. Anti-microbial activity of *C. roseus* ethanol extracts.

S. No	Organism	Plant Part			
		Leaf	Stem	Root	Flower
1	*E. coli*	+	+++	+	+
2	*P. aeruginosa*	++	+	+	+
3	*S. marcescens*	+	+	+	++
4	*S. typii*	+	+	++	+
5	*S. aureus*	++++	+++	+++	+++

6	*S. pyogens*	+++	++	+	++
7	*B. cereus*	-	-	++	++
8	*B. subtilis*	++	++	++++	-

(Growth analysis: +++ = abundant; ++ = normal; + = less; - = no)

Table 3. Anti-microbial activity of *C. roseus* methanol extracts.

S. No	Organism	Plant Part			
		Leaf	Stem	Root	Flower
1	*E. coli*	-	-	-	-
2	*P. aeruginosa*	+	+	-	-
3	*S. marcescens*	+	+	-	-
4	*S. typii*	+	+	++	+
5	*S. aureus*	+++	++	++	+
6	*S. pyogens*	++	+	+	++
7	*B. cereus*	-	-	++	+
8	*B. subtilis*	++	++	++++	-

(Growth analysis: +++ = abundant; ++ = normal; + = less; - = no)

Table 4. Anti-microbial activity of *C. roseus* aqueous extracts.

S. No	Organism	Plant Part			
		Leaf	Stem	Root	Flower
1	*E. coli*	-	-	-	-
2	*P. aeruginosa*	-	-	-	-
3	*S. marcescens*	-	-	-	-
4	*S. typii*	-	-	-	-
5	*S. aureus*	-	-	-	-
6	*S. pyogens*	-	-	-	-
7	*B. cereus*	-	-	-	-
8	*B. subtilis*	-	-	-	-

(Growth analysis: +++ = abundant; ++ = normal; + = less; - = no)