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Antibiogram of *Catharanthus roseus* Extracts

[1]S. Sathiya, [1]B. Karthikeyan, [2,3]Cheruth Abdul Jaleel, [4,5]M.M. Azooz and [6]Muhammad Iqbal

[1]Department of Microbiology, Faculty of Agriculture, Annamalai University,
Annamalainagar 608 002, Tamilnadu, India
[2]Stress Physiology Lab, Department of Botany, Annamalai University,
Annamalainagar 608 002, Tamilnadu, India
[3]DMJM International (AECOM Middle East Ltd.), Consultant of Gardens Sector Projects,
Alain Municipality and Eastern Emirates, P.O. Box 1419, Al-Ain, Abu Dhabi, United Arab Emirates
[4]Department of Botany, Faculty of Science, South Valley University, 83523 Qena, Egypt
[5]Department of Biology, Faculty of Science, King Faisal University,
P.O. Box: 380, Al-Hassa 31982, Saudi Arabia
[6]Department of Botany, Govt. College University, Faisalabad, Pakistan

Abstract: The present study was conducted to find out the antibiogram of different extracts of two varieties of *Catharanthus roseus* (L.) G. Don. "rosea" and "alba". The plant parts, leaves, stems, roots and flowers were separately tested for their antibiogram by using different solvents (methanol, acetone and ethyl acetate). Among the three solvents used for antibiogram, ethyl acetate extracts of different plant parts were found to induce best antibiogram followed by methanol and acetone extracts. Of the two varieties tested, "rosea" had better antibiogram than "alba". Extracts of all parts of both varieties of *C. roseus* like root, stem, leaf and flower were found to cause the largest antibiogram towards *Bacillus subtilis* followed by *Klebsiella* sp. while least antibiogram was observed against *Streptococcus* sp. The *Staphylococcus aureus* was moderately sensitive to different solvent extracts of the plant. The best antibiogram of ethyl acetate could be attributed to high solubility of the active compounds of *Catharanthus* in this solvent compared to other solvents.

Key words: *Staphylococcus aureus*, *Klebsiella* sp., *Streptococcus* sp., *Catharanthus roseus, Bacillus subtilis*, Antibiogram, Antibiotic

INTRODUCTION

Medicinal plants provide basic raw materials for different industries such as pharmaceutical, cosmetic, perfumery and food, etc. The medicinal plants are referred to plants that are used for their therapeutic or medicinal values.The whole plant or its different parts may be valued for its therapeutic, medicinal, aromatic or savory qualities. They also play vital role as an antimicrobial agent. There has been resurgence in the consumption and demand for medicinal plants. Many plants components are now synthesized in large laboratories for the use in pharmaceuticals preparations. Resistant to antimicrobial agents such as antibiotics is emerging worldwide of variety of organisms and multiple drug resistant organisms thus pose serious threats to treating infectious diseases. Hence, plant derived antimicrobial agents have received considerable attention in recent years.

India is endowed with about 47000 species of plants and ranks 8th in the world biodiversity. Out of these, 8000 species are known to be medicinal. Indian system of medicine uses around 2500 plant species belonging to more than 1000 genera. About 8000 species are used by industries of which approximately 25% are presently cultivated. Similarly, some 1300 plant species are known to contain aromas but only about 2% are grown. Both India and Pakistan, being countries with wide geographical variations, have vast potential to grow these plants. Moreover cultivation of these plants can offer a wide scope for small farmers to improve their

Corresponding Author: Dr. C.A. Jaleel, DMJM International (AECOM Middle East Ltd.),
Consultant of Gardens Sector Projects, Alain Municipality and Eastern Emirates,
P.O. Box 1419, Al-Ain, Abu Dhabi, United Arab Emirates

living standards. There is an urgent need for development of medicinal and aromatic plants through organized cultivation to meet the growing national and international demands for the quality raw material, in substantial quantities, to support user industry and dispensaries especially based on Indian system of medicine. There has been an upsurge in demand for pharmaceutical, raw medicinal plant materials, herbs and aromatic plants of the subcontinent origin in western countries.

The tropical plant Madagaskar Periwinkle (*Catharanthus roseus*) (L.) G. Don. is an important medicinal plant of family Apocynaceae. It is cultivated mainly for its alkaloids, which are having anticancer activities [1]. The two classes of active compounds in Vinca are alkaloids and tannins. *Catharanthus roseus* produces more than 100 mono terpenoids indole alkaloids (TIA) in different organs [2]. The leaves and stems are the sources of dimeric alkaloids, vinacristine and vinblastine that are indispensable cancer drugs, while roots have antihypertensive, ajmalicine and serpentine [3]. The major alkaloid is vincamine and its closely related semi-synthetic derivative widely used as a medicinal agent, known as ethyl-apovincaminate or vinpocetine has vasodilating, blood thinning, hypoglycemic and memory-enhancing actions [4]. The extracts of Vinca have demonstrated significant anticancer activity against numerous cell types [5]. Although the multi-step TIA are quite complex and their production is strictly under molecular regulation [6,7], few medicinal cultivars of *C. roseus* with high alkaloids traits have also been obtained [8].

The antibiogram of 10 indole alkaloids and 4 semi synthetic variables obtained from three plants, *C. roseus, Vallesia antillana* Wood and *Ervatamia coronaria* Staph, of the family Apocynaceae growing in Cuba was assessed in vitro. The alkaloids and the variables used were catharantine, vindoline, vindolinine, perivine, reserpine, tabernaemontanine, tetrahydroalstonine, aparicine, vindolinic acid, reserpic acid and vindolininol. These were faced to 40 bacterial strains from the genera *Salmonella, Shigella, Proteus, Escherichia, Pseudomonas, Staphylococcus* and *Coryneb acterium* as well as to fungi and yeasts from the genera *Aspergillus, Cunnighamella, Candida* and *Saccharomyces.* The method involving cylindric sections in a double agar layer was applied and after 24-48 h of incubation at 25°C for fungi and yeasts and 37 °C for bacteria, inhibition zones are reported in millimeters [9].

Antibiogram have been reported in some plants [10,11]. However, in spite of extensive survey of literature on *C. roseus* shows various aspects of studies [12,21], information on its antibiogram is scarce. Experiments were conducted on the antibiogram of *C. roseus* on *B. cereus* and *B. megaterium* and it was found that among various extraction procedures with different solvents, crude ethyl acetate extract was very effective antimicrobial where as same extract from the plants grown in sandy soil did not show any antibiogram. From these observations, it is clear that soil condition play a major role in determining phytochemical constituents [22].

The present research study was carried out for evaluation of plant extracts (leaf, stem, root and flower) of two varieties of *Catharanthus* using methanol, ethyl acetate and acetone solvents for their *in vitro* antibiogram against *Staphylococus aureus*, *Streptococcus* sp., *Bacillus subtilis* and *Klebsiella* sp.

MATERIALS AND METHODS

Plant Materials: The fresh and healthy leaves, stem, root and flower from two different varieties of *C. roseus,* "rosea" and "alba" were collected from the Herbal Garden of Department of Botany, Annamalai University, India. The plant materials were washed thoroughly with tap water and then with sterilized distilled water. The plant materials were dried in shade at room temperature (28±2°C) for about 1 h and used as the raw materials for the extraction of antimicrobial compounds from the plant.

Preparation of Catharanthus Plant Extracts: The dried leaf, stem, root and flower (1.0 kg) were powdered by electrical blender. Three litres of methanol, acetone and ethyl acetate separately were used for the extraction of 1.0 kg in the soxhlet apparatus followed by the standard procedure [23]. The plant material was loaded in the inner tube of the soxhlet apparatus and then fitted into a round-bottomed flash containing methanol, acetone and ethyl acetate separately. The solvents were boiled gently (40°C) over a heating mantle using the adjustable rheostat. The extraction was continued for 8 h and the solvent was removed at the reduced pressure with the help of rotary vacuum evaporator to yield a viscous dark green residue (12.5g) of each of methanol, acetone and ethyl acetate of root, stem, flower and leaf extracts.

Cultures Used: Microorganisms for the study were collected from the laboratory of Department of Microbiology, Annamalai University, India. They were sub cultured and identified for their sensitivity. The strains of bacteria used are *Bacillius subtilis*, *Klebsiella* sp., *Staphylococcus aureus* and *Streptococcus* sp.

Plate 1: *Catharanthus roseus* "Rosea" Variety

Plate 2: *Catharanthus roseus* "Alba" Variety

Plate 3: Inhibitory effect of ethyl acetate root extracts of *Catharanthus roseus* "rosea" variety against *Bacillus subtilis*

The stocks cultures of microbial strains were maintained in nutrient agar slants at 4°C in refrigerator. All the chemicals and reagents used in the present investigation were of analytical grade.

Plate 4: Inhibitory effect of ethyl acetate root extract of *Catharanthus roseus* "rosea" variety against *Klebsiella* sp

Plate 5: Inhibitory effect of ethyl acetate root extract of *Catharanthus roseus* "rosea" variety against *Staphylococcus* sp

Preparation of Media for Antibiogram Testing: The following media were prepared to test the antibiogram of "rosea" and "alba" varieties of *C. roseus*. beef 3g, peptone 5 g, sodium chloride 5 g and agar 15g. These ingredients were dissolved in one litre warm water. The pH was adjusted to 7.2 and filtered. The media was sterilized at 121 °C for 15 min. at 15 psi and poured in sterile *Petri* dishes in laminar air flow hood.

Evaluation of Antibiogram of Catharanthus Species Extracts: Antibiogram of medicinal compounds was tested through disc diffusion method (Kirby-Bauer Method). that is most commonly employed method to evaluate the antibiogram.

The organisms to be used for the *in-vitro* test were maintained and preserved on nutrient agar slopes by refrigeration at 4 °C. Subcultures were maintained at regular intervals. The inoculums were prepared using bacterial suspension containing $1x10^6$ cells by MacFardian standard tubes. The MacFardian tubes were calibrated as to having the turbidity with 10^6 cells using 0.2 ml of $BaCl_2$ to which 9.8 ml of sulphuric acid (H_2SO_4) was added. The turbidity was compared

with that of the bacterial suspension of various clinically isolated bacteria suspended in sterile distilled water.

Discs of 5 mm diameter were prepared using Whatman filter paper No. l. These were sterilized in the hot air oven at 160 °C for 1 h. The discs were impregnated with different solvent extracts of two varieties of *Catharanthus roseus* and stored for at 4 °C for the further use. Control paper discs were prepared by using 1% dimethylsulphoxide.

Antibiogram of Catharanthus Species Extract: Disc diffusion was carried out for the bacterial suspension containing 10^6 cells. Plates with nutrient agar were seeded and then drained off. They were desiccated at room temperature for 15-20 min. The disc prepared from different concentrations (250, 500 and 1000 mg/L) of herbal extract was placed in quadrangular manner in Petri dishes. Then Petri dishes were incubated at 30 ± 2 °C for 24 h. After 24-48 h, the results were noted for the zone of inhibition diameter which measured in mm.

RESULTS AND DISCUSSION

The results of the experiments carried out on the antibiogram effect of leaf, stem, root and flower extracts of *C. roseus* varieties "rosea" and “alba” by using different solvents like methanol, ethyl acetate and acetone.

Antibiogram of methanolic extracts of C. roseus against B. subtilis, Klebsiella sp., Staphylococcus aureus and Streptococcus sp.

Among the two varieties, methanolic extract of "rosea" was found to be more effective against all microorganisms (Table 1-4). The root extract was found to induce maximum inhibitory effect against these microorganisms followed by leaf and flower. While the methanol extract of stem of both varieties did not show any antibiogram. However, the leaf and flower of *C. roseus* "rosea" variety also produced appreciable antibacterial property compared with “alba” variety. The higher concentration (1000 mg/L) of different plant parts produced highest inhibitory activity against these microorganisms. The maximum inhibitory effect of 7.50, 5.60, 4.54 and 3.26 mm inhibition zone was recorded against *Bacillus subtilis*, *Klebsiella* sp., *Staphylococcus aureus* and *Streptococcus* sp., respectively in root extracts of C. *roseus* "rosea" followed by the leaf extract. These results support earlier results by Satyan *et. al*. [22] who found antibacterial activity of leaf more than flower

Table 1: Impact of methanol extract of *Catharanthus roseus* against *Bacillus subtilis* measured by the area of the zone of inhibition (mm)

Catharanthus varieties	Plant parts	Concentration in mg/L 250	500	1000
Rosea	Leaf	3.00	5.66	6.74
	Stem	0.00	0.00	0.00
	Root	4.44	6.25	7.50
	Flower	1.66	2.00	2.25
Alba	Leaf	2.44	3.14	4.07
	Stem	0.00	0.00	0.00
	Root	3.16	4.20	5.00
	Flower	0.90	1.21	1.54

Table 2: Impact of methanol extract of *Catharanthus roseus* against *Klebsiella* sp. measured by the area of the zone of inhibition (mm)

Catharanthus varieties	Plant parts	Concentration in mg/L 250	500	1000
Rosea	Leaf	2.80	3.20	4.14
	Stem	0.00	0.00	0.00
	Root	3.24	4.00	5.60
	Flower	1.60	2.40	2.90
Alba	Leaf	1.90	2.74	3.77
	Stem	0.00	0.00	0.00
	Root	2.17	3.50	4.27
	Flower	0.70	1.10	1.50

Table 3: Impact of methanol extract of *Catharanthus roseus* against *Streptococcus aureus* measured by the area of the zone of inhibition (mm)

Catharanthus varieties	Plant parts	Concentration in mg/L 250	500	1000
Rosea	Leaf	2.21	2.50	3.00
	Stem	0.00	0.00	0.00
	Root	2.50	2.75	3.26
	Flower	0.50	0.65	0.80
Alba	Leaf	2.00	2.26	2.87
	Stem	0.00	0.00	0.00
	Root	2.00	2.14	3.00
	Flower	0.40	0.50	0.70

Table 4: Impact of methanol extract of *Catharanthus roseus* against *Streptococcus* sp. measured by the area of the zone of inhibition (mm)

Catharanthus varieties	Plant parts	Concentration in mg/L 250	500	1000
Rosea	Leaf	2.50	3.00	3.86
	Stem	0.00	0.00	0.00
	Root	2.80	3.25	4.54
	Flower	0.50	0.65	0.80
Alba	Leaf	1.80	2.77	3.24
	Stem	0.00	0.00	0.00
	Root	2.50	3.10	3.60
	Flower	0.40	0.60	0.70

Table 5: Impact of ethyl acetate extract of *Catharanthus roseus* against *Bacillus subtilis* measured by the area of the zone of inhibition (mm)

Catharanthus varieties	Plant parts	Concentration in mg/L 250	500	1000
Rosea	Leaf	9.34	11.62	16.40
	Stem	7.20	9.78	13.68
	Root	12.32	15.84	18.24
	Flower	2.56	5.24	7.78
Alba	Leaf	3.33	5.49	8.83
	Stem	2.11	4.51	7.83
	Root	6.25	8.63	10.98
	Flower	1.70	3.30	4.50

Table 6: Impact of ethyl acetate extract of *Catharanthus roseus* against *Klebsiella* sp. measured by the area of the zone of inhibition (mm)

Catharanthus varieties	Plant parts	Concentration in mg/L 250	500	1000
Rosea	Leaf	7.20	9.42	11.66
	Stem	5.15	7.33	9.50
	Root	11.53	13.45	15.90
	Flower	0.90	1.14	1.34
Alba	Leaf	3.36	5.55	7.61
	Stem	1.20	3.25	5.45
	Root	5.36	7.71	9.89
	Flower	0.80	1.00	1.20

Table 7: Impact of ethyl acetate extract of *Catharanthus roseus* against *Staphylococcus aureus* measured by the area of the zone of inhibition (mm)

Catharanthus varieties	Plant parts	Concentration in mg/L 250	500	1000
Rosea	Leaf	4.52	6.27	8.66
	Stem	3.27	4.86	7.39
	Root	6.74	8.40	10.32
	Flower	0.45	0.80	1.20
Alba	Leaf	3.00	4.21	5.66
	Stem	2.44	3.39	4.30
	Root	4.24	7.00	7.78
	Flower	0.36	0.57	0.80

Table 8: Impact of ethyl acetate extract of *Catharanthus roseus* against *Streptococcus* sp. measured by the area of the zone of inhibition (mm)

Catharanthus varieties	Plant parts	Concentration in mg/L 250	500	1000
Rosea	Leaf	2.29	3.51	5.60
	Stem	1.24	2.37	3.43
	Root	3.44	5.66	7.12
	Flower	0.50	0.60	0.60
Alba	Leaf	3.11	5.22	7.30
	Stem	1.08	2.19	3.25
	Root	5.22	3.71	9.42
	Flower	0.45	0.45	0.50

extracts of *C. roseus* grown under different soil conditions. In the present studies, greater antibiogram were found in root extracts of both varieties that could be due to the presence of active compounds like ajamalicine, vindolininic acid and vindolininol present in excess in root compared to leaf and flower [9]. The leaf extracts of both varieties of *C. roseus* had maximum inhibition zone (6.74 mm) againsl *B. subtilis* followed by *Klebsiella* sp., *S. aureus* and *Streptococcus* sp. Similar results were reported by Geetha [24] which might be due to active compounds (vincristine and vinblastine) present in the leaf extract. The stem extracts of *C. roseus* of both varieties did not show any antimicrobial properties. Similar effect was observed by Satyan *et. al*., [22] in stem extracts of *C. roseus*.

Antibiogram of ethyl acetate extracts of C. roseus against B. subtilis, Klebsiella sp., Staphylococcus aureus and Streptococcus sp.

Among different concentrations, 250, 500 and 1000 mg/L of different plant parts of two *Catharanthus* varieties tested, 1000 mg/L produced highest inhibitory activity against all the microorganisms (Table 5-8). The inhibitory effect was directly proportional to the concentration of the plant extracts tested. However, the maximum inhibitory effect in terms of the area of the zone of. inhibition.was 18.24, 15.90, 10.32 and 7.12 mm in the root extract of “rosea” variety against *B. subtilis*, *Klebsiella* sp., *Staphylococcus aureus* and *Streptococcus* sp., respectively followed by leaf extract of “rosea” at 1000 mg/L. The ethyl acetate extracts of stems and flowers of the two varieties showed minimum inhibition zones against all microorganisms. Inhibitory effect against bacteria has earlier been reported by Satyan *et al*., [22] in stem extracts of *C. roseus*.

Antibiogram of acetone extracts of C. roseus against B. subtilis, Klebsiella sp., Staphylococcus aureus and Streptococcus sp.

The acetone extracts of "rosea" variety of *C. roseus* was found to be more effective against different microorganisms in comparison to "alba" (Table 9-12). The higher concentration of acetone extract of root was found to induce maximum inhibitory effect against all microorganisms followed by leaves, stem and flowers of both varieties. The maximum inhibition zone was 6.00, 5.00, 4.77 and 4.17 mm against *B. subtilis*, *Klebsiella* sp., *Staphylococcus aureus* and *Streptococcus* sp., respectively in root extract of acetone of "rosea" followed by “alba” variety.

Table 9: Impact of acetone extract of *Catharanthus roseus* against *Bacillus subtilis* measured by the area of the zone of inhibition (mm)

Catharanthus varieties	Plant parts	Concentration in mg/L 250	500	1000
Rosea	Leaf	3.50	4.84	5.82
	Stem	2.80	3.66	4.12
	Root	4.00	5.26	6.00
	Flower	0.00	0.00	0.00
Alba	Leaf	3.00	4.50	5.29
	Stem	2.20	3.11	3.90
	Root	3.26	5.14	5.87
	Flower	0.00	0.00	0.00

Table 10: Impact of acetone extract of *Catharanthus roseus* against *Klebsiella* sp. measured by the area of the zone of inhibition (mm)

Catharanthus varieties	Plant parts	Concentration in mg/L 250	500	1000
Rosea	Leaf	3.00	3.90	4.50
	Stem	2.75	3.29	3.99
	Root	3.22	4.27	5.00
	Flower	0.00	0.00	0.00
Alba	Leaf	2.50	3.66	3.89
	Stem	2.00	3.00	3.14
	Root	3.00	3.54	4.20
	Flower	0.00	0.00	0.00

Table 11: Impact of acetone extract of *Catharanthus roseus* against *Staphylococcus aureus* measured by the area of the zone of inhibition (mm)

Catharanthus varieties	Plant parts	Concentration in mg/L 250	500	1000
Rosea	Leaf	2.56	3.41	4.21
	Stem	2.21	3.00	3.90
	Root	3.00	3.84	4.77
	Flower	0.00	0.00	0.00
Alba	Leaf	2.11	3.14	3.76
	Stem	2.00	2.77	2.97
	Root	2.76	3.74	4.26
	Flower	0.00	0.00	0.00

Table 12: Impact of acetone extract of *Catharanthus roseus* against *Staphylococcus* sp. measured by the area of the zone of inhibition (mm)

Catharanthus varieties	Plant parts	Concentration in mg/L 250	500	1000
Rosea	Leaf	2.44	3.20	4.00
	Stem	2.14	3.00	3.50
	Root	2.84	3.60	4.17
	Flower	0.00	0.00	0.00
Alba	Leaf	2.01	2.89	3.14
	Stem	2.00	2.44	2.75
	Root	2.66	3.27	3.84
	Flower	0.00	0.00	0.00

In conclusion, among the three solvents used for extraction, ethyl acetate extracts of different plant parts were found to possess best antibiogram followed by methanol and acetone extracts. This may be due to high solubility of active compounds of *Catharanthus* varieties with ethyl acetate during the extraction process compared to other solvents. The extracts of root, stem, leaf and flower with different solvents of both varieties were found to cause best antimicorbial activity towards *B. subtilis* followed by *Klebsiella* sp. while least antibiogram was observed in *Streptococcus* sp. However, *S. aureus* was found to be moderately sensitive against different solvent extracts of the plant.

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