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Study of Antimicrobial Activity of Catharanthus roseus L.

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Abstract

India boasts one of the largest biodiversity regions in Asia, characterized by a wealth of medicinal and aromatic plant diversity. The therapeutic applications of these plants reflect a longstanding tradition across various cultures. The genus Catharanthus has some medicinal properties, herbs known for enormous potentialities and to be employed for the treatment of cancer in some parts of the world paving a way for replacing the allopathic treatments with the natural ones. The dissolvable concentrates of Catharanthus roseus indicated huge antibacterial movement against Bacillus subtilis, Escherichia coli, Pseudomonas, Staphylococcus aureus and so on. Leave and flower concentrate of Catharanthus roseus recorded noteworthy action against all the test microorganisms.

Keywords: Catharanthus roseus, Antimicrobials, Human Pathogenic Bactria, Well Diffusion Method

Introduction

Plant-derived substances have recently become of great interest owing to their versatile applications. Recently, contemporary science and society have acknowledged the therapeutic benefits of traditional plants as an alternative to synthetic allopathic healthcare medications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs. Medicinal plants are the nature's gift to human being to make disease free healthy life. It plays a vital role to preserve our health. India is one of the most medico culturally diverse countries in the world where the medicinal plant sector is part of a time honored tradition that is respected even today. Traditional medicines derive their scientific heritage from rich experiences of ancient civilization. Hence, it is not surprising that traditional medicines claim comes for several "difficult to cure" diseases. India is well known for its rich traditional systems of medicine i.e. Siddha, Ayurveda, Unani and Amchi (Tibetan) besides a vast reservoir of living traditions in ethno-medicine. Currently, the emergence of resistance in microbial communities to the standard medications employed for their treatment poses a significant challenge in the healthcare sector. (Alviano and Alviano, 2009). Plants contain spectrum of bioactive compounds viz., secondary metabolites i.e., alkaloids, flavonoids, phenols, polyphenols, saponins, steroids and tannins derivatives, that could potentially be utilized as potential antimicrobial agent against pathogenic bacterial strains due to their biological efficacy (Lisec et al., 2015). These naturally derived bioactive agents have played a significant role in the pharmacological sector, contributing to the development of a variety of healthcare products, such as antibacterial, anti-tumor, and anti-parasitic drugs (Gortzi et al., 2007). The active ingredients extracted from medicinal flora by means of organic solvents are more significant (Mitra and Sur, 1997). The interface between active secondary metabolites might have induced the remedial properties of medicinal plant extracts (Jaiswal and Jain, 2017). Research on

medicinal plants is being used by contemporary pharmaceutical companies to both uncover possible bioactive therapeutic ingredients and synthesize medications based on their chemical composition ((Meskin et al., 2002; Unnikrishnan, 2010). As compared to the enormous potential of earth's plants, only a small fraction has been explored till now (Chidozie et al., 2014). Hence, more organized efforts are required based on bioassay determination of natural products from medicinal plants.

Catharanthus roseus, commonly known as bright eyes, Cape periwinkle, graveyard plant, Madagascar periwinkle, old maid, pink periwinkle, rose periwinkle, is a species of flowering plant in the family Apocynaceae. It is native and endemic to Madagascar, but grown elsewhere as an ornamental and medicinal plant, a source of the drugs vincristine and vinblastine, used to treat cancer. It was formerly included in the genus Vinca as Vinca rosea (Falcao et al., 2017). This plant is one of the generally utilized restorative plant and decorative plants (Magnotta et al., 2006). These plants are disseminated all through the biosphere creating under a wide extent of climatic circumstances and they are known to overwhelm or to convey accommodating feed and food things. Research scholars identified the occurrence of alkaloids in C. roseus, this plant was thoroughly examined with drug associations (Falcao et al., 2017). The seeds and leaves of C. roseus are applied as normal medications and antimicrobial activities (Raja et al., 2018).

Keeping the above facts in mind, the present study was designed and proceeded accordingly.

Material and Method:

Collection of Plant Sample and Processing

Catharanthus roseus plant were collected from Centre of Excellence on MAPs (Medicinal and Aromatic Plants) and NTFP (Non Timber Forest products), Indira Gandhi Krishi Vishwavidyalaya, Raipur, C.G. Plant sample was identified by Dr. K. K. Shukla, Professor, SoS in Biotechnology. Pt. R.S. University, Raipur, (C.G.). The leaves of collected plant material were then processed viz., oven dried and pulverized into powder. The extracts were prepared by cold percolation method. The required fraction of grinded powder was soaked in (50% w/v) acetone, methanol, ethanol and distilled water for 72 h. The prepared mixtures were stirred using a sterile glass rod at 24 h interval. The extracts were filtered by Whatmann filter paper no 1(Dulger and Gonuz, 2004). The filtrates were then concentrated in water bath.

Determination of Antimicrobial

The antimicrobial profile of each extract was evaluated against a set of Gram positive and negative pathogenic bacteria using Kirby – Bauer method determined by NCCLS standards (National Committee for Clinical Laboratory Standards, 2002). Well diffusion method was utilized to assess antibacterial action of Catharanthus roseus leave and flower extract as portrayed by (Bauer et al., 1966). Both well diffusion and disc diffusion method sterile petri plates were set up with 20 ml of Muller Hinton Agar. The standard inoculums of bacterial suspension acclimated to 0.5 McFarland turbidity standard which is comparable to $1x10^8$ CFU/ml of microorganisms were cleaned on the seeded media and permitted to dry for 15 min. Then a well or a cup was formed in the seeded agar with the help of a borer having inner diameter of 6 mm. 40µl of aliquot of different concentration of essential oil ranging from 100 mg/ml and then positioned onto the inside of the hardened agar medium. Petri plates were then brooded for 24 h at 35 ± 1^0C. At last the zone of restraint shaped by Catharanthus roseus

leave and flower was recorded after 24 h hatching at 35±1^{0}C. The impacts were contrasted and that of standard, streptomycin (positive control). Concentrate was tried in triplicate alongside streptomycin (1mg/circle). The plates were kept at 4°C for 1h for dispersion of concentrate, from that point were hatched at 37±2°C for 24 h. Zone of hindrance (IZ) or discouraged development of microorganisms was estimated and the 'Activity Index' (AI) for each concentrate was determined.

$$\text{Activity index (AI)} = \frac{\text{Inhibition Zone of the}}{\text{Inhibition Zone of the standard}}$$

Test organisms used for the analysis

MTCC bacterial isolates viz., Aeromonas hydrophilla (MTCC 966), B. cereus (MTCC 633), B. brevis*, Enterobacter aerogenes*, E. facalis*, Escherichia coli (MTCC 1591), Klabsiella pneumoniae (MTCC 2405), Salmonella typhi (MTCC 531), Vibrio cholerae (MTCC 1168), S. dysentery *, Candida albicans (MTCC 1022), Saccharomyces cerevisiae (MTCC1732).

Result

Antibacterial activity using well diffusion method

Results of antimicrobial activity of extracts of the test plant through well diffusion method are presented in Table 2 & 3. It is clearly indicates that ethanolic and methanolic fractions of test plant leaves showed very strong inhibitory activity against Enterobacter aerogenes at 100µg of concentration. It was followed by B. brevis, B. cereus and E. coli. Inhibition was significantly declined against K. pneumoniae and S. typhi at same concentration. More or less similar trend was also recorded with methanolic fraction against B. brevis, E. coli. and A. hydrophilla while failed to inhibit S. dysentery. The aqueous extract of test plant leaves was also showed inhibitory activity against bacteria which was maximum against B. brevis followed by E. coli while very less effective against S. typhi.

Significant inhibitory effect of extracts of test plant leaves against yeast had also recorded during the present investigation. Extracts were highly effective against S. cervisiae but mo activity was recorded against C. albicans (Table 2). In comparison to aqueous fraction methanolic fraction showed maximum sensitivity against these yeasts similar trends were also recorded with ethyl acetate and ethanol fraction in case of S. cervisiae. No effect was recorded against C. albicans (7.80 and 13.80mm). Data recorded in table 2 were statistically significant.

It is evident from the data recorded in Table 3 that test plant flower extracts were also significantly effective against test bacterial strains. Methanolic fraction exhibited maximum antibacterial effect which was followed by acetone, ethanol, ethyl acetate and distilled water. The maximum inhibition was observed against B. cereus in methanolic fraction followed by B. brevis, Enterobacter aerogenes and E. coli. Minimum inhibitory effect was recorded against K. pneumoniae. Acetone and ethanolic fraction also gave highest activity against Enterobacter aerogenes and B. brevis, respectively and minimum against S. typhi. There was no appreciable activity recorded against Klabsiella pneumonia

and S. dysentery.

Extracts of test plant flower extracts were also found to be most effective against yeast cells than test plant leaves. They showed significant level of inhibition against S. cerevisiae, and C. albicans. Both methanolic and ethanolic fraction of test plant leaves showed maximum activity amongs all the fractions against, S cerevisiae which was followed by C. albicans. No remarkable activity was recorded with other test extract of same plant except methanolic and ethanolic fraction which was moderately effective against C. albicans.

Table 1. Antimicrobial activity of Catharanthus roseus leave extract against some bacteria and yeast through WDM

S.N.	Tested microorganism	Acetone	MeOH	EtOAc	EtOH	D/W	Ref.
1.	Aeromonas hydrophilla (MTCC 966)	11.30±0.25	13.50±0.21	13.00±0.00	15.83±0.16	11.00±0.32	30.20 ± 0.04
2.	B. cereus (MTCC 633)	25.50±0.09	21.50±0.06	26.00±0.05	30.50±0.74	14.00±0.00	29.50 ± 0.07
3.	B. brevis *	24.00±0.17	29.83±0.11	24.00±0.04	30.67±0.15	20.50±0.04	31.60 ± 0.07
4.	Enterobacter aerogenes *	25.16±0.11	25.50±0.35	18.50±0.02	31.00±0.01	16.20±0.20	30.10 ± 0.02
5.	E. facalis *	19.00±0.02	21.00±0.04	16.00±0.00	22.00±0.05	13.50±0.04	34.50 ± 0.02
6.	Escherichia coli (MTCC 1591)	21.50±.0.20	29.16±0.21	18.00±0.06	26.63±0.05	18.00±0.00	26.18±0.05
7.	Klabsiella pneumoniae (MTCC 2405)	ND	-	8.67 ±0.07	19.60±0.18	-	27.10±0.02
8.	Salmonella typhi (MTCC 531)	11.67±0.35	-	11.00±	17.00±0.05	7.30±0.01	28.00±0.15
9.	Vibrio cholerae (MTCC 1168)	15.33±0.01	15.20±0.12	10.50±0.05	18.50±0.06	-	27.50±0.16
10.	S. dysentery *	-	-	-	-	-	25.00±0.01
11.	Candida albicans (MTCC 1022)	-	-	-	-	-	NT
12.	Saccharomyces cerevisiae (MTCC1732)	14.50±0.06	18.83±0.25	11.50±0.02	16.50±0.03	12.67±0.06	NT

- Data are multiple of three
- Values ± standared error (SE)
- WEM: Well diffusion method replicates

- Ref.: Reference antibiotic (Gentamycin)
- *ND: Not detectable, -: No activity, NT: Not tested

Table 2. Antimicrobial activity of Catharanthus roseus flower extract against some bacteria and yeast through WDM

S.N.	Test Microorganism	Acetone	MeOH	EtOAc	EtOH	D/W	Ref
1	Aeromonas hydrophilla (MTCC 966)	9.5 ± 0.00	15.5± 0.30	9.67± 0.16	12.63± 0.24	11.00± 0.00	30.20 ± 0.04
2	B. cereus (MTCC 633)	26.20±0.06	31.00±0.05	20.00±0.00	21.50±0.05	18.00±0.25	29.50 ± 0.07
3	B. brevis *	20.83±0.10	24.00±0.05	19.00±0.09	21.83±0.11	18.00±0.09	31.60 ± 0.07
4	Enterobacter aerogenes *	19.01±0.20	25.00±0.01	22.10±0.30	24.0±0.09	13.00±0.08	30.10 ± 0.02
5	E. facalis *	15.51±0.09	18.20±0.00	15.00±0.09	14.00±0.015	13.00±0.05	34.50 ± 0.02
6	Escherichia coli (MTCC 1591)	21.16±0.00	25.83±0.00	19.83±0.16	21.00±0.00	16.0± 0.33	26.18±0.05
7	Klabsiella pneumoniae (MTCC 2405)	10.63±0.30	9.50±0.05	-	-	-	27.10±0.02
8	Salmonella typhi (MTCC 531)	10.5±0.06	13.67±0.00	7.83±0.05	11.83±0.02	8.00±0.01	28.00±0.15
9	Vibrio cholerae (MTCC 1168)	10.00±0.05	14.00±0.05	ND	11.00±0.09	-	27.50±0.16
10	S. dysentery *	-	ND	-	-	-	25.00±0.01
11	Candida albicans (MTCC 1022)	13.00 ± 0.36	15.10 ± 0.06	-	11.50 ± 0.36	-	NT
12	Saccharomyces cerevisiae (MTCC1732)	18.16 ± 0.06	19.67 ± 0.04	17.00 ± 0.07	13.63 ± 0.04	17.83 ± 0.10	NT

- Data are multiple of three replicates
- Values ± standared error (SE)
- WEM: Well diffusion method
- Ref.: Reference antibiotic (Gentamycin)
- *ND: Not detectable, -: No activity, NT: Not tested

Discussion

Plants belonging to species Catharanthus roseus (L.) have long been used in various traditional medicinal practices. This information has been thus employed in the present research. Similar results were also demonstrated by Wagay et al, 2013, whose reports coincided with Aslam et al, 2010. As observed in Table 1 and 2, all the extracts exhibited notable antimicrobial properties but methanolic and ethanolic extracts both from leave and flower showed best activity against bacteria and yeasts. Similar results were obtained by Madureira et al. (2012). Activities observed against B. cereus, B. subtilis, S. aureus, P. aeruginosa, E. coli, were in accordance with studies conducted by Falcao et al., 2017.

Conclusion

The Catharanthus roseus plants were selected due to their vast pharmacological applications, also they are predominant in Chhattisgarh region of central India. The present research work was focused on the assessment of antimicrobial efficacy of Catharanthus roseus. It was identified as bioactive compound present in Catharanthus roseus that act as antimicrobial agent. This finding supports the use of root and stem extract of Catharanthus roseus in the treatment of bacterial pathogens by alternative systems of medicine. The present findings could further potentially be contributed as to serve better candidate for the drug development application i.e. antibiotic, in pharmaceutical sector.

Conflict of interest:

The authors declare none.

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