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INVITRO ANTIBACTERIAL ACTIVITY OF METHANOLIC LEAF EXTRACT DERIVED FROM *CATHARANTHUS ROSEUS*

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ABSTRACT

In the present study, the methanolic leaf extract of *Catharanthus roseus* was extracted by soxhlet extraction process and the presence of alkaloid was detected using the thin layer chromatography. Further, the predominant compounds in the leaf extract were identified by GC-MS. The antibacterial activity of the extract was performed for various pathogenic bacteria such as *Staphylococcus aureus, Eschericia coli, Bacillus megaterium, Bacillus subtilis, Bacillus flexus, Bacillus* sp. SSKSD01 (KF751671)*, Bacillus* sp. SSKSD05 (KF751673)*, Bacillus* sp. SSKSD08 (KF751675*), Pseudomonas* sp. SSKSD06 (KF751674)*, Staphylococcus* sp. SSKSD17 (KF751681)*, Klebseilla pneumoniae, Pseudomonas stutzeri, Pseudomonas fluorescens, Proteus vulgaris, Enterobacter aeruginosa* by agar well diffusion method and the obtained results were compared with streptomycin as a positive control. From the present study, it was clear that the methanol extract of *C. roseus* leaf possessed antibacterial property.

Key Words: *Catharanthus roseus,* methanolic leaf extract*,* Antibacterial activity

INTRODUCTION

Medicinal plants are a boon to the world which is widely used directly and indirectly (Magnotta et al. 2006). They are a source of large amounts of drugs, which have the properties of anti-inflammatory, anti-bacterial, anti-fungal, anti-diabetic, anti-cancer, anti-oxidant, anti-hypertensive and anti-mitotic activities the antibiotic properties and have no side effects. Plant based natural metabolites, becomes more popular throughout the world nowadays, such as alkaloids, terpenoids, flavanoids, saponins, tannins and glycosides are derived from various parts of plants like bark, leaves, flowers, roots, fruits, seeds, etc. (Magnotta et al. 2006; Jaleel et al. 2006; Jaleel and Pannerselvam, 2007). *Catharanthus roseus* is a medicinal plant, which is cultivated as an ornamental plant almost throughout the tropical and subtropical world (Rahman et al. 1984; Moreno et al. 1993). The plant has been used for centuries to treat a variety of diseases (Handa et al. 2008). The plant has more than 70 types of alkaloids which exhibit antibacterial, antiproliferation and anti-metastasis effects (Rahman et al. 1983; Parekh et al. 2006). The anticancer drugs vincristine and vinblastine are synthesized from alkaloids of *C. roseus* (Parekh et al. 2006; Das et al. 2010). In this study, the methanolic leaf extract was characterized by GC-MS and the antibacterial activity (for various bacteria) was studied.

MATERIALS AND METHODS

2.1. Sample collection

Catharanthus roseus leaves were collected from Vivekanandha Educational Institutions,

Tiruchengode, Tamil Nadu. The leaves were washed to remove the dirt and outside debris with distilled water and dried in shade. The air dried leaves were powdered using mechanical blender and stored in air tight container for further use.

2.2. Soxhlet extraction method

Fifty grams of air dried sample was taken in a thimble of the soxhlet apparatus for extraction using 80% methanol as solvent. The chlorophyll was removed by centrifugation the crude extract with equal volume of dichloromethane at 4000 rpm for 10 mins. The final concentrated leaf extract was left for evaporation of methanol and stored in the sterile tubes for further studies.

2.3. Thin layer chromatography analysis

Thin layer chromatography (TLC) was performed on silica gel (0.5mm thickness) plates with ethyl acetate: methanol:water (70:20:10) as solvent system. A drop of a methanolic leaf extract of *Catharanthus roseus* was applied onto the TLC plate and allowed for migration. After migration of sample, the plate was left for air drying and sprayed with cerium ammonium sulphate (CAS) as a chromogenic reagent (1% CAS in 85% phosphoric acid).

2.4. Gas chromatography-mass spectrometry (GC-MS) analysis

The GC-MS analysis of methanolic leaf extract was quantitatively performed by Gas Chromatograph Mass Spectrometer (JEOL GCMATEII). Hp5-Ms column was used. High pure helium was the carrier gas and the flow rate was 1 mL/min. Temperature at the front inlet was 220°C and at the oven was 50°C to 250°C, gradually raised at 10°C/min. Ion chamber and GC interface temperature was maintained at 250°C. With the aid of National Institute of Standards and Technology library the peaks were compared and identified.

2.5. Antibacterial activity

Conventional spread plate technique was performed using 100 µL of various pathogenic bacteria such as *Staphylococcus aureus*, *Eschericia coli*, *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus flexus*, *Bacillus* sp. SSKSD01 (KF751671), *Bacillus* sp. SSKSD05 (KF751673), *Bacillus* sp. SSKSD08 (KF751675*)*, *Pseudomonas* sp. SSKSD06 (KF751674), *Staphylococcus* sp. SSKSD17 (KF751681), *Klebseilla pneumoniae*, *Pseudomonas stutzeri*, *Pseudomonas fluorescens*, *Proteus vulgaris*, *Enterobacter aeruginosa.* Then, the agar plates were incubated for 5 mins in an inverted position and the wells were made using gel puncture on the agar plates. 20 µL of the methanolic leaf extract was added into the wells. Similarly, 20 µL of streptomycin was also added into the wells of plates containing different bacterial strains as positive controls. All the plates were incubated at 37ºC for 24 h.

RESULTS AND DISCUSSIONS

3. 1. Extraction of Catharanthus roseus Extract

The crude extract of *Catharanthus roseus* leaves was extracted by soxhlet apparatus and the chlorophyll was removed by using dichloromethane (Fig. 1). CAS reagent has shown the pink around the yellow colour band on TLC plate. In thin layer chromatography, R_f value was calculated for the identified band as 0.84. R_f values for alkaloid standards were compared with that calculated R_f value for the methanolic leaf extract and was found that value related with vincristine and vindoline alkaloid (Wagay et al. 2013). The previous researchers have reported more than 130 alkaloids present in *C. roseus* plant. The alkaloids present in the aerial parts of *C. roseus* were well extracted by methanol as solvent according to Shams et al. (2009) modified method. The calculated R_f was same as in the report of Wagay et al. (2013).

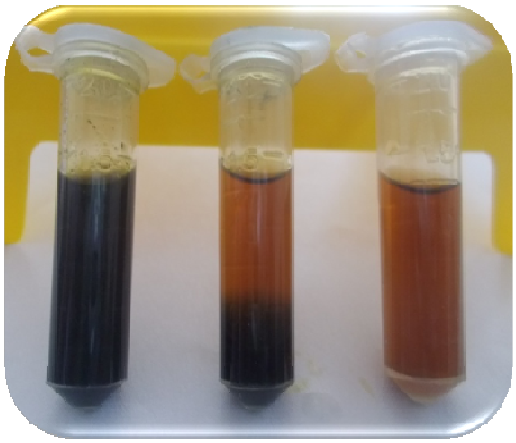

Figure 1
Stages of chlorophyll removal

3. 2. GC-MS Analysis

The GC-MS analysis of methanolic leaf extract revealed that a variety of bioactive compounds and compositions were present in the plant extract (Table 1, Fig. 2). The GC-MS analysis revealed the presence of five compounds namely Vitamin d_3, 14-Hydroxy-14-methyl-hexadec-15-enoic acid methyl ester, Ethaneperoxoic acid 1-cyano-1-[2-(2-phenyl-1, 3-dioxolan-2-yl)ethyl]pentyl ester, 10-Octadecenoic acid methyl ester, Dasycarpidan-1-methanol acetate (ester) and was confirmed by the comparison of results with National Institute of Standards and Technology library. In which 10-Octadecenoic acid methyl ester being the major compound in *C. roseus*. The composition (%) of identified compounds was given in Table 1. The present work of GC-MS analysis of methanolic leaf extract of *C. roseus* was in good agreement with results Asghar et al. (2011) where they carried out the GC-MS analysis for the plant extract of *Iris germanica* but they used petroleum ether as solvent to prepare the plant extract.

3.3. Antibacterial Activity

The zone of inhibition of the methanolic leaf extract against various bacteria was measured and compared with streptomycin as positive control (Table 2).

Table 1
GC-MS analysis of methanolic leaf extract of C. roseus

Chemical compounds	Retention time (mins)	Peak area	Composition (%)
Vitamin d_3	12.28	3933992	11.99
14-Hydroxy-14-methyl-hexadec-15-enoic acid methyl ester	15.27	4806024	14.65
Ethaneperoxoic acid 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl)ethyl]pentyl ester	15.78	6318744	19.26
10-Octadecenoic acid methyl ester	16.98	11214888	34.20
Dasycarpidan-1-methanol acetate (ester)	22	2731808	8.33

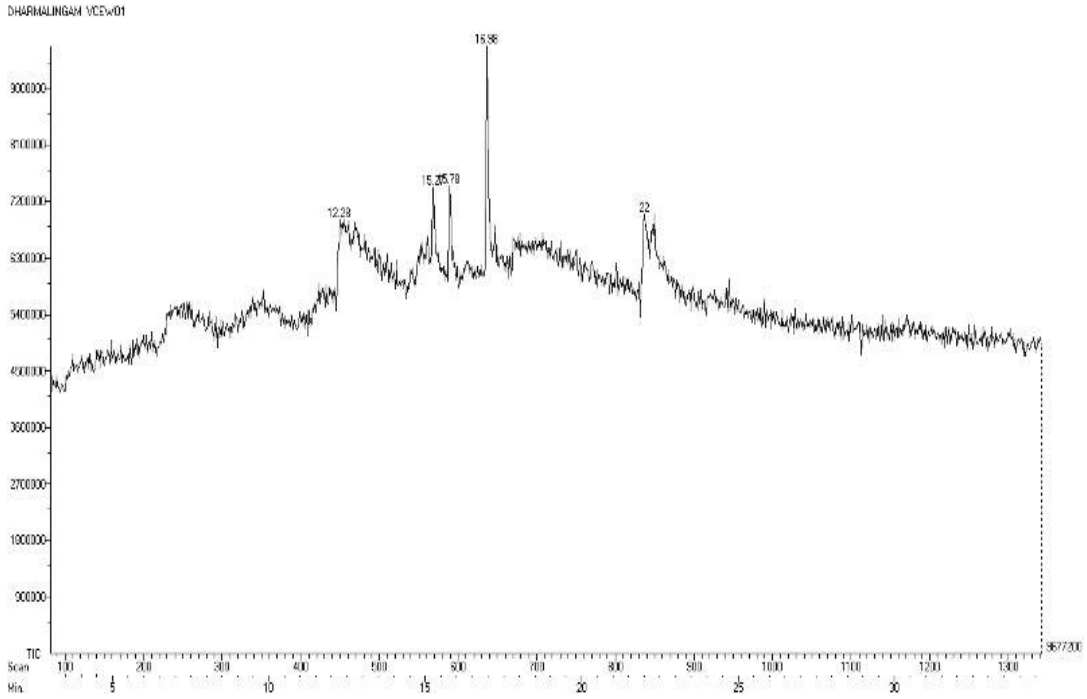

Figure 2
GC-MS chromatogram of bioactive compounds in the methanolic leaf extract of C. roseus

The identified biocompounds of methanolic leaf extract of *C. roseus* greatly contributed to antibacterial in the present study. In the present study, the extract showed significantly more activity against *Staphylococcus* sp. SSKSD17 (KF751681) with the zone of inhibition of 34 mm for 10 µl of methanolic leaf extract when compared to standard streptomycin (500 µg/mL) with a zone of inhibition of 38 mm followed by *Bacillus* sp. SSKSD01 (KF751671) (32 mm), *Bacillus* sp. SSKSD05 (KF751673) (30 mm), *Bacillus* sp. SSKSD08 (KF751675) (29 mm), *Staphylococcus aureus* (29 mm), *Pseudomonas stutzeri* (28 mm), *Bacillus megaterium* (27 mm), *Bacillus flexus* (24 mm), *Escherichia coli* (21 mm), *Pseudomonas fluorescens* (19 mm), *Bacillus subtilis* (18 mm), *Proteus vulgaris* (17 mm), *Pseudomonas* sp. SSKSD06 (KF751674) (12 mm), *Klebseilla pneumonia* (10 mm) and *Enterobacter aeruginosa* (04 mm).

Table 2
The zone of inhibition of methanolic leaf extract of C. roseus against various bacteria

Bacterial isolates	Zone of inhibition (Including well diameter 5mm)	
	Streptomycin (500 µg/mL) (Diameter in mm)	Methanolic leaf extract (Diameter in mm)
Staphylococcus aureus	32	29
Escherichia coli	26	21
Bacillus megaterium	35	27
Bacillus subtilis	29	18
Bacillus flexus	26	24
Klebseilla pneumonia	25	10
Pseudomonas stutzeri	32	28
Proteus vulgaris	26	17
Pseudomonas fluorescens	28	19
Enterobacter aeruginosa	12	04
Bacillus sp. SSKSD01 (KF751671)	39	32
Staphylococcus sp. SSKSD17 (KF751681)	38	34
Pseudomonas sp. SSKSD06 (KF751674)	18	12
Bacillus sp. SSKSD05 (KF751673)	37	30
Bacillus sp. SSKSD08 (KF751675)	31	29

Govindasamy and Srinivasan (2012) reported that *C. roseus* had antibacterial activity against *S. typhi* where the maximum inhibition zone was obtained for methanolic leaf extract as 21.2 mm and from the methanolic leaf extract of as 15.6 mm. In the same way, Dash et al. (2011) stated the antibacterial activity against *Pseudomonas sp. Shigella dysentiriae, Salmonella typhi and E. coli* using with methanolic and acetone extract from *T. foenum* and *C. sativum*. The antibacterial activity for *Melia azedarach* against *S. aureus, Staphylococcus epidermidis, Streptococcus sp, Enterococcus faecalis, B. subtilis, E. coli, Edwardsiella tarda, Klebsiella pneumonia, Proteus mirabilis*, *Proteus vulgaris*, *P. aeruginosa*, *S. typhi*, *Shigella boydii, Shigella dysenteriae, Shigella flexneri* and *Plesiomonas shigelloides* was also reported (Hussain et al. 2011). Subbaiyan et al. (2013) analysed the antibacterial activity for various pathogenic bacteria for the different solvent extracts (methanol, petroleum ether, aqueous and chloroform) of *Catharanthus pusillus.* The maximum inhibition zone was observed in the methanolic extract of *C. pusillus* against *Streptococcus faecalis* (24.6±0.39) and *Klebsiella pneumonia* (23.6±0.32) This work suggests the significant antibacterial activity of the methanolic leaf extract of *C. roseus* which find its way applications in secondary infections associated with chemotherapy.

CONCLUSION

In the present study, only five compounds were found in the plant extract by GC-MS analysis, in which 10-Octadecenoic acid, methyl ester being the major compound in *C. roseus*, is one of the most important components and possessed better antibacterial activity. It was clear that the methanol extract of *C. roseus* leaf possessed antibacterial property which of importance for the development of new therapeutic agents. Further works will need to be done in the future to correlate the specific compound with its biological property.

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