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Research Article

PHYSICO-PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL PROSPECTIVE OF *CATHARANTHUS PUSILLUS* (MURRAY) G. DON -AN IMPORTANT ANTICANCER MEDICINAL PLANT

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ABSTRACT

Objective: The current study described that physico-chemical parameters, preliminary phytochemical screening and anti-bacterial activity of the whole plant extract of *Catharanthus pusillus*.

Methods: All the crude extracts were analyses to know the phytochemical constituent by standard procedure and antibacterial activity by using disc diffusion method.

Result: The results of the present investigation phytochemical constituents revealed that alkaloids, tannin, saponins, coumarin, phenol, terpenoids and cardiac glycosides as major compounds present in methanolic extract. Whereas phytosterols, flavonoids showed only in the presence of chloroform extract. The antibacterial activity showed the highest zone of inhibition in methanol extract (24.6±0.39) against *Streptococcus faecalis*, *Klebsiella pneumonia* (23.6±0.32) and chloroform extract had moderate activity against tested pathogenic bacteria. The aqueous and petroleum ether extract have least activity against tested pathogens.

Conclusion: The plant extract of *Catharanthus pusillus* have more phytochemical constituents could be responsible for their prospective antibacterial activity.

Keywords: *Catharanthus pusillus*, Anticancer, Coumarin and Antibacterial activity

INTRODUCTION

Medicinal plants provide basic raw materials for different industries such as pharmaceutical, cosmetic and food, etc. The medicinal plants are referred to plants that are used for their therapeutic or medicinal values. The most of the plant or its different parts could be valued for its therapeutic, medicinal, aromatic or savory qualities [1]. World Health organization estimated that 70-80% of people worldwide rely chiefly on traditional knowledge of herbal medicine to meet their primary healthcare needs [2]. The global demand for herbal medicine is not only large, but it is growing every day [3]. The plant based traditional medicines are used to treat gastrointestinal disorder, fever, malaria, pain and diabetes [4]. The Human pathogenic bacteria like *Escherichia coli* and *Staphylococcus aureus* are intestinal bacteria often implicated in several gastrointestinal disorders. Gastrointestinal diseases caused by *E. coli* are the most frequent causes of death in developing countries [5].

Catharanthus pusillus belong to the family Apocyanaceae. It is widely used as various treatments of diseases and traditionally used as herbal medicine [6]. The roots, leaves and latex of these plants are used to treat skin and liver diseases, leprosy, dysentery, worms, ulcers, tumor and ear aches [7]. In modern medicine the alkaloids as chemotherapeutic agents from *Catharanthus pusillus* are known for their anticancer pain-relieving properties. Hence, the present investigation evaluate the preliminary phytochemical analysis and antibacterial activity were tested all the crude extracts of *Catharanthus pusillus*.

MATERIALS AND METHODS

Plant material

In this opportunity the plants of *Catharanthus pusillus* were collected from field at foothills of Madukarai hills, Western Ghats of Coimbatore region, Tamilnadu, India. The samples of flowering plants were identified self and binomially by Botanical Survey of India (Southern part Coimbatore, Tamilnadu, India) and voucher specimens were deposited at the Herbarium Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamilnadu, India.

Physico-chemical parameters

Total ash

About 5g accurately weighed powder was incinerated in a silica dish put in a muffle furnace at the temperature not exceeding 450˚C until it become free from CO_2. It was then cooled and weighed.

Water soluble ash

Ash was dissolved in distilled water and the insoluble parts were collected on an ash less filter paper and ignited at 450˚C to a constant weight. By subtracting the weight of insoluble part from that of the ash, the weight of soluble part of ash could be obtained. Percentage of water soluble ash was calculated with reference to the air dried ash (drug).

Acid insoluble ash

Ash was boiled with 25ml dilute HCl (6N) for five minutes. The insoluble matter collected on as ash less filter paper, washed with hot water and ignited at a temperature not exceeding 450˚C to a constant weight.

Preparation of extracts

The whole plant parts of *Catharanthus pusillus* were shade dried for 15 days. The different shade dried plant materials were powered in mixer grinder. Soxhlet apparatus was used for the extraction. The liquid extracts were evaporated to dryness by vacuum distillation [8].

Aqueous extract

25g of whole plant part powder were dissolved in 100 mL hot distilled water containing conical flask that were kept on a rotary shaker for 12 hours under 80rpm and the residues were filtered using No. 1 Whatman filter paper. Then collected the residues were dried first on a hot water bath to remove wetness and then in an oven. After drying, the residues were weighed and scraped out and different aliquots were dissolved in 5ml sterile water and were stored at 4°C

Preliminary phytochemical investigation

The extracts were screened for the presence of alkaloids, tannins, phlobatannins, saponins, flavonoids, steroids, terpenoids, cardiac glycosides and reducing sugar [9-11].

Alkaloids

0.2g of extract was warmed with 2% H_2SO_4 for two minutes. It was filtered and few drops of Dragendroff's reagent were added. Formation of orange red precipitate indicates the presence of alkaloids.

Tannins

Small quantity of extract was mixed with water and then heated on a water bath. The mixture was filtered and ferric chloride was added to the filtrate. Development of a dark green solution indicates the presence of tannins.

Phlobatannins

About 0.5g of each plant extract was dissolved in distilled water and filtered. The filtrate was boiled with 2% HCl solution. Red precipitate formation shows the presence of phlobatannins.

Saponins

About 0.2g of the extract was shaken well with 5ml of distilled water and then heated to boil. Frothing evolution shows the presence of saponins.

Flavonoids

About 0.2g of each plant extract was dissolved in diluted NaOH and HCl. Yellow solution that turns into colourless indicates the presence of flavonoids.

Steroids and Phytosteroids

To 0.5 ml of the plant extract equal volume of chloroform was added and subjected with few drops of concentrated sulfuric acid. Appearance of brown ring indicates the presence of steroids and appearance of bluish brown ring indicated the presence of Phytosteroids.

Cardiac glycosides

Keller-killani Test:

About 0.5g of each extract was treated with 2ml of glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1ml of concentrated tetraoxosulphate acid to give a brown ring formation at the interface.

Glycosides

Small amount of alcoholic extract of samples was dissolved in 1ml of water and then aqueous NaOH solution was added. Formation of yellow colour indicates the presence of glycosides.

Reducing sugar

The crude extracts were shaken well with 5ml of distilled water and filtered. The filtrate was boiled with drops of Fehling's solution A and B for 2 minutes, an orange red precipitate development indicates the reducing sugar.

Detection of amino acid

Ninhydrin test: Two drops of ninhydrin solution (5 mg of ninhydrin in 200 ml of acetone) are added to two ml of aqueous filtrate. The color change was observed. A characteristic purple color indicated the presence of amino acids.

Detection of coumarin

10% NaOH (1ml) was added to 1 ml of the plant extracts. The yellow color formation was indicated the presence of coumarin.

Antibacterial activity:

The following bacterial pathogens were used in this study viz., *Pseudomonas aeruginosa, Bacillus thuringiensis, Euterococcus faecalis, Serratia marcescens, Salmonella paratyphi, Salmonella paratyphi* A, *Salmonella paratyphi* B, *Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus faecalis*. The bacterial activity of all the extracts was impregnated with inoculated culture of agar nutrient medium [12]. The inoculation of bacterial culture was incubated over night at 28˚C for 24 h. Each pathogen was tested in triplicate and after incubation; the diameter of inhibition zones was measured [13].

Statistical Analysis

Triplicates were maintained in all the experiments and means were segregated using Duncan's Multiple Range Test (DMRT). Significant differences were recorded at 5% level (*P<0.05*).

RESULT

The present study of physico-chemical parameters from plant material were recorded in Table 1. The above studies enable the identification of the material for future investigation and form an important aspect of drug studies.

Table 1: Yield of total ash content and extracts obtained from the whole parts of *Catharanthus pusillus*

Plant parts	Solvent	Time of extraction	Colour of extraction	Yield %	Physical parameters	Yield %
Whole parts	Petroleum either	24	Light green	26.66	Total ash	8.5
	Chloroform	48	Golden yellow	40	Acid insoluble ash	2.25
	Methanol	48	Dark green	60	Water soluble ash	3.5
	Aqueous	12	Light brown	13.33		

Yield % = (Yield weight/Sample) × 100

The results of phytochemical analysis of various solvent extracts of *Catharanthus pusillus* were recorded in Table 2. From this analysis, methanol extracts of whole plant parts like root, stem and leaf were found to be more chemical constituents compared to other extracts.

Table 2: Preliminary phytochemical constituents of different solvent extracts of *Catharanthus pusillus*

Phytochemical Constituents	Whole plant parts			
	Petroleum either	Chloroform	Methanol	Aqueous
Tannins	-	+	++	+
Saponins	+	+	++	++
Alkaloids	-	+	++	+
Flavonoids	-	+	+	-
Phlobatannins	-	-	-	-
Steroids	+	+	-	-
Terpenoids	-	+	+	-
Phenol	-	+	+	+
Cardiac glycosides	-	-	+	+
Glycosides	-	-	+	-
Reducing sugar	-	-	-	-
Amino acid	-	-	-	-
Phytosteroids	+	-	-	+
Coumarin	-	+	++	-

-, Absent and +, Present

Antibacterial activity

The result of the antibacterial activity suggested that, the maximum zone of inhibition was observed in methanolic extract against *Streptococcus faecalis* (24.6±0.39) and *Klebsiella pneumonia* (23.6±0.32) and minimum zone of inhibition was observed in *Bacillus thuringiensis* (17.6±0.01) and *Serratia marcescens* (16.1±0.11). The highest zone of inhibition showed in chloroform extract against *Klebsiella pneumoniae* (16.2**±**0.96) and *Pseudomonas aeruginosa* (14.33±0.69). The lowest zone of inhibition was recorded in *Euterococcus faecalis* (10.1±1.24). The higher inhibition was observed in petroleum ether extracts against *Klebsiella pneumonia* (15.6±0.12) and *Staphylococcus aureus* (15.6±0.54) and lowest inhibition was recorded against *Salmonella paratyphi* A (10.1**±**1.63) and *Streptococcus faecalis* (9.3±0.05). The moderate zone of inhibition observed in aqueous extract against *Salmonella paratyphi* (12.1±1.63) and least of inhibition was obtained in against *Salmonella paratyphi* B (7.6±0.47).

Table 3: *In vitro* antibacterial activities of different solvent extracts of *Catharanthus pusillus*

Bacterial pathogens	Zone of inhibition (mm)				
	Petroleum ether	Chloroform	Methanol	Aqueous	Tetracycline
Streptococcus faecalis	9.3±0.05	11.0±0.26	24.6±0.39	9.3±1.24	23.0±0.09
Klebsiella Pneumoniae	15.6±0.12	16.2±0.96	23.6±0.32	9.6±1.24	24.1±1.63
Bacillus thuringiensis	11.6±0.85	13.3±0.98	17.6±0.01	9.0±1.41	28.6±0.62
Pseudomonas aeruginosa	10.6±0.85	14.3±1.69	18.6±0.90	8.6±1.69	19.6±0.24
Salmonella paratyphi	10.6±0.09	14.0±1.63	23.6±0.02	12.1±1.63	24.6±1.24
Salmonella paratyphi A	10.1±1.63	11.1±0.16	21.0±0.48	8.0±1.63	24.3±0.05
Salmonella paratyphi B	11.6±1.69	11.3±0.39	23.6±0.05	7.6±0.47	23.3±0.86
Serratia marcescens	10.3±1.24	11.0±0.81	16.1±0.11	8.6±0.94	27.6±0.09
Streptococcus aureus	15.6±0.54	11.2±0.68	22.0±0.32	10.3±1.69	29.0±0.81
Euterococcus faecalis	12.3±0.05	10.1±1.24	20.1±0.32	8.3±0.47	8.3±0.47

Values are mean±SD (*n*=3); Mean values followed by different superscripts in a column are significantly different (*P<0.05*) according to Duncan's multiple range tests (DMRT).

DISCUSSION

The present study also made an effort to identify the phytochemical constituent's analysis and the results revealed that the presence of soluble sugars, reducing sugar, amino acid, proteins, lipids, chlorophyll, phenol, ortho-dihydroxy phenols and these phytochemical constituents previously reported with several biological properties [14-15]. Similarly, Govndasamy [13] reported the phytochemical analyses of *Catharanthus roesus* were showed the presence of soluble sugar, reducing sugar, protein, amino acids, lipids, total chlorophyll, phenol and ortho-dihydroxyphenols in the ethanolic extract. Hussain *et al.* [16] reported that the phytochemical analysis of *Ranunculus arvensis, Equisetum ravens, Carathamus lanatus and Fagonia critica* showed more phytochemical constituents. Thenmozhi *et al.* [17] reported the phytochemical screening with the *Catharanthus roseus* was showed that presence of tannin, flavonoids, alkaloids, saponins and terpenoids. In the Present investigation the methanol and chloroform extract showed more number of phytoconstituents of *Catharanthus pusillus*.

Antibacterial activity was identified the methanolic extract of *Catharanthus pusillus* showed potential activity of against tested pathogens, due do their more phytochemical constituents. Similarly the ethanolic extract *Catharanthus roseus* showed highest zone of inhibition against *Salmonella paratyphi* and lowest zone of inhibition was observed against *Staphylococcus aureus* and *E. coli.* [13]. Dash *et al*. [18] reported that the potential antibacterial activity of with methanol and acetone extract of *Trigonella foenum* and *Coriandrum sativum, Pseudomonas sp. Shigella dysentiriae, Salmonella typhi and E. coli*. Khan *et al.* [19] reported that the antibacterial activity of *Melia azadirachta* extract were tested against *Staphylococcus 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*. Thenmozhi *et al.* [17] reported that the antibacterial activity of *Catharanthus roseus* ethanol extract were tested against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Streptococcus pyogenes.*

CONCLUSIONS

The tested all crude extract of *Catharanthus pusillus* showed some of their phytochemical constituents such as flavonoids, alkaloids saponins, tannins and coumarin and terpenoids. There is no doubt that these plants are reservoir of potentially useful chemical compounds which serve as drugs, provide newer leads and clues for modern drug design. Due to its many medicinal properties there is enormous scope of future research on *Catharanthus pusillus*. Further investigation of pharmacological study should be conduct to the unexploited potential of these plants.

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