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. World Health organization estimated that 70-80% of people worldwide rely chiefly on traditional knowledge of herbal medicine to meet their primary healthcare needs. The global demand for herbal medicine is not only large, but it is growing every day. The plant based traditional medicines are used to treat gastrointestinal disorders, fever, malaria, pain and diabetes. 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.

Catharanthus pusillus belong to the family Apocynaceae. It is widely used as various treatments of diseases and traditionally used as herbal medicine. The roots, leaves and latex of these plants are used to treat skin and liver diseases, leprosy, dysentery, worms, ulcers, tumor and earaches. 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. Then it was 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.

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 scrapped 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.

Alkaloids

0.2g of extract was warmed with 2% H2SO4 for two minutes. It was filtered and few drops of Dragendorff'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 0.5 g 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 0.2 g of the extract was shaken well with 5 ml of distilled water and then heated to boil. Frothing evolution shows the presence of saponins.

Flavonoids
About 0.2 g 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 0.5 g of each extract was treated with 2 ml of glacial acetic acid containing a drop of ferric chloride solution. This was underplayed with 1 ml of concentrated tetraoxosulphate acid to give a brown ring formation at the interface.

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

Reducing sugar
The crude extracts were shaken well with 5 ml 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 (1 ml) 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, Eutecoccus faecalis, Serratia marcescens, 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 overnight 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

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

Yield % = (Weight of Sample/Weight) × 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

<table>
<thead>
<tr>
<th>Phytochemical Constituents</th>
<th>Whole plant parts</th>
<th>Petroleum either</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tannins</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Reducing sugar</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Amino acid</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Coumarin</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

- 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 pneumoniae (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 (14.3±0.69). The lowest zone of inhibition was recorded in Eustercoccus 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 Salmonellaparatyphi A (10.1±0.63) and Streptococcus faecalis (9.3±0.05). The moderate zone of inhibition observed in aqueous extract against Salmonellaparatyphi A (12.1±1.63) and least of inhibition was obtained in against Salmonella paratyphi B (7.6±0.47).

<table>
<thead>
<tr>
<th>Bacterial pathogens</th>
<th>Zone of inhibition (mm)</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>Methanol</th>
<th>Aqueous</th>
<th>Tetracycline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus faecalis</td>
<td>9.3±0.05</td>
<td>11.0±0.26</td>
<td>24.6±0.39</td>
<td>9.3±1.24</td>
<td>23.0±0.09</td>
<td></td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>15.6±0.12</td>
<td>16.2±0.96</td>
<td>23.6±0.32</td>
<td>9.6±1.24</td>
<td>24.1±1.63</td>
<td></td>
</tr>
<tr>
<td>Bacillus thuringiensis</td>
<td>11.6±0.85</td>
<td>13.3±0.98</td>
<td>17.6±0.01</td>
<td>9.0±1.41</td>
<td>28.6±0.62</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>10.6±0.85</td>
<td>14.3±1.69</td>
<td>18.6±0.90</td>
<td>8.6±1.69</td>
<td>19.6±0.24</td>
<td></td>
</tr>
<tr>
<td>Salmonella paratyphi</td>
<td>10.6±0.09</td>
<td>14.0±1.63</td>
<td>23.6±0.2</td>
<td>12.1±1.63</td>
<td>24.6±1.24</td>
<td></td>
</tr>
<tr>
<td>Salmonella paratyphi A</td>
<td>10.1±1.63</td>
<td>11.6±0.16</td>
<td>21.0±0.48</td>
<td>8.0±1.63</td>
<td>24.32±0.05</td>
<td></td>
</tr>
<tr>
<td>Staphylococcus epidermidis</td>
<td>11.3±0.90</td>
<td>11.6±0.98</td>
<td>23.6±0.05</td>
<td>7.6±0.47</td>
<td>23.32±0.86</td>
<td></td>
</tr>
<tr>
<td>Serratia marcescens</td>
<td>10.3±0.81</td>
<td>12.4±1.80</td>
<td>16.1±0.11</td>
<td>8.6±0.94</td>
<td>27.6±0.09</td>
<td></td>
</tr>
<tr>
<td>Streptococcus auresus</td>
<td>15.6±0.54</td>
<td>11.2±0.68</td>
<td>22.0±0.32</td>
<td>10.3±1.69</td>
<td>29.0±0.81</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>12.3±0.05</td>
<td>10.1±1.24</td>
<td>20.1±0.32</td>
<td>8.3±0.47</td>
<td>8.3±0.47</td>
<td></td>
</tr>
</tbody>
</table>

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 test (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 analyses by reported several biological properties [14-15]. Similarly, Govindasamy [13] reported the phytochemical analysis 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 alka, saponins and terpenoid was reported the phytochemical constituents. Thenmozhi et al. [17] reported the phytochemical screening with the Catharanthus roesus showed that presence of tannin, flavonoids, alkaloids, saponins and terpenoids. In the present investigation the methanol and chloroform extract showed more number of phytochemicals of Catharanthus pullus.

Antibacterial activity was identified the methanolic extract of Catharanthus pullus showed potential activity of against tested pathogens, due do their more phytochemical constituents. Similarly the ethanolic extract Catharanthus roesus showed highest zone of inhibition against Salmonella paratyphi and lowest zone of inhibition was observed against Staphylococcus aureus and E. coli [13]. Dass et al. [18] reported that the potential antibacterial activity of with methanol and acetone extract of Trigonella foenum and Coriandrum sativum, Pseudomonas sp. Shigella dysentiae, Salmonella typhi and E. coli. Khan et al. [19] reported that the antibacterial activity of Melia azadirachta extract were tested against Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, P. aeruginosa, S. typhi, Shigella boydii, Shigella dysenteriae, Shigella flexneri and Plesiomona shigelloides. Thenmozhi et al. [17] reported that the antibacterial activity of Catharanthus roesus ethanol extract were tested against Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae and Streptococcus pyogenes.

CONCLUSIONS

The tested all crude extract of Catharanthus pullus 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 pullus. Further investigation of pharmacological study should be conduct to the unexploited potential of these plants.

REFERENCE


