INTRODUCTION

Benoda is located in Warud tahsil of Amravati district in Maharashtra State of India. This area is very rich in flora and fauna having various types’ medicinal plants as it is present in the ranges of Satpuda Mountain. The different parts of Catharanthus roseus A. are used for medicinal uses. Mainly Leaves, flowering tops is used for extraction of oil. This oil has been found to have anti-bacterial, anti-yeast action. Researchers have found that it can kill some intestinal parasites and have mind antibiotic effects. It is also observe that leaves of it are used extensively in folk medicine for decreasing sugar level of blood and showed significant anti-hyperglycemic effect. Due to
Recently in this laboratory enormous work on natural products was carried out by one research group of D.T. Tayade\textsuperscript{11-19} especially on proximate, phytochemical, physiochemical and spectroscopical analysis of various plants and as a part of research work, presently being undertaken in this laboratory on natural products, hence it appeared sufficient interesting to carry out phytochemical analysis of the leaves of \textit{Catharanthus roseus}A.\textsuperscript{A}

Preparation of Sample

First the site for leaves collection was decided. The whole leaves were collected from same region of Benoda. Before picking the whole plant, the soil was moistened. The collection of sample was done in between 30\textsuperscript{th} July 2011 to 10\textsuperscript{th} August 2011. The leaves of \textit{Catharanthus roseus}A. were separated by scissor and were shed dried. They were dried at room conditions. The leaves were pulverized in grinding mill having a screen of 5mm diameter hole to achieve particle size 40-60 mesh. This fine powder was treated as a sample powder for various analyses. All chemicals used are of A.R. grade.

Determination of Mg, Ca, S and Fe elements

Leaves sample was taken and kept in furnace at 400°C for 12 hours. Then there is a formation of ash. The ash was transferred into conical flask to it 20% hydrochloric acid was added, the reaction mixture was continuously shaken vigorously for 1 hour and it was filtered. The filtrate was taken to determine Mg, Ca, S and Fe elements. Qualitative analysis was carried out by given literature method\textsuperscript{18} and result is given in Table No.1

Determination of Na and Cl elements

Leaves sample was taken and kept in furnace for 12 hours at 400°C. Then there was formation of ash. This ash was transferred in a conical flask to it 20% nitric acid was added, the reaction mixture was continuously shaken for 1 hour and it was filtered. The filtrate was taken for qualitative analysis to determine Na and Cl elements. Qualitative analysis was carried out by given literature method\textsuperscript{18} and result is given in Table No.1

Test for Glucoside

Leaf sections of \textit{Catharanthus roseus}A. were taken and immersed in picric acid, washed with distilled water and placed in sodium carbonate. These sections then placed in gave red colour with hydrochloric acid.

Test for Cyogenic glucoside

Juice of \textit{Catharanthus roseus}A. was extracted in water. It was taken in test tube to it a mixture of distilled water, toluene and chloroform was added. The reaction mixture was kept for 30 minutes. Previously treated strip of filter paper with picric acid and sodium carbonate was fixed in this test tube and allowed to stand for four days. Strip of filter paper becomes red in colour.

Test for Acubin type of glucoside

Leaf sample was refluxed with dil. HCl. It was cooled and filtered. In filtrate isoamyl alcohol was added and vigorously shook, amyl alcohol layers developed red colour.

Test for Phenol

Leaf sample was taken in a test tube to it ethanol was added, shaked vigorously and kept for overnight. It was filtered and in filtrate 2 to 3 drops of aqueous ferric chloride was added. Violet colour developed.

Test for Flavonols

Leaf sample was refluxed with aqueous ethanol, to it chloroform is added and shaked
vigorously. It was filtered and in filtrate pinch of boric acid and few drops of acetic acid was added. The reaction mixture was further shake green florescence with yellow shade was developed.

**Test for Amino acid**
Leaf sample was refluxed with ethanol to it chloroform was added, shaken vigorously and filtered. This filtrate was spotted on Whatman's No.1 chromatographic paper and separation was achieved by using phenol and a mixture of n-butanol, acetic acid and water. Chromatograms were sprayed with ninhydrin in acetone. Violet and pink spots developed.

**Test for Syringic Acid**
Leaf section was taken it was mounted by 50% aqueous sulphuric acid and studied under microscope, section developed bluish colour.

**Test for Alkaloids**
Leaf sample was refluxed with acetic acid in ethanol. It was concentrated and cooled on water bath. It was filtered and in filtrate Mayer's reagent was added. Gray colour was obtained.

**Test for Steroids**
Leaf sample was refluxed with ethanol; to it petroleum ether was added. The reaction mixture was shaking vigorously and then chloroform was added, it was filtered. In filtrate sodium sulphate was added and again filtered, to the filtrate Libbermann-Buccard reagent was added. Blue colour was obtained.

**Test for Tannin**
Leaf sample was taken; it was dipped in alkaline aqueous ferric chloride solution. Bluish green colour developed.

**Test for Polysaccharide, Pectin and Hemicellulose**
Weighed amount of dried leaf sample was taken in filter paper. It was folded and put in soxhlet extractor and it was extracted with benzene. The powder left in the filter paper was dried and again weighed. This powder was refluxed with water. It was cooled, filtered and to the filtrate ethanol was added, whitish precipitate was obtained indicating presence of polysaccharide.

The previously dried residue was refluxed with aqueous ammonium oxalate solution. It was filtered and acidified with dilute H₂SO₄ by using as indicator. After acidification ethanol was added in excess. Pale yellowish precipitate obtained indicating presence of pectin.

Previously treated residue after the extraction of pectin is refluxed with aqueous NaOH. The reaction mixture was cooled, filtered and in filtrate acetic acid was added till it gave smell of acetic acid. To it excess of ethanol was added, white colour precipitate was obtained indicating that hemicellulose is present.

**Test for Fats**
Leaf section was taken; this section was immersed in alcoholic acid Sudan IV dye. Rinsed with aqueous alcohol and mounted in glycerin blue colour obtained.

**Test for Volatile Oil**
Leaves sample was taken in a filter paper.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Element/Compound</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Magnesium</td>
<td>Absent</td>
</tr>
<tr>
<td>2</td>
<td>Calcium</td>
<td>Absent</td>
</tr>
<tr>
<td>3</td>
<td>Sulphur</td>
<td>Present</td>
</tr>
<tr>
<td>4</td>
<td>Iron</td>
<td>Present</td>
</tr>
<tr>
<td>5</td>
<td>Sodium</td>
<td>Present</td>
</tr>
<tr>
<td>6</td>
<td>Chlorine</td>
<td>Present</td>
</tr>
<tr>
<td>7</td>
<td>Glucosides</td>
<td>Present</td>
</tr>
<tr>
<td>8</td>
<td>Acubine type of</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>Glucosides</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Cyanogenic Glucosides</td>
<td>Present</td>
</tr>
<tr>
<td>10</td>
<td>Phenol</td>
<td>Present</td>
</tr>
<tr>
<td>11</td>
<td>Flavanol</td>
<td>Present</td>
</tr>
<tr>
<td>12</td>
<td>Amino acids</td>
<td>Present</td>
</tr>
<tr>
<td>13</td>
<td>Syringic acid</td>
<td>Present</td>
</tr>
<tr>
<td>14</td>
<td>Alkaloides</td>
<td>Present</td>
</tr>
<tr>
<td>15</td>
<td>Steroides</td>
<td>Present</td>
</tr>
<tr>
<td>16</td>
<td>Tannin</td>
<td>Present</td>
</tr>
<tr>
<td>17</td>
<td>Polysaccharides</td>
<td>Present</td>
</tr>
<tr>
<td>18</td>
<td>Pectin</td>
<td>Present</td>
</tr>
<tr>
<td>19</td>
<td>Hemicellulose</td>
<td>Present</td>
</tr>
<tr>
<td>20</td>
<td>Fats</td>
<td>Present</td>
</tr>
<tr>
<td>21</td>
<td>Volatile Oil</td>
<td>Present</td>
</tr>
</tbody>
</table>
The filter paper was folded and put in soxhlet extractor and extracted with benzene. The extract was taken and concentrated with distillation it give oil. In this way the Phytochemical analysis of leaves samples of *Catharanthus roseus* was carried out and results obtained during work was given in Table 1

REFERENCES