ABSTRACT
Miluusa velutina var. deviyarina (Family- Annonaceae ) was reported from southern ghat of India and has also reported from Chandrapur district of Vidarbha region (Maharashtra State). The objective of this paper was to determine phytochemical screening of ethanol extract of Miluusa velutina var. deviyarina leaf along with biological activity against gram positive and gram negative microorganism. The extract was also tested for antifungal activity.

Keywords: M. Velutina var. deviyarina, phytochemical, biological activity

Introduction:
The medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents. Phytochemicals are naturally occurring in the medicinal plants, leaves, vegetables and roots that have defense mechanism and protect from various diseases. Phytochemicals are primary and secondary compounds. Chlorophyll, proteins and common sugars are included in primary constituents and secondary compounds have terpenoid, alkaloids and phenolic compounds. Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anticanter, anti-malarial, inhibition of cholesterol synthesis, anti-viral and anti-bacterial activities. Terpenoids are very important in attracting useful mites and consume the herbivorous insects. Alkaloids are used as anaesthetic agents and are found in medicinal plants. The Annonaceae family includes 80 genera and about 850 species distributed in tropical and subtropical areas of America, Africa and Asia [1]. The plants belonging to family Annonaceae are used as antibacterial, anticancer, antihelminthic, antiparasitic and pesticidal agents [2]. Leaf oil obtained from different species of Miluusa is also reported and varying amount of constituents is present in it [3]. Miluusa tomentosa oil has been found to have both antibacterial [4] and analgesic properties [5]. From Maharashtra only two species of Miluusa (M. tomentosa and M. velutina) have been reported. Recently, new variety of Miluusa was identified named as M. velutina var. deviyarina [6]. This research paper deals with phytochemical screening and biological study of ethanol extract of leaf of this new variety of Miluusa.

Materials and method
Plants collection-The present work was carried out at Department of Chemistry, J.M.V. Chandrapur, Gondwana University. The plant named Miluusa velutina var. deviyarina was collected from Chandrapur forest region. Their botanical identity of plant was determined and authenticated from literature available in Department of Botany, J.M.V. Chandrapur. The leaves of Miluusa velutina var. deviyarina was thoroughly washed with water and dried under shade for about ten days. The dried plant sample was ground well into a fine powder in a mixture grinder. The powder was stored in an air sealed polyethylene bag at room temperature before extraction.

Preparation of Extract:-
The powdered plant material was extracted using Soxhlet apparatus with organic solvents ethanol. The extract was concentrated. The extract was stored in air tight glass container at 4°C.

Micro-organism collection-
The microorganism used in the study: Gram-negative E-coli, Gram-positive S-aureus and Nizer fungus Aspergillus were obtained from stock culture in the Department of Microbiology, J.M.V. Chandrapur.

Antimicrobial sensitivity testing of extracts against selected microorganism:-
Susceptibility test were carried out. The modified agar well diffusion method [7, 8] to test the antimicrobial activity of the extract. The medium employed was diagnostic sensitivity agar. The culture were prepared in triplicate and incubated at 37°C for 24 to 72 h. 0.2 ml of the broth culture of the test organism was put in a sterile Petri-dish and 18 ml of sterile molten diagnostic sensitivity agar was added. Well were bored into the medium using 0.1 ml of the extracts. Streptomycin and Chloramphenicol were used as the standard antimicrobial agents at a concentration of 10 mcg/disk, 30 mcg/disk respectively. The plates were kept in sterilized inoculation chamber for 2 h to facilitate diffusion of the antimicrobial agents into the medium. The plates were then incubated at 37°C for 24 h and the diameter of zone of inhibition of microbial growth were measured in the plates in millimeters.

Phytochemical analysis:- The extracts were analyzed for the presence of Alkaloid, Terpenoid, Tannine, Saponin, Flavonoid, Phlobatannin, Anthraquinone, Reducing Sugar, Glycoside and Cardiac glycoside[9 to 12 ].

Alkaloid
About 0.2 g of the extract was warmed with 2% H2SO4 for two minutes. It was filtered and few drop of Dragenclof's reagent was added. Orange
red precipitated indicates the presence of alkaloids.

**Tannin**
Small quantity of extract was mixed with water and heated on water bath. The mixture was filtered and ferric chloride was added to the filtrate. A dark green solution indicate the presence of tannins.

**Anthraquinones**
About 0.5 g of the extract was boiled with 10% HCl for few minutes in a water bath. It was filtered and allowed to cool. Equal volume of CHCl₃ was added to the filtrate. Few drop of 10% NH₃ was added to the mixture and heated. Formation of rose-pink colour indicates the presence of anthraquinones.

**Glycoside**
The extract was hydrolyzed with HCl solution and neutralized with NaOH solution. A few drop of Fehling's solution A and B were added. Red precipitate indicates the presence of glycoside.

**Reducing Sugars**
The extract was shaken with distilled water and filtered. The filtrate was boiled with drop of Fehling's solution for few minutes. An orange red precipitate indicates the presence of reducing sugar.

**Saponins**
About 0.2 g of the extract was shaken with 5 ml of distilled water and then heated to boil. Frothing (appearance of creamy mist of small bubbles) shows the presence of saponins.

**Flavonoids**
Extract of about 0.2 g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colourless, indicates the presence of flavonoids.

**Phlobatannins**
The extract (0.5 g) was dissolved in distilled water and filtered. The filtrate was boiled with 2%HCl solution. Red precipitated show the presence of phlobatannins.

**Terpenoids (Salkowski test)**
0.2 g of extract was mixed with 2 ml Chloroform (CHCl₃) and concentrated H₂SO₄ (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to indicate positive results for the presence of terpenoids.

**Cardiac glycosides**
Five ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underplayed with 1 ml of concentrated H₂SO₄. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brow ring, while in acetic acid layer, a greenish ring may form just gradually throughout thin layer.

**Results**
Phytochemical screening of ethanol extracts of Miliusa velutina Var. deviyarina leaf is shown in table 1. The susceptibility of test microorganism to the crude extracts of Miliusa velutina Var. deviyarina leaf is shown in table 2.

<table>
<thead>
<tr>
<th>Chemical Components</th>
<th>Ethanol Extract</th>
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<tbody>
<tr>
<td>Alkaloid</td>
<td>-</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>Anthraquin-ones</td>
<td>-</td>
</tr>
<tr>
<td>Glycoside</td>
<td>-</td>
</tr>
<tr>
<td>Reducing Sugars</td>
<td>-</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
</tr>
<tr>
<td>Phlobatannins</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>-</td>
</tr>
<tr>
<td>Cardiac- glycosides</td>
<td>-</td>
</tr>
</tbody>
</table>

(+ = Present, - = Absent)

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Microorganism</th>
<th>Aspergillus Niger</th>
<th>Gram+ve z (S-aureus)</th>
<th>Gram-ve (E-coli)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>09 mm</td>
<td>12 mm</td>
<td>10 mm</td>
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<table>
<thead>
<tr>
<th>Table 1. Phytochemical screening of extracts.</th>
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**Discussion**
The qualitative analysis of extracts from leaf of Miliusa velutina Var. deviyarina showed the presence of phytochemical constituents such as tannin and saponin in the ethanol extract and need further investigation to find out remaining bioactive constituents. The results are summarized in table 1 and 2. The above results indicate that, the ethanol extract of leaves of plant investigated are rich in saponins, tannins. Extracts of leaf were tested against Gram positive S-aureus and gram negative E-coli. Extracts also tested for antifungal activity against Aspergillus Niger and showed the inhibition of growth. Ethanol extract was found to be less sensitive against Gram positive s-aureus and gram negative E-coli (with zone of inhibition below 13 mm less sensitive) due to absence of remaining components in ethanol extract. Further studies are going on this plant in order to isolate, identify, characteristics and elucidate the structure of bioactive compounds and their activity.

**Conclusion**
The plant studied here can be seen as a potential source of useful drugs. Further studies are going on this plant in order to isolate, identify, characteristics and elucidate the structure of bioactive compounds.

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**References**


