PHYTOCHEMICAL SCREENING OF ETHANOL EXTRACTS OF NEW VARIETY OF MILIUSA VELUTINA LEAVES IN CHANDRAPUR FOREST REGION

Pravin S. Jogi

Department of Chemistry, Janata Mahavidyalaya, Chandrapur, Maharashtra, India

jogipravin@yahoo.in

ABSTRACT

Miliusa 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 *Miliusa 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, anticancer, 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, anthelmintic, antiparasitic and pesticidal agents [2]. Leaf oil obtained from different species of *Miliusa* is also reported and varying amount of constituents is present in it [3]. *Miliusa tomentosa* oil has been found to have both antibacterial [4] and analgesic properties [5]. From Maharashtra only two species of *Miliusa* (*M. tomentosa* and *M. velutina*) have been reported. Recently, new variety of *Miliusa* 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 *Miliusa*.

Materials and method

Plants collection: The present work was carried out at Department of chemistry, J.M.V. Chandrapur, Gondwana University. The plant named *Miliusa 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 *Miliusa 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 Dragencloffs 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 CHCl_3 was added to the filtrate. Few drop of 10% NH_3 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 mass 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 (CHCl_3) and concentrated H_2SO_4 (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 H_2SO_4 . 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 1. Phytochemical screening of extracts.

Chemical Components	Ethanol Extract
Alkaloid	-
Tannin	+
Anthraquin-ones	-
Glycoside	-
Reducing Sugars	-
Saponins	+
Flavonoids	-
Phlobatannins	-
Terpenoid	-
Cardiac- glycosides	-

(+ = Present, - = Absent)

Table 2. Antimicrobial sensitivity testing of ethanol extract.

Extracts	Microorganism		
	Aspergillus Niger	Gram+ve z (S-aureus)	Gram-ve (E-coli)
Ethanol	09 mm	12 mm	10 mm

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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