



PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *MILIUSA TOMENTOSA* (ROXB.) J. SINCLAIR BY USING VARIOUS ORGANIC SOLVENTS

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ABSTRACT

The present study was to evaluate the phytochemical compound from six different solvent of dried leaves and stem of *Miliusa tomentosa* belongs to family Annonaceae. Qualitative preliminary phytochemical analysis of this plant contains carbohydrate, protein, cardiac glycoside, glycosides, alkaloids, flavonoids, saponins, steroids, anthraquinones, tannins, quinines and inorganic compound. The presence of these phytochemicals can be correlated with medicinal potential of these plants.

KEYWORDS: *Miliusa tomentosa*, Phytochemical screening; Quantitative analysis.

INTRODUCTION

Higher and aromatics plants have traditionally been used in folk medicine as well as to extend the shelf life of foods, medicinal plant use for inhibition against bacteria, fungi and yeasts. Annonaceae is a pantropical family of shrubs, trees and lianas. The family consists of about 130 genera and 2300 species. Although the position of Annonaceae within the Angiosperms and order Magnoliales and its family circumscription is clear and undisputed.^[1] The plants belonging to family Annonaceae are used as antibacterial, anticancer, anthelmintic, antiparasitic and pesticidal agents.^[2] The genus *Miliusa* (Annonaceae family) consists about 40 species which grows in tropical rainforest of India, Thailand, South China and North Australia.^[3] The different species of *Miliusa* are invariably small to large trees and are found in a wide range of rainforest communities. Only three species of Genus *Miliusa* occur in Australia, which are endemic to there and contain two essential oils.^[4] The plant is used in folk medicine for different symptom such as gastropathy and glomerulonephropathy.^[5] In Chinese traditional medicine *Miliusa tomentosa* oil has been found to have both antibacterial and analgesic properties.^[6]

Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances.^[7] Two new isoquinoline alkaloids, 2,10- dimethoxy-3,11-dihydroxy-5,6-dihydroprotober -berine and 1,9-dihydroxy-2,11 -dimethoxy-4,5- dihydro-7-oxoaporphine, together with thirteen known alkaloids, were isolated from the ethanolic extracts of the stem and leaves of *M. cuneata* (Graib).^[8] Since *Miliusa tomentosa* (Roxb.) J Sinclair is

one of them, its traditional uses are not reported but its fruits are eaten in some parts of India and its tree yields a pale yellow gum known as karee gum.^[9] Thus, main objective of this research work is to consider the photochemical screening of the content which is present in different crude extracts.

MATERIAL AND METHODS

Collection of plant materials

Fresh plant parts of *Miliusa tomentosa* (Roxb.) J. Sinclair were collected from naturally growing populations located in Melghat region Maharashtra, India, during February-March 2016. The samples were identified at the herbarium section of the Department of Botany, Vidya Bharati Mahavidyalaya, Amravati. The fresh plant part samples were washed by tap water, air dried in shadow at room temperature milled well into a fine powder in a mixer grinder and stored until for further analysis.

Preparation of plant extracts in aqueous and different organic solvent

5 gm of dried powder was macerated with 100 ml of aqueous, petroleum ether, chloroform, toluene, butanol and benzene in a conical flask and shaken at room temperature for 24 hours and filtered through Whatman No.1 filter paper. The concentrated extracts were taken in colour amber bottles and kept in refrigerator for further analysis using the standard methods.^[10]

Calculation of Extraction Yield (% Yield)

The yield (% w/w) from dried extract was calculated as:
Yield (%) = $(W_1 \times 100) / W_2$

W_1 is the weight of the extract after lyophilization of solvent and W_2 is the weight of the powdered material.

Phytochemical screening

Phytochemical screening tests for the identification of protein, sugar, amino acid, alkaloids, flavonoids, steroids, cardiac glycosides, tannins, phenols, quinines & inorganic tests were carried out for all the extracts by the standard methods.^{[11][12]}

Tests for carbohydrates

Fehling's Test

1ml Fehling's A solution and 1 ml of Fehling's B solution were mixed and boiled for one minute. Now the equal volume of test solution was added to the above mixture. The solution was heated in boiling water bath for 5-10 minutes. First a yellow, the brick red precipitate was observed.

Benedict's reagent

Equal volumes of Benedict's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Solution appeared green showing the presence of reducing sugar.

Molisch's test

Equal volumes of Molisch's reagent and test solution were mixed in a test tube. The mixture was heated in boiling water bath for 5 minutes. Appearance of violet or purple colour ring showing the presence of reducing sugar.

Standard Test for combine sugar

In 1 ml extract 5 ml conc. HCl added and hydrolyzed by boiling and neutralized by adding NaOH and repeat the fehling test, Brick red ppt indicate result.

Keton's test

2 ml extract add with few crystal of resorcinol and equal volume of conc. HCl and heat over spirit lamp observed rose coloration indicate presence of keton.

Test for proteins

Burette Test

To the small quantity of extract 1-2 drops of burette reagent was added. Formation of violet colour precipitate showed presence of proteins.

Million's Test

To the small quantity of extract 1-2 drops of Million's reagent was added Formation of white colour precipitate showed presence of proteins.

Test for Xanthoprotein

In 2ml of test solution a few drops of conc. nitric acid and 3ml of ammonia were added appearance of red precipitate indicate the presence of xanthoprotien.

Tests for Anthroquinone glycosides

Borntrager's Test

To the 3 ml of extracts, dil. H_2SO_4 was added. The solution was then boiled and filtered. The filtrate was cooled and to it equal volume of benzene was added. The solution was shaken well and the organic layer was separated. Equal volume of dilute ammonia solution was added to the organic layer. The ammonia layer turned pink showing the presence of glycosides.

Tests for Cardiac glycosides

Keller-Killiani test

5ml of extract, 1ml of conc. H_2SO_4 , 2 ml of Glacial acetic acid and 1 drop of $FeCl_3$ solution was added. Appearance of brown ring shows the presence of cardiac glycosides.

Test for steroids

Salkowski test

To 2 ml of extract, 2ml of chloroform and 2ml of conc. H_2SO_4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Test for alkaloids

Hager's test

2-3 ml of filtrate, few drops of dil. HCl and Hager's reagent was added and shake well. Yellow precipitate was formed showing the presence of alkaloids.

Mayer's test

2-3 ml of filtrate, dil. HCl and Mayer's reagent was added and shake well. Yellow precipitate was formed showing the presence of alkaloids.

Dragendroff's test

2-3 ml of filtrate, few drops of dil. HCl and Dragendroff's reagent was added and shake well. Formation of orange-brown precipitate showed the presence of alkaloids.

Wagners test

2 ml extract add the reagent reddish brown ppt show indicates the presence of alkaloids.

Tests for flavonoids

Lead Acetate test

To the small quantity of extract lead acetate solution was added. Formation of yellow precipitate showed the presence of flavonoids.

Alkaline test

To the test solution add few drops of NaOH solution formation of intense yellow color which turn to colorless solution after addition of dil. Acetic acid indicate presence of flavonoids.

NaOH test

Small amount of extract was treated with NaOH and HCl observe formation of yellow Orange color.

H₂SO₄

Fraction of extract treated with Conc.H₂SO₄ and observe formation of orange color.

Test for Tannins and Phenolics compound**FeCl₃ solution Test**

On addition of 5% FeCl₃ solution to the extract, deep blue black colour appeared.

Lead Acetate test

On addition of lead acetate solution to the extract white ppt appeared.

Phlobotannins

2 ml extract add dil.HCl observe red ppt.

Gelatin test

To the test solution add few drops of 10% gelatin solution white ppt indicate the result.

Test for Saponin**Foam Test**

1 ml extract 20 ml distilled water was added and shakes well in measuring cylinder for 15 min. Then 1 cm layer of foam was formed.

Frothing test

3 ml extract with 10 ml D/W in test tube and plug the Stoppard and shake vigorously for 5 min. it allow to stand 30 min observe the honey comb forth indicate result.

Test for Glycosides

The extract was mixed with a little anthrons on a watch glass. One drop of conc. sulphuric acid was added and made into a paste and warmed gentle over the water bath. Dark green coloration indicates the presence of glycosides.

Test for Quinone

2 ml test solution was treated with a few drops of conc. H₂SO₄ or aq. NaOH solution. Colour formation indicates the presence of quinoid compound.

Test for fixed oil

A small quantity of powder was pressed between the filter paper. Formation of grease spot indicates the presence of fixed oil and fats.

RESULT

The preliminary phytochemical analysis of leaf and stem of *Miliusa tomentosa* in this study revealed the presence of glycosides, steroid, saponins, flavonoids, tannins, alkaloids, phenolic compounds, quinine, anthraquinone and inorganic test (Table-1 and Table-2). Using different solvent (aqueous, benzene, butanol, chloroform, toluene, and petroleum ether) extract. This study revealed the presence of glycosides maximum in benzene, butanol and petroleum ether, where as steroids were present in all solvents except water. Alkaloids presence maximum in water and chloroform. Flavonoids minimum presence in benzene, butanol, toluene and water while phenolic compounds found in butanol and water extracts only but tannins were absent in all extracts. Saponins were maximum present in chloroform and anthraquinone absent in all extracts while as inorganic tests showed positive results in chloroform extracts. In contrast to all this carbohydrate are maximum in butanol extract and protein in aqueous solvent extracts of leaf.

The phytochemical analysis of stem extracts show the presence of glycosides, saponins maximum in chloroform extract while alkaloids, phenol, inorganic test in butanol extract. Flavonoids and steroid recorded in petroleum extract only. Interestingly, tannin and anthraquinone absent in all extract (Table-1 and Table-2). Extractive values of leaf and stem extract by various solvents shown in (Table -3). Extractive values are varies according to solvents systems.

Table 1: Phytochemicals analysis in the stem extracted by various solvents

Sr.No	Phytochemicals	Test Name	Benzene	Butanol	Chloroform	Toluene	Petroleum Ether	Water
1	Carbohydrate	Fehling	-	-	+	-	+	-
		Benedict	-	-	-	-	-	+
		Molisch	-	-	-	-	-	-
		Combine Sugar	-	+	-	-	-	+
		Keton	+	+	-	-	-	+
2	Proteins	Biurett	-	-	-	-	-	-
		Millions	-	-	+	-	-	-
		Xanthoprotein	-	+	-	-	-	+
3	Glycosides	Cardiac	+	+	+	+	+	+
		Keller-Killani	-	-	+	-	-	-
5	Steroid	Salkowski test	-	-	-	+	+	+
		Lieberman-Buchnard	-	-	-	-	-	-
		H ₂ SO ₄	-	-	+	-	-	-
6	Alkaloids	Hager's	-	-	-	-	-	+
		Mayer's	-	+	+	+	-	-

		Dragendroff's	+	+	-	-	-	+
		Wagner's	+	-	-	+	+	+
7	Flavonoids 8	Lead acetate	+	+	+	+	+	+
		Alkaline	+	-	-	+	+	-
		NaOH	-	-	-	-	-	-
		H ₂ SO ₄	-	+	+	-	+	+
8	Phenolic	FeCl ₃	-	+	-	-	-	+
		Lead acetate	+	+	+	+	+	+
9	Tannin	Gelatin	-	-	-	-	-	-
		Phlobotannin	-	-	-	-	-	-
10	Saponin	Foam	-	-	+	+	+	+
		Frothing	-	-	+	-	-	+
11	Quinone	quinone	+	+	+	+	-	-
12	Anthraquinone	Anthraquinone	-	-	-	-	-	-
13	Inorganic	Sulphate test	+	+	+	+	-	-
		Carbonate test	+	+	+	+	+	-

(+) Present (-) Not detected

Table 2: Phytochemicals analysis in the Leaf extracted by various solvents

Sr.No	Phytochemicals	Test Name	Benzene	Butanol	Chloroform	Toluene	Petroleum Ether	Water
1	Carbohydrate	Fehling	+	+	+	-	+	+
		Benedict	+	+	+	-	+	+
		Molisch	-	-	-	-	-	-
		Combine Sugar	-	+	-	-	-	+
		Keton	-	+	-	-	-	+
		Biurett	-	-	-	-	-	-
2	Proteins	Millions	-	-	-	-	-	+
		Xanthoprotein	-	+	-	+	+	+
		Cardiac	+	+	-	-	+	+
3	Glycosides	KellerKillaiani	+	+	-	+	+	-
		Salkowski	+	+	+	+	+	-
5	Steroid	Lieberman-Buchnard	+	+	+	+	+	-
		H ₂ SO ₄	+	+	+	-	+	-
		Hager's	-	-	-	-	-	-
6	Alkaloids	Mayer's	-	-	+	-	-	-
		Dragendroff's	-	-	-	-	-	+
		Wagner's	+	+	+	+	+	+
		Lead acetate	+	+	+	+	+	+
7	Flavonoids	Alkaline	-	-	-	-	-	-
		NaOH	-	-	-	-	-	-
		H ₂ SO ₄	+	+	-	+	-	+
		FeCl ₃	-	+	-	-	-	+
8	Phenolic	Lead acetate	+	+	+	+	+	+
		Gelatin	-	-	-	-	-	-
9	Tannin	Phlobotannin	-	-	-	-	-	-
		Foam	+	-	+	-	-	+
10	Saponin	Fothing	-	-	+	-	-	-
		quinone	+	+	+	+	+	-
12	Anthraquinone	Anthraquinone	-	-	-	-	-	
13	Inorganic	Sulphate test	-	-	+	+	+	-
		carbonate test	-	+	+	-	+	-

(+) Present (-) Not detected

Table 3: Extractive values of leaf and stem extract by various solvents

Sr. No.	Solvent Name	Leaf extract colour	Extractive of Leaf	Stem extract colour	Extractive value of Stem
1	Benzene	Blackish green	0.13	Green	0.07
2	Butanol	Green	0.04	Light green	0.45
3	Chloroform	Dark Green	0.08	Light green	0.012
4	Toluene	Yellowish Green	0.09	Light green	0.01
5	Pet. Ether	Yellowish Green	0.07	Light green	0.11
6	Water	Orange	0.19	Red Orange	0.11

DISCUSSION

The phytochemical screening revealed the presence glycosides, steroids, alkaloids, flavonoids, phenolics, tannins, saponins, quinines, anthraquinones and inorganic compounds. The leaf extract detected the phytochemicals including Flavonoids, Steroids, Saponins and Alkaloids which have been supported by some other workers also.^[13] The crude extract of leaf and stem showed the absence of phytochemical constituents such as tannins and anthraquinones. Similar results have been found by other workers^[14] hence supporting present work. Alkaloids tend to be organic and natural ingredients that have nitrogen and are also physiologically active together with sedative and analgesic roles.^[15] Phenolic compounds are some of the most widespread molecules among plant secondary metabolites, are known to act as natural antioxidants.^[16] Cardiac glycosides are the compounds used to treat congestive heart failure and cardiac arrhythmia. These compounds work by inhibiting the Na⁺/K⁺ pump.^[17] Saponins are high molecular weight compounds in which a sugar molecule is combined with triterpene or steroid aglycon, so there are two major groups of saponins; triterpene saponins and steroid saponins. These are therapeutically important as they show hypolipidemic and anticancer activity of cardiac glycosides.^[18] Flavonoids enhance the effects of Vitamin C and function as antioxidants. They are also known to be biologically active against liver toxins, tumors, viruses and other microbes.^[19] The yield of extracts depending on the solvent and plant material used.^[20] Hence the preliminary phytochemical investigation are actually obliging in finding chemical ingredients in the plant that may help to their quantitative evaluation and also in locating the source of pharmacologically active principle.

CONCLUSION

The results of the phytochemical analysis showed the leaves and stem extracts indicate their potential as a source of bioactive principles that may supply drugs for modern medicines. Further studies are therefore required to validate their antimicrobial, antihyperglycemic, anti-inflammatory and anthelmintic activities. In addition, isolation, purification and characterization of the active chemicals are necessary to ensure that the plant has novel chemicals which will be helpful in future studies.

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