PHYTOCHEMICAL INVESTIGATION AND DETERMINATION OF TOTAL PHENOLS, FLAVONOID AND ALKALOIDS CONCENTRATION IN LEAVES EXTRACT OF *MILIUSA TOMENTOSA*

Abstract

Genus *Miliusa* (Annonaceae) comprises about 60 species and is widely native throughout India and Bhutan to Australia and New Guinea, but mostly found in many Asia countries such as Vietnam, Thailand and China. The increasing interest in powerful biological activity of secondary metabolites outlined the necessity of determining their contents in medicinal plants. *Miliusa tomentosa* (Roxb.) J. Sinclair (*M. tomentosa*, Annonaceae) commonly known as hoom, kari. It is a large deciduous tree, growing up to 20 m tall. Bark is blackish brown. Leaves are thick leathery, ovate, oblong, 4-10 cm long, 2-5.5 cm broad, smooth above, softly hairy below, base rounded, margin entire, tip pointed, leaf-stalk 2-5 mm. They are burnt and the smoke is allowed to pass over the body of lad after delivery to reduce body swelling. Fruits are given to children to cure the weakness in summer. The aim of the present study is to examine leaf of *M. tomentosa* for phytochemical profile. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenolics, flavonoids and alkaloids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent method and aluminium chloride method respectively. Phytochemical analysis revealed the presence of alkaloids, glycosides, flavonoids, phenols, proteins, carbohydrate and saponins. The present study concluded that the crude extract of *M. tomentosa* is a rich source of secondary phytoconstituents which impart significant antioxidant potential. The findings of the present study will be helpful to phytochemists, pharmacologists and pharmaceutical industries.

Keywords: *Miliusa tomentosa*, Annonaceae, Phytochemical, Folins ciocalteau reagent.

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Introduction

Drugs from the plants are easily available, less expensive, safe and efficient and rarely have side effects [1]. According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. Large sections of the population in developing countries still rely on traditional practitioners and herbal medicines for their primary care [2]. Medicinal plants are plants in which one or more of their organs contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs. WHO consultative group that formulated this definition stated also that, such a description makes it possible to distinguish between medicinal plants whose therapeutic properties and

constituents have been established scientifically and plants that are regarded as medicinal but which have not yet been subjected to a thorough scientific study [3]. Such plants should be investigated to better understand their properties, safety and efficacy. The medicinal properties of plants are due to some chemical constituents that produce certain pharmacological action on the humans. The qualitative analysis of phytochemicals of a medicinal plant is reported as vital step in any kind of medicinal plant research. Screening of plants constituents accurately can be done by employing chromatographic techniques [4]. Quantification usually employs the use of gravimetric and spectroscopic methods with several advanced approaches now available [5]. 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 [6]. The plants belonging to family Annonaceae are used as antibacterial, anticancer, anthelmintic, antiparasitic and pesticidal agents [7]. The genus *Miliusa* (Annonaceae) consist about 40 species which grows in tropical rainforest of India, Thailand, South China and North Australia [8].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 [9]. The plant is used in folk medicine for different symptom such as gastropathy and glomerulonephropathy[10]. In Chinese traditional medicine *M. tomentosa* oil has been found to have both antibacterial and analgesic properties [11]. Knowledge of the chemical constituents of plants is desirable because such information will be valuable for synthesis of complex chemical substances [12]. 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) [13]. Since *M. tomentosa* 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 [14]. Thus, main objective of this research work is to consider the photochemical screening of the content which is present in different crude extracts.

MATERIALS AND METHODS

Plant material

Leaves of *M. tomentosa* were collected from rural area in month of December 2019. The leaves plant sample were separated and washed with sterile distilled water to remove the adhering dust particles and other unwanted materials. The leaf was air dried under room

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temperature. The dried plant samples were cut and grinded to make it in powder form.The powdered samples were stored in clean, dry and sterile container for further use.

Chemical reagents

All the chemicals used in this study were obtained from HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals used in this study were of analytical grade.

Defatting of plant material

Powdered leaves of *M. tomentosa* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by soxhletion method

100 gram of powdered leaves of *M. tomentosa* was exhaustively extracted with hydroalcoholic solvent (ethanol: water: 80:20) by soxhletion method. The extract was evaporated above their boiling points. Finally, the percentage yields were calculated of the dried extracts [15].

Qualitative phytochemical analysis of plant extract

The *M. tomentosa* extracts obtained was subjected to the preliminary phytochemical analysis following standard methods by Khandelwal and Kokate [16, 17]. The extract was screened to identify the presence or absence of various active principles like phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, fats or fixed oils, protein and amino acid and tannins.

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso *et al* [18]. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso *et al* [18]. 1ml of 2% $AlCl_3$ methanolic solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

Total alkaloids determination

The plant extract (1mg) was dissolved in methanol, added 1ml of 2 N HCl and filtered [19]. This solution was transferred to a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4 ml chloroform by vigorous shaking and collected in a 10-ml volumetric flask and diluted to the volume with chloroform. A set of reference standard solutions of atropine (40, 60, 80, 100 and 120µg/ml) were prepared in the same manner as described earlier. The absorbance for test and standard solutions were determined against the reagent blank at 470 nm with an UV/Visible spectrophotometer. The total alkaloid content was expressed as mg of AE/100mg of extract.

Results and discussions

The crude extracts so obtained after each of the successive soxhletion extraction process were concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the leaves of the plants using petroleum eather and hydroalcoholic as solvents are depicted in the Table 1. The results of qualitative phytochemical analysis of the crude powder of leaf of *M. tomentosa* were shown in Table 2. Hydroalcoholic extracts of *M. tomentosa* showed the presence of alkaloids, glycosides, flavonoids, saponins, phenols, proteins, saponins and carbohydrate.Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.011X+0.011$, $R^2= 0.998$,** where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: **$Y=0.032X + 0.018$, $R^2=0.998$**, where X is the quercetin equivalent (QE) and Y is the absorbance. Total alkaloid content was calculated as atropine equivalent mg/100mg using the equation based on the calibration curve: **$Y=0.007X+ 0.024$, $R^2=0.995$**, where X is the Atropine equivalent (AE) and Y is the absorbance. The total phenolic,

flavonoids and alkaloid estimation of hydroalcoholic extracts of leaves of *M. tomentosa* showed the content values of 0.478, 1.057 and0.692 respectively Table 3.

Table 1: Results of percentage yield of leaf extracts

S. No.	Extract	% Yield (W/W)
1	Pet. ether	5.69
2	Hydroalcoholic	9.14

Table 2: Result of phytochemical screening of extracts of *M. tomentosa*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids** Hager's Test:	+ve
2.	**Glycosides** Legal's Test:	+ve
3.	**Flavonoids** Lead acetate Test:	+ve
4.	**Diterpenes** Copper acetate Test:	-ve
5.	**Phenol** Ferric Chloride Test:	+ve
6.	**Proteins** Xanthoproteic Test:	+ve
7.	**Carbohydrate** Fehling's Test:	+ve
8.	**Saponins** Froth Test:	+ve

Table 3: Estimation of total phenolic, flavonoids and alkaloid content of *M. tomentosa*

S. No.	Extract	Total phenolic content (mg/100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)	total alkaloid content (mg/ 100 mg of dried extract)
1	Hydroalcoholic	0.478	1.057	0.692

CONCLUSION

Qualitative and quantitative analysis of phenolics and flavonoids from leaves extract of *M. tomentosa* was achieved first time in this work. The observed level of phytoconstituents revealed that *M. tomentosa* is a rich source of antioxidant compounds. Currently available synthetic antioxidants are suspected to cause or prompt negative health effects, hence strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, the plant parts may be used as an alternative source for flavonoids and phenols for traditional remedies. Further phytochemical studies are also required to isolate and characterize active ingredients that are responsible for its

antioxidant and others activity and to explore the existence of synergism if any, among the compounds.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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