Journal of Pharmaceutical Research International

33(64B): 597-601, 2021; Article no.JPRI.85005
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Phytochemical Investigation and Determination of Total Phenols, Flavonoid and Alkaloid Concentration in Leaves Extract of *Miliusa tomentosa*

Nikhil Brijendra Kumar Shukla [a*], Neeraj Sharma [a] and Shailesh Jain [a]

[a] Madhyanchal Professional University (MPU), Educational Institute Bhopal, (M.P.), India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i64B35996

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/85005

Original Research Article

Received 25 October 2021
Accepted 27 December 2021
Published 30 December 2021

ABSTRACT

The genus Miliusa (Annonaceae) has over 60 species that are native to India, Bhutan, Australia, and New Guinea, but are mostly found in numerous Asian nations such as Vietnam, Thailand, and China. The growing interest in secondary metabolites' potent biological action highlighted the need of assessing their presence in therapeutic plants. *Miliusa tomentosa* (Roxb.) J. Sinclair (Annonaceae, *M. tomentosa*) is also known as hoom, kari. It is a huge deciduous tree that may reach a height of 20 metres. The bark is a dark brown. The leaves are thick leathery, ovate, oblong, 4-10 cm long, 2-5.5 cm broad, smooth above, gently hairy below, base rounded, border whole, tip pointy, and the leaf-stalk is 2-5 mm long. They are burned, and the smoke is allowed to travel over the baby's body after birth to minimise swelling. In the summer, children are fed fruits to help them recover from their frailty. The current study's goal is to look at the phytochemical profile of *M. tomentosa* leaf. The well-known test procedure available in the literature was used to determine the qualitative analysis of various phytochemical elements as well as the quantitative analysis of total phenolics, flavonoids, and alkaloids. The phenolic and flavonoids were quantified using the Folins Ciocalteau reagent technique and the aluminium chloride method, respectively. The presence of alkaloids, glycosides, flavonoids, phenols, proteins, carbohydrates, and saponins was discovered by phytochemical study. 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.

**Corresponding author: E-mail: shuklanikhil1990@gmail.com;*

Keywords: Miliusa tomentosa; annonaceae; phytochemical; folins ciocalteau reagent.

1. INTRODUCTION

They are burned, and the smoke is allowed to travel over the baby's body after birth to minimise swelling. In the summer, children are fed fruits to help them recover from their frailty. The current study's goal is to look at the phytochemical profile of M. tomentosa's leaf [1,2]. The well-known test procedure available in the literature was used to determine the qualitative analysis of various phytochemical elements as well as the quantitative analysis of total phenolics, flavonoids, and alkaloids. The phenolic and flavonoids were quantified using the Folins Ciocalteau reagent technique and the aluminium chloride method, respectively. The presence of alkaloids, glycosides, flavonoids, phenols, proteins, carbohydrates, and saponins was discovered by phytochemical study. The WHO consultative group that developed this definition also stated that such a description allows for the distinction between medicinal plants whose therapeutic properties and constituents have been scientifically established and plants that are regarded as medicinal but have not yet been subjected to a thorough scientific study [3]. Such plants should be studied in order to have a better understanding of their qualities, safety, and efficacy. Plants' therapeutic capabilities are attributable to chemical compounds that have pharmacological effects on humans. The qualitative examination of a medicinal plant's phytochemicals is regarded as an essential stage in any type of medicinal plant research. Chromatographic methods can be used to correctly screen plant components [4]. Gravimetric and spectroscopic techniques are often used for quantification, however various sophisticated methodologies are now accessible [5]. Annonaceae is a pantropical plant family that includes shrubs, trees, and lianas. There are around 130 genera and 2300 species in the family. Although Annonaceae's location within the Angiosperms and order Magnoliales, as well as its family circumscription, is unambiguous and undisputed [6]. Annonaceae plants are utilised as antibacterial, anticancer, anthelmintic, antiparasitic, and pesticidal agents [7]. The genus *Miliusa* (Annonaceae) has around 40 species that grow in the tropical rainforests of India, Thailand, South China, and North Australia [8]. Miliusa species range in size from tiny to huge trees and may be found in a variety of rainforest ecosystems. In Australia, only three species of the genus Miliusa exist, all of which are indigenous to the country and contain two essential oils [9]. The herb is used in traditional medicine to treat a variety of symptoms, including gastropathy and glomerulonephropathy [10]. *M. tomentosa* oil has been discovered to have antibacterial and analgesic effects in Chinese traditional medicine [11]. Knowledge of plant chemical ingredients is desirable since it will be useful in the production of complicated chemical molecules [12]. Two novel isoquinoline alkaloids, 2,10-dimethoxy-3,11-dihydroxy-5,6-dihydroprotober -berine and 1,9-dihydroxy-2,11-dimethoxy-4,5-dihydro-7-oxoaporphine, were recovered from ethanolic preparations of M. cuneata (Graib) stem and leaves, together with thirteen recognized alkaloids [13]. *M. tomentosa* is one of them, and while its traditional uses are unknown, its fruits are eaten in some parts of India, and its tree produces a pale yellow gum known as karee gum [14]. Thus, the primary goal of this research is to explore photochemical screening of the substance contained in various crude extracts.

2. MATERIALS AND METHODS

2.1 Plant Material

M. tomentosa leaves were gathered in a rural region in December 2019. To eliminate clinging dust particles and other undesired contaminants, the leaves plant sample was separated and washed with sterile distilled water. The leaf was dried by air at ambient temperature. The dried plant samples were chopped and ground into powder form. For future usage, the powdered samples were kept in a clean, dry, and sterile container.

2.2 Reagents for Chemistry

HiMedia Laboratories Pvt. Ltd. (Mumbai, India), Sigma Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem Ltd. (Mumbai, India), and SRL Pvt. Ltd. supplied all of the chemicals utilised in this investigation (Mumbai, India).

This study's substances were all of analytical grade.

2.3 Defatting of Plant Material

M. tomentosa powdered leaves were shade dried at room temperature. The shade dried plant material was coarsely pulverized and extracted

with petroleum ether by maceration. The extraction was maintained until the material had been defatted.

2.4 Soxhletion Extraction Technique

Soxhletion was used to extract 100 g of powdered *M. tomentosa* leaves using a hydroalcoholic solvent (ethanol: water: 80:20). The extract was evaporated at temperatures above their boiling points. Finally, the dried extracts' percentage yields were computed [15].

3. RESULTS

3.1 Qualitative Phytochemical Analysis of Plant Extract

The *M. tomentosa* extracts were submitted to preliminary phytochemical analysis using established procedures developed by Khandelwal and Kokate [16,17]. The extract was tested for the presence or absence of phenolic compounds, carbohydrates, flavonoids, glycosides, saponins, alkaloids, lipids or fixed oils, protein and amino acids, and tannins.

3.2 Total Phenol Analysis

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

3.3 Total Flavonoids Determination

Olufunmiso et al. [18] used the Olufunmiso technique to determine the total flavonoid content. 1 mL of 2% $AlCl_3$ methanolic solution was added to 3 mL of extract or standard and allowed to stand for 15 minutes at room temperature before measuring absorbance at 420 nm with a UV/visible spectrophotometer. The flavonoid content was determined using a typical quercetin graph, and the findings were represented as quercetin equivalent (mg/100mg).

3.4 Determination of Total Alkaloids

The plant extract (1mg) was dissolved in methanol, 1ml of 2 N HCl was added, and the mixture was filtered [19]. In a separating funnel, 5 ml of bromocresol green solution and 5 ml of phosphate buffer were added. The mixture was vigorously agitated with 1, 2, 3, and 4 mL of chloroform before being collected in a 10-mL volumetric flask and diluted to volume with chloroform. In the same manner as previously described, a series of atropine reference standard solutions (40, 60, 80, 100, and 120g/ml) were created. An UV/Visible spectrophotometer was used to measure the absorbance of test and standard solutions against the reagent blank at 470 nm. The total alkaloid content was given in milligrammes of AE per 100mg of extract.

4. DISCUSSIONS AND OUTCOMES

The crude extracts produced after each consecutive soxhletion extraction step were concentrated on a water bath by totally evaporating the solvents to get the real extraction yield. Table 1 shows the yield of extracts produced from plant leaves using petroleum ether and hydroalcoholic as solvents. Table 2 displays the findings of a qualitative phytochemical study of *M. tomentosa* leaf crude powder. *M. tomentosa* hydroalcoholic extracts contained alkaloids, glycosides, flavonoids, saponins, phenols, proteins, saponins, and carbohydrate. TPC was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the calibration curve equation: Y = 0.011X+0.011, R2= 0.998, where X is the gallic acid equivalent (GAE) and Y is the absorbance. Total flavonoids content was determined as quercetin equivalent (mg/100mg) using the calibration curve equation: Y=0.032X + 0.018, R2=0.998, where X represents the quercetin equivalent (QE) and Y represents the absorbance. The total alkaloid content was determined as atropine equivalent mg/100mg using the calibration curve equation: Y=0.007X+ 0.024, R2=0.995, where X is the Atropine equivalent (AE) and Y is the absorbance. Table 3 shows that the total phenolic, flavonoids, and alkaloid content of hydroalcoholic extracts of *M. tomentosa* leaves were 0.478, 1.057, and 0.692, respectively.

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

5. CONCLUSION

The qualitative and quantitative analysis of phenolics and flavonoids from *M. tomentosa* leaves extract was accomplished for the first time in this study. The amount of phytoconstituents examined suggested that *M. tomentosa* is a rich source of antioxidant chemicals. Because currently available synthetic antioxidants are suspected of causing or precipitating harmful health consequences, tight limitations on their usage have been imposed, and there is a tendency to replace them with naturally occurring antioxidants. Furthermore, the plant components might be employed as an alternate source of flavonoids and phenols in traditional medicines. More phytochemical research is needed to isolate and describe the active substances responsible for the antioxidant and other action, as well as to investigate the occurrence of synergism, if any, among the compounds.

DISCLAIMER

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.

CONSENT

It's not applicable.

ETHICAL APPROVAL

It's not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yadav RNS, Agarwala M. Phytochemical analysis of some medicinal plants. Journal of Phytology. 2011; 3(12):10-14.
2. WHO. Traditional medicine-growing needs and potentials. WHO Policy Perspectives Med; 2002.
3. WHO. Traditional Medicine and Modern Health Care Progress Report by the Direct General World Health Organization, Document No A-144110; 1991.
4. Sneader W, The discovery of aspirin: A reappraisal, BMJ (Clinical research ed. 2000;321(7276):1591-1594.
5. Aguoru CU, Ameh SJ, Olasan O, Comparative phyochemical studies on the presence and quantification of various bioactive compounds in the three major organs of okoho plant (cissus popunea guill & perr) in Benue State, North Central Nigeria, West Africa, European Journal of Advanced Research in Biological and Life Sciences. 2014; 2(2):16-22.
6. Qiu YL, Lee J, Bernasconi-Quadroni F, Soltis DE. Et al. Phylogeny of basal angiosperms: Analysis of five genes from three genomes" International Journal of Plant Science. 2000;161(6):3–27.
7. Jumana S, Hasan CM, Rashid MA. Antibacterial activity and cytotoxicity of *Miliusa velutina.* Fitoterapia. 2000; 71:559-561.
8. Sawasdee KT, Chaowas KU, Likhitwitayawuid K. New Neolignans and a Phenylpropanoid Glycoside from twigs of *Miliusa mollis*. Molecules. 2010;15:639-648.
9. Jessup LW. The genus *Miliusa Leschen*, ex A. DC. (Annonaceae) in Australia. Austrobaileya, 1988;2:517-523.
10. Kamperdick C, Van NH, Sung TV. Constituents from *Miliusa balansae* (Annonaceae) Phytochemistry. 2002;61: 991-994.
11. Huong DT, Van NTH, Kamperdick C, Anh NTH, Sung TV. Two New Bis-styryl Compounds from *Miliusa balansae.* ChemInform. 2008;10:1002.
12. Mojab F, Kamalinejad M, Ghaderi N, Vanidipour HR. Phytochemicals screening of some species of Iranian plants. Iran Journal of Pharmacy and Research. 2003;3:77-82.
13. Jessup LW. The genus *Miliusa Leschen*, ex A. DC. (Annonaceae) in Australia. Austrobaileya, 1988;2:517-523.
14. Anonymous. The Wealth of India (Raw material) Publication of Information Directorate. Council of Scientific and Industrial Research New Delhi. 1991:377-8.
15. Mukherjee PK. Quality Control of Herbal Drugs, 2nd Edition, Business Horizons. 2007;2-14.
16. Khandelwal KR. Ed. Practical Pharmacognosy Technique and Experiments, 23rd Edn. 2005;15.
17. Kokate CK. Ed. Practical Pharmacognosy, 4th Edn. Vallabh Prakashan. 1994;112:120.
18. Olufunmiso OO, Afolayan AJ. Phenolic Content and antioxidant property of the bark extract of *Ziziphus mucronata* wild. Subsp. Mucronata wild, BMC, Complementary and alternative medicine. 2011;11:130.
19. Fazel S, Hamidreza M, Rouhollah G, Mohammadreza V. Spectrophotometric determination of total alkaloids in some. Iranian medicinal plants. Thai J Pharm Sci. 2008;32:17-20.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/85005