Current Trends in Pharmacology and Clinical Trials
ISSN: 2642-0848

Academic Strive
Researcher's perception is our reality

Research Article *Volume 8 Issue 1*

Phytochemical Screening, Total Flavonoid and Total Phenolic Content and Antioxidant Activity of Different Parts of *Madhuca longifolia*

Khare P[1]* and Khare N[2]

[1]Department of Pharmacy, Shri Ram Murti Smarak, India

[2]Department of Pharmacy, M.J.P. Rohilkhand University, India

***Corresponding author:** Pragati Khare, Department of Pharmacy, Shri Ram Murti Smarak, C.E.T., Bareilly, UP, India, ORCID: 0000000292038561; Email: pragatikhare10@gmail.com

Received Date: December 23, 2024; **Published Date:** January 08, 2025

Abstract

Background: *Madhuca longifolia* has historically been used to treat various diseases in India but there is still limited study of different parts of this plant.

Objective: The purpose of this study was to evaluate the phytochemicals, the total flavonoid and total phenolic material, as well as the antioxidant activity of *Madhuca longifolia* ethanolic root, stem, leaf and seed extract.

Methods: Using 70 percent ethanol as the solvent for 2 h, each part of the plant was extracted by reflux and repeated 3 times. The total flavonoid content was determined by a colorimetric assay of aluminium chloride at 415 nm. The total phenolic content was calculated using a micro-plate reader with Folin-Ciocalteu 1:4 at 765 nm. Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenger technique, antioxidant activity was calculated.

Results: Phytochemical screening has shown that flavonoids and saponin are present in all samples. The total content of flavonoids was highest in leaves and lowest in roots, while the total content of phenols was highest in leaves and lowest in seed kernels. The crude extracts showed the highest value of DPPH free radical scavenging activity in leaf extract, followed by root, stem and seed kernel.

Conclusion: The 70 percent ethanol leaf extract of *Madhuca longifolia* showed the highest yield, total flavonoid content and total phenolic content. In addition, leaf extract has the highest activity of DPPH free radical scavenging (75.802 µg/ml), which may be correlated with its higher phenolic content.

Keywords: Mahua; Phytochemical Screening; DPPH; Total Flavonoid; Total Phenolic Content

Abbreviations

M.longifolia: *Madhuca longifolia*; GAE: Gallic Acid Equivalent; QE: Quercetin Equivalent; DPPH: 1,1-Diphenyl-2-Picrylhydrazyl; IC50: Concentration of Samples Resulting in 50% Inhibition.

Introduction

Madhuca longifolia belongs to family Sapotaceae and is also called Mahua [1]. The term Madhuca is derived from "Madhu" (Sanskrit word) which means honey. It is also known as Indian butter tree. Mahua is a deciduous and

Copyright © 2024 Khare P and Khare N.

medium-sized tree found in India, Nepal, and Srilanka [2]. All the parts of Mahua possess many medicinal qualities. Fruits - refrigerant, aphrodisiac, tonic, and antiulcer. Leaf - wound healing, anthelmintic, emollient, and rheumatism. Flower - refrigerant, liquor, increase milk production in woman, diuresis, antihelmenthic, hepatoprotective. Bark - tonsillitis, stomachache, antivenom in snake poisoning. Oil - laxative, hemorrhoids, piles [3].

It is composed of various phytoconstituents which include flavonoids, triterpenoids, glycosides, saponins, and steroids [4]. *M. longifolia* can produce about 20-200 kg of seeds/year [5].

Antioxidants could be used to control certain pathophysiological conditions involving free radicals, such as cardiovascular and neurodegenerative diseases [6]. DPPH scavenging activity was one in vitro method developed to assess antioxidant activity. In addition, this technique is rapid, does not require many steps and reagents, and compares with other techniques inexpensively [7].

Flavonoid and phenolic compounds can be useful as antioxidant from natural sources. The distribution of phenolic compounds varies between different parts of the plant [8]. The research was carried out to measure the phytochemicals and to determine the total flavonoid content, the total phenolic content and the antioxidant activity of the ethanolic extract of the root, stem, leaf and seed kernels.

Material and Methods

Chemicals

Sodium carbonate, Foline-Ciocalteu reagent, Gallic acid, ascorbic acid, quercetin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), aluminium chloride, ethanol and sodium acetate was purchased from Sigma Aldrich.

Plant Materials

The parts of *Madhuca longifolia* were collected from the College Campus of Shri Ram Murti Smarak (College of Engineering and Technology), Bareilly (Uttar Pradesh) and identified (specimen number- RU/PS/2016/415) by Prof. A.K. Jaitly, Head, Department of Plant Science, Mahatma Jyotiba Phule Rohilkhand University, Bareilly, Uttar Pradesh.

Preparation of Samples

The plant materials were gathered and washed, dried at room temperature, crushed into powder and placed in an airtight jar made of glass. Using 70 percent ethanol as the solvent for 2 h, 50 g of powdered samples were extracted by reflux and repeated 3 times. The extracts were then evaporated using a rotary vacuum evaporator and dried at 50°C in a water bath.

Determination of Percentage Yield (%)

The percentage yield of the extract was determined using the dry weight of extract (a) and soaked samples material (b) using the formula:

$$Percentage\ yield\ \ (\%) = \frac{a}{b} \times 100$$

The extraction yield was calculated for each extract.

Phytochemical Screening

The qualitative phytochemical test was conducted according to Bandiola [9]. Alkaloid test was performed with Bouchardat, Mayer and Dragendorff reagents; flavonoid test with Shinoda test; tannin test with gelatin test, gelatin-salt test, and test with ferrous (III) chloride; saponin test with froth test; quinone with Borntrager test; terpenoid/steroid test with Liebermann-Burchard reagent.

Determination of Total Flavonoid Content

Total flavonoid content was determined with minor modification by an aluminium chloride colorimetric assay adapted from Chatatikun, et at. [10] and Sandip, et al. [11]. In 96% ethanol, a standard solution of quercetin was prepared at a concentration of 30,40,50,60,70,80,90,100 µg/ml. 10 µl of 10 percent aluminium chloride solution was applied to 50 µl of extracts (1 mg/ml) or normal solution, followed by 150 µl of 96 percent ethanol. In a 96 well plate, 10 µl of 1 M sodium acetate was applied to the blend. As a blank reagent, 96 percent ethanol was used. All the reagents were combined and incubated at room temperature and shielded from light for 40 minutes. With a microplate reader, the absorbance was measured at 415 nm (Versamax Microplate Reader, USA). The total flavonoid content per g of plant extract was expressed as mg of Quercetin Equivalents (QE).

Determination of Total Phenolic Content

With some modifications, the microplate total phenolic content method was based on the 96-well microplate Folin-Ciocalteu method adapted from Ahmad, et al. [12]. Absolute diluted extract of 25 µL for each portion of *M.longifolia* was mixed and shaken for 60 seconds in a flat-bottom 96-well microplate with 100 µL of 1:4 diluted Folin-Ciocalteu reagent. The mixture was left for 240 seconds, then added 75 µL of sodium carbonate solution (100 g/L) and shaken for 1 min at medium continuous velocity. The absorbance was measured at 765 nm using the microplate reader after 2 h at room temperature (Versamax Absorbance Microplate Reader). The absorption of the same reaction was subtracted from the absorbance of the reaction with the sample with ethanol instead of the extract or normal. Dilutions of gallic acid (10–200 mg/L) were used as calibration criteria. Total phenolic contets were expressed as mg Gallic Acid Equivalents (GAE)

per g of plant extract.

Antioxidant activity

The DPPH scavenging assay was used to determine each extract's antioxidant activity. According to Zahratunnisa, et al. [13]. testing was performed with minor modification on a 96-well plate. In each well plate, 20 µL stock solution of extracts at various concentrations (100, 500, 1000, 1500, 2000 ppm) and 180 µL of 0.147 mM DPPH solution were added. After 30 minutes of room temperature incubation in a dark room, the absorbance was read at 517 nm using the Versamax Microplate Reader. Methanol was used as blank. The scavenging ability (%) was calculated as follows:

$$\%\text{inhibition} = \frac{\text{absorbtion of sandard} - \text{absorbtion of crude extract}}{\text{absorbtion of standard}} \times 100$$

Ascorbic acid was used as positive standard. All tests were performed in triplicate. Concentration of samples resulting in 50 percent inhibition on DPPH (IC50 value) were calculated (Figure 1).

Results

Percentage Yield of Extract

The results showed the highest yield was associated with leaves (33.8 percent), followed by seed kernel (28.26 percent), root (18.52 percent) and then stem (10.48 percent) as seen on Figure 2.

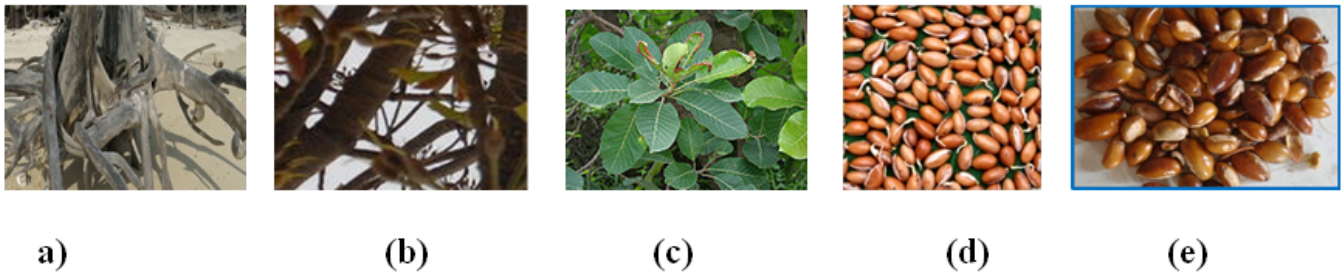

Figure 1: *Madhuca longifolia* (a) root; (b) stem; (c) leaf; (d) seed; (e) seed kernel.

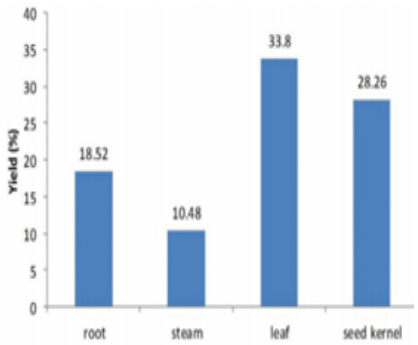

Figure 2: Percentage of yield.

Phytochemical screening

The extracts were further examined to establish phytochemical compound in the sample. The typical phytochemicals from plant such as flavonoid, alkaloid, terpenoid, steroid, tannin, and saponin have identified (Table 1).

Phytochemical Constituents	Alkaloids	Saponin	Flavonoid	Steroid	Triterpenoids	Quinone	Tannin
Root	-	+	+	-	+	-	+
Leaf	-	+	+	+	-	-	+
Stem	-	+	+	-	-	-	+
Seed kernel	-	+	+	+	+	-	+

Table 1: Phytochemical screening of the extracts.

Total Flavonoid Content

The result of the total flavonoid content is given in Table 2 of the four crude extracts. Quercetin normal calibration curve equation was y = 0.0291x-0.0397, R2 = 0.9904. Leaves contained the highest sum of total flavonoid compounds (32.05 ± 0.35mgQE/g) among the four crude extracts, followed by stem (24.82 ± 0.46), seed kernel (16.21 ± 1.35) and then root (15.55 ± 0.08 mgQE/g).

Sample Total	Flavonoid Content (mgQE/gram)
Root	15.55 ± 0.08
Stem	24.82 ± 0.46
Leaf	32.05 ± 0.35
Seed kernel	16.21 ± 1.35

Table 2: Total Flavonoid Content.

Total Phenolic Content

The consequence of the complete determination of phenolic material from 70% ethanolic extracts of different parts of *M.longifolia* in Table 3 is shown. The gallic acid calibration curve showed maximum absorbance at a wavelength of 765 nm (equation y = 0.0531x + 0.0003, R2 = 0.9951). Gallic acid equivalents have been recorded for the total phenol content of the four crude extracts determined by the Folin Ciocalteu process. Among the four crude extracts, the leaves contained the highest amount of total phenolic compounds (149.64 ± 3.94 mgGAE/g), followed by the stem (147.42 ± 16.05), the root (89.81 ± 3.00) and the seed kernel (73.34 ± 10.59 mgGAE/g).

Sample Total	Phenolic Content (mgGAE/gram)
Root	89.81 ± 3.00
Stem	147.42 ± 16.05
Leaf	149.64 ± 3.94
Seed kernel	73.34 ± 10.59

Table 3: Total Phenolic Content.

Antioxidant Activity

Table 4 demonstrates the capacity of the extract to scavenge the DPPH radical. The leaf is more capable than other components to scavenge free radicals.

Sample	IC50 (µg/mL)
Root	133.778
Stem	166.92
Leaf	75.802
Seed kernel	> 200
Ascorbic acid	2.28

Table 4: Antioxidant DPPH Scavenging Activity.

Discussion

The extraction was conducted using 70% ethanol as a solvent. This solvent is preferred for the extraction of phenolic compounds from plants, according to Jing, et al [14]. In order to maximise the phenolic and flavonoid material, the Reflux method was selected as an extraction method. To prevent any degradation of phenolic and flavonoid as a targeted compound, temperature not exceeding 70°C was maintained [15]. Different plant parts may have different phytochemical compounds, which could lead to different pharmacological effects of each component. Phytochemical screening showed that *Madhuca longifolia* root, stem, leaf and seed extracts contain flavonoid and saponin, and are devoid of alkaloid and quinone. Tannin, one of the polyphenols that can donate hydrogen and function as an antioxidant, was only found in the leaf [16]. The total flavonoid content was measured using the aluminium chloride process. Aluminum chloride can form a stable complex in flavonols and flavones with the carbonyl group at C4 and hydroxyls at C3 (flavonols) and C5. It could also form labile acid complexes with ortho-position hydroxyls in B rings of flavonoids [17]. In the present analysis, the total phenol content of four different crude extracts was evaluated. The leaf contained the highest quantity of phenolic compounds (149.64 mg of GAE/g of crude extract) and the lowest quantity of seed kernel extract (73.34 mg of GAE/g). The leaves also have the highest total flavonoid content (32.05 ± 0.35 mgQE/g) and the lowest root flavonoid content (15.55 ± 0.08 mgQE/g). By the delocalisation of the spare electron over the molecule, the molecule DPPH is a free radical. The electron's delocalization increases to a deep violet. In absorption, the amount of decreased DPPH was estimated at 517 nm [18]. In the current study, ascorbic acid was used as a well-known potent antioxidant as a positive control for the scavenging activity of DPPH. Compared to other plant components calculated at the lowest IC50 value, the leaf portion showed the highest potential to scavenge DPPH activity, but it has lower antioxidant capacity compared to ascorbic acid. The phenolic content of the leaf will contribute to the antioxidant effect through the ability to donate hydrogen. In this research, the antioxidant activity of the leaf and seed kernel of *Madhuca longifolia* was found to be different from that of the study in another country [19,20]. The difference could be caused by different phytogeographic regions and plant nutrition, which could alter the plant's secondary metabolites and due to different extraction methods and polarities of the solvents [21].

Conclusion

Compared to the other plant parts the 70% ethanol leaf extract of *Madhuca longifolia* showed the highest yield, total flavonoid content and total phenolic content. In addition, leaf extract has the highest activity of DPPH free radical scavenging (75.802 µg/mL), which may be correlated with

its higher phenolic content.

Acknowledgement

We are thankful to the Management of Department of Pharmacy, Shri Ram Murti Smarak, C.E.T., Bareilly, Uttar Pradesh, India, for providing chemicals and other infrastructure for doing this research work. The work is dedicated to my guide and coguide.

Conflict of Interest

No conflict of interest.

References

1. Dahake AP, Chakma CS, Chakma RC, Bagherwal P (2010) Antihyperglycemic activity of methanolic extract of *Madhuca longifolia* bark. Diabetologia Croat 39(1): 3-8.

2. Annalakshmi R, Uma R, Chandran GS, Sahayam CS, Charles A (2012) Evaluation of physico-chemical constants and phytochemical analysis of *Madhuca longifolia.* Int J Nat Prod Res 1(3): 64-66.

3. Akhil M, Sarma SK, Poornachandrarao GV, Jyothi CH, Kumar RD, et al. (2014) Evaluation of anthelminthic activity of leaves of *Madhuca longifolia*. Int J Pharmacol Toxicol 4(2): 99-104.

4. Kumar KP, Vidyasagar G, Ramakrishna D, Reddy IM, Gupta VS, et al. (2011) Screening of *Madhuca indica* for antidiabetic activity in streptozotocin and streptozotocin - Nicotinamide induced diabetic rats. Int J Pharmtech Res 3(2): 1073-1037.

5. Datta A, Pal A, Bandyopadhyay A (2016) A study on the effect of habitual consumption of *Madhuca longifolia* drinks on the prevalence of diabetes and dyslipidemia among Santhal tribals. Int J Basic Clin Pharmacol 5(3): 1108-1111.

6. Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Rev 4(8): 118-126.

7. Alam MN, Bristi NJ (2013) Review on in vivo and in vitro methods evaluation of antioxidant activity. Saudi Pharmaceutical Journal 21(2): 143-152.

8. Ghalem M, Merghache S, Belarbi M (2014) Study on the antioxidant activities of root extracts of Zizyphus lotus from the western region of Algeria. Pharmacognosy J 6(4): 32-42.

9. Bandiola TMB (2018) Extraction and Qualitative Phytochemical Screening of Medicinal Plants: A Brief Summary. Int J Pharm 8(1): 137-143.

10. Chatattikun M, Choabchalard A (2013) Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (Daucus carota Linn) root crude extracts. J Chem and Pharm Res 5(4): 97-102.

11. Sandip P, Shreewardhan R, Sandeephan M, Abhay C, Debjani D (2004) Phytochemical evaluation and free radical scavenging potential of Hugonia Mystax (L) Leaf Extract. Bionano Frontier 7(2).

12. Ahmad I, Yanuar A, Mulia K, Mun'im A (2017) Application of ionic liquid as a green solvent for polyphenolics content extraction of Peperomia Pellucida (L) Kunth Herb. J Young Pharm 9(4): 486-490.

13. Zahratunnisa N, Elya B, Noviani A (2017) Inhibition of alpha glucosidase and antioxidant test of stem bark extracts of Garcinia fruticosa Lauterb. Pharmacogn J 9(2): 273-275.

14. Jing L, Ma H, Fan P, Gao R, Jia Z (2015) Antioxidant potential, total phenolic and total flavonoid contents of Rhododendron anthopogonoides and its protective effect on hypoxia-induced injury in PC12 cells. Bio Med Central Complementary and Alternative Medicine 15(1): 287.

15. Ghasemzadeh A, Jaafar H (2014) Optimization of reflux conditions for total flavonoid and total phenolic extraction and enhanced antioxidant capacity in pandan (pandanus amaryllifolius roxb.) using response surface methodology. The Scientific World Journal pp: 1-10.

16. Zhang S, Lin Y, Zhou H, Wei S, Lin G, et al. (2010) Antioxidant tannins from stem bark and fine Root of Casuarina equisetifolia, Molecules 15(8): 5658-5670.

17. Chang CH, Yang MH, Wen HM, Chern JC (2002) Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10(3): 178-182.

18. Sharma A, Bhardwaj S, Mann AS, Jain A, Kharya MD (2007) PhCog Rev: Review article screening methods of antioxidant activity: An overview. Pharmacognosy Review 1(2): 1-7.

19. Mandal S, Hazra B, Sarkar R, Biswas S, Mandal N (2011) Assessment of the antioxidant and reactive oxygen species scavenging activity of methanolic extract of Caesalpinia crista Leaf. Evidence-Based Complementary and Alternative Medicine pp: 1-11.

20. Shukla S, Mehta A, John J, Singh S, Mehta P, et al. (2009)

Antioxidant activity and total phenolic content of ethanolic extract of Caesalpinia bonducella seed kernels. Food and Chemical Toxicology. 47(8): 1848-1851.

21. Zhang X, Zhao Y, Guo L, Qiu Z, Huang L, et al. (2017) Differences in chemical constituents of Artemisia annua L from different geographical regions in China. PLoS ONE 12(9): e0183047.