RESEARCH ARTICLE

Comparative Extraction and Quantification of Myricetin from Leaves of *Madhuca longifolia* Using RP-HPLC

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ABSTRACT

Background: This study's purpose was to develop a sensitive, repeatable reverse-phase high-performance liquid chromatography (RP-HPLC) method for extracting the main flavonoid Myricetin from the leaves of *Madhuca longifolia* and quantifying it.

Materials and Methods: Different leaves extracts were prepared using various solvent systems, including ethanol, ethanol: water (50:50), and water, through various extraction methodologies, including hot and cold maceration and soxhlet-assisted extraction, in order to optimize the best solvent system and ideal extraction methodology (SAE). Myricetin was separated by chromatography using a C_{18} column.

Results: According to the findings, the hydroalcoholic extract had the greatest content of myricetin at 2.524 ng. This study reports the presence of flavonoid content in *M. longifolia* leaves extract that can be extracted using, water:ethanol and ethanol. The best solvent system was water:ethanol, and the best extraction technique was cold maceration.

Conclusion: *M. longifolia* extracts were produced by hot and cold maceration, SAE techniques, and their flavonoid concentrations were measured using HPLC-ESI-MS/MS. Water: Cold maceration and ethanol were shown to be promising solvent systems. While trying to extract myricetin from *M. longifolia* leaves, the extraction approach was more effective than other traditional extraction methods.

Keywords: *Madhuca longifolia*, myricetin, maceration, soxhlet assisted extraction, RP-HPLC.

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INTRODUCTION

Extraction is a crucial step in investigations involving the discovery and isolation of active chemicals from plant materials. The process of making crude plant extracts is the first step in the separation and refinement of chemical components found in plant tissues. There has been an increase in public awareness of possible benefits of plant phytochemicals in preserving health in recent years. Cancer, cardiovascular disease, and age-related neurological illnesses are just a few of the serious health problems that have been connected to reactive oxygen species (ROS), which are harmful free radicals.[1]

The technique of choice for isolating and quantifying polyphenolics in plants is high-performance liquid chromatography (HPLC).[2] RP-HPLC techniques for polyphenols usage chromatographic conditions that utilize binary solvent system, Diode array detector (DAD) and almost solely an RP-C_{18} column make up a crucial, dependable approach for characterizing phenolic chemicals because of its accuracy and relative economic significance.[3] Yet, it is unlikely that a single method will be discovered to separate several polyphenols, even within a single extract, given the quantity of phenolics and variety of extraction solvents and techniques. Most of these techniques were created to assess several polyphenolic groups in single plant or one or two groups in various plant sources, many of which are non-food plants.[4]

RP-HPLC is a frequently used chromatographic method to determine secondary metabolites in plants. It has several uses in a variety of sectors, including the separation of active molecules and the evaluation of their quality and quantity.[5-13]

Myricetin (3, 3', 4', 5, 5', 7-Hexahydroxyflavone) is a member of the flavonoid class of polyphenolic compounds. It is also mentioned in *Madhuca longifolia* (mahua), a member of the Sapotaceae family and well-known as Honey tree and Butter-nut tree. Mahua's phytochemical analysis reveals that it contains a variety of nutrients, including sugar, vitamins, protein, alkaloids, glycosides, tannins, flavonoids, steroids, saponins, terpenoids and phenolic compounds. These compounds are responsible for a variety of pharmacological

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effects, including spasmolytic, anti-inflammatory, analgesic, antioxidant, anticonvulsant, antihyperglycemic, and hepatoprotective.[14] Given the significance of myricetin, it is necessary to improve the extraction yield and efficiency as key characteristics. In light of this, the present study effort was conducted using straightforward comparative conventional extraction procedures to increase extraction percent yield and concentration of pure myricetin.

The main objective of this research was to develop optimized procedure of Soxhlet assisted extraction (SAE), Hot maceration and cold maceration techniques for preparation of extracts from *M. longifolia* leaves and to compare these extraction techniques. Further, the quantification of bioactive compound myricetin was done using RP-HPLC technique. It is anticipated that these data will help find the optimal extraction method of flavonoid extraction from *M. longifolia* leaves.

MATERIALS AND METHODS

General

With a mixer grinder, the dried, verified leaves of *M. longifolia* were reduced to powder (Havells India Ltd., Delhi, India). Leaf powder was filtered in sieve shaker for 20 minutes (CIP Machineries, Ahmedabad, GJ, India) to choose a uniform particle size. The powder was put through sieves with varied mesh sizes to achieve this (12, 24, 45, 85, and 120 mesh, Swastika Electric and Scientific Works, Ambala, HR, India). The leaf powder that made it through 120 mesh sieves was gathered and put to use in additional extraction tests. From Sigma-Aldrich, recommended myricetin was purchased, purified to 98% by HPLC (St-Louis, MO, USA). All solvents were analytical grade (Finar Chemicals Ltd., Ahmedabad, GJ, India) and HPLC grade (MERCK Specialty Pvt. Ltd., Mumbai, MH, India), respectively, and were utilized for both extraction and the chromatographic purpose. The extraction procedures involved use of accelerated solvent extractor (E-914, Buchi India, Bombay, MH, India) and ultrasonic bath (Model: USB 6.5L (H), power: 230VPCi Analytics, Thane, MH, India). The extracts were newly made and kept in vacuum-sealed desiccators (Riviera Glass Pvt. Ltd., Mumbai, MH, India) until analysis.[15]

Cold Maceration

In the study of medicinal plants, maceration is a widely used traditional extraction method. The simplest and most straightforward extraction method is this one. It entails soaking powdered plant material in solvent-filled container with an appropriate stopper. A 60 g of dried powdered leaves were used in the maceration extraction procedure (n = 5), which was carried out for 24 hours while being occasionally stirred. After 24 hours, the combinations were further filtered, RP-HPLC determined their myricetin content, and they were then concentrated using a rotary vacuum evaporator.[16]

Hot Maceration

This is one of the main methods of the maceration process in which gentle heat was used for the extraction process. The temperature factor had a positive effect on the extraction yield and myricetin content. The goal of the maceration procedure is to soften and fracture the plant's cell wall in order to release soluble phytochemicals. 60 g of dried powdered leaves used in the hot maceration extraction method (n = 5) were macerated with water for 2 hours before being kept in a container for 24 hours. After 24 hours, the combinations were further filtered, RP-HPLC determined their myricetin content, and they were then concentrated using a rotary vacuum evaporator.

Soxhlet Assisted Extraction technique (SAE)

The *M. longifolia* leaves were treated with SAE to extract myricetin as much as possible. A Soxhlet apparatus was used to extract (n = 5) 60 g of powdered leaves using 300 mL of ethanol as the extraction solvent. The porous bag or thimble was created from filter paper and put in a thimble chamber (Borosil, Mumbai, MH, India). The round bottom flask is used to heat the extraction solvent, which vaporizes into the thimble chamber before condensing into the condenser. Seven hours were spent repeating the process. Condensed vapors transform into liquid as they approach the cooled siphon tube arm and drip back into the flask with a circular bottom. Following the end of the extraction procedure, round bottom flask's sample was collected, vacuum rotary evaporator-concentrated, and its myricetin concentration was calculated using RP-HPLC.[15]

Identification and Quantification of Myricetin in Extracts using HPLC

Aqueous, Hydroalcoholic and ethanolic leaves Extracts of different samples of *M. longifolia* by Hot maceration, Cold maceration and SAE were analyzed using HPLC at 370 nm. The C18, 250 x 4.6 mm, 5 um (X-Bridge, Waters) was used for the chromatographic separation, with an oven temperature of 37°C. The mobile phase was prepared up of acetonitrile and water (30:70, v/v), which were diluted to pH of 2.3 using 20 mM citric acid at a 1.0 mL/min flow rate for total run time of 20 minutes.[5]

RESULTS AND DISCUSSION

HPLC Analysis

Extraction of *M. longifolia* leaves using aqueous extract in hot maceration, hydroalcoholic extract (Water: Ethanol, 50:50%) in cold maceration and ethanolic extract in soxhlet assisted extraction (SAE) were performed. The standard linearity curve was plotted to determine the concentration of bioactive myricetin in *M. longifolia* leaves using HPLC. To perform the linearity, different calibration standards of myricetin viz, 2, 4, 6, 8, 10 and 12 ng/mL were made, and Figure 1 shows myricetin standard calibration curve made using a pre-calibrated HPLC process. From the standard curve of myricetin, regression equation y = 82726x + 2979.3 was obtained along with regression coefficient R^2 value 0.9992 indicating the plotted graph's linearity. HPLC fingerprint of standard myricetin was shown in Figure 2.

Maceration

The plant cell wall is softened and broken down using this fundamental approach, which releases the phytochemicals into

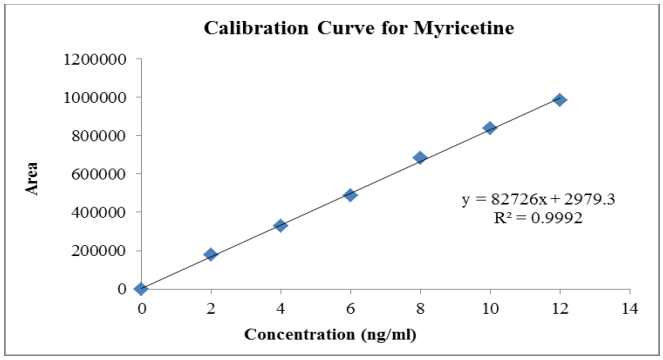

Figure 1: Calibration curve for myricetin

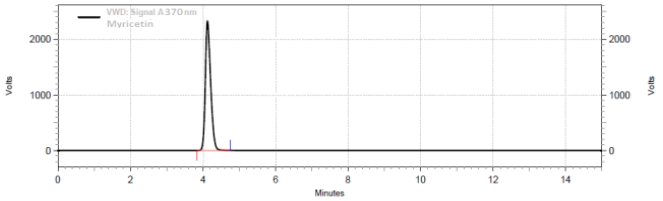

Figure 2: HPLC fingerprinting of standard myricetin

the extraction solvent. Convection and conduction are used to transport heat during CME, and the type of substance that is recovered from the plant material depends on the solvent used. Water and ethanol, two separate extraction solvents, were used in this study project's maceration extraction technique (50:50). The highest yield was obtained when *M. longifolia* was extracted using ethanol and water as the solvent. Ethanol:water was selected as the superior extraction solvent based on a percent extraction yield. In Table 1, the % yield for each solvent was calculated.

In comparison to other extracting solvents, the HPLC assessment of myricetin in *M. longifolia* employing ethanol: water as the extracting solvent revealed a greater quantity of myricetin. The concentration of aqueous and hydroalcoholic extract of myricetin obtained from hot and cold maceration was found to be 2.267 and 2.524 ng, respectively. HPLC fingerprint of aqueous and hydroalcoholic extract obtained was shown in Figures 3 and 4, respectively.

Soxhlet Assisted Extraction Technique (SAE)

Compared to the maceration process, this technique only needs a little solvent. With this procedure, a number of variables, including temperature and solvent sample ratio, must be taken into account. Using ethanol as the extraction solvent, soxhlet aided extraction of *M. longifolia* powder was carried out.

Table 1: Percent yield of extraction of *M. longifolia* by different extraction methods

Extraction Method	*Solvent system used for extraction*	*Mean% yield (%)*
Hot maceration	Aqueous (Water)	12.0 ± 0.21
Cold maceration	Hydroalcoholic (Water: Ethanol, 50:50 %)	19.4 ± 0.35
Soxhlet extraction	Alcoholic (Ethanol)	10.4 ± 0.28

*The extraction was performed separately in terms of batch (n = 5)

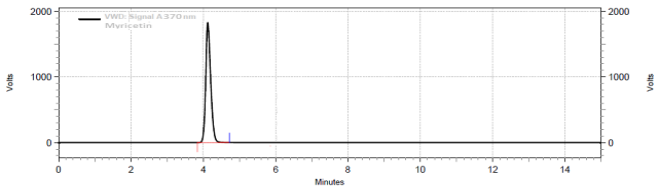

Figure 3: HPLC fingerprinting of myricetin by using hot maceration in aqueous phase.

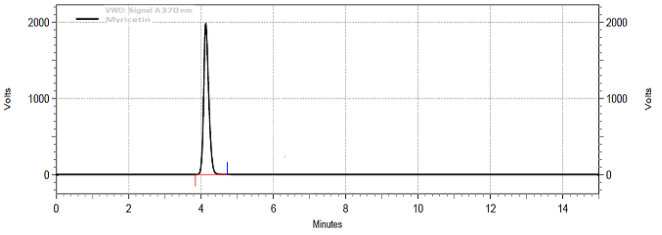

Figure 4: HPLC fingerprinting of myricetin by using cold maceration in ethanol: water (50:50)

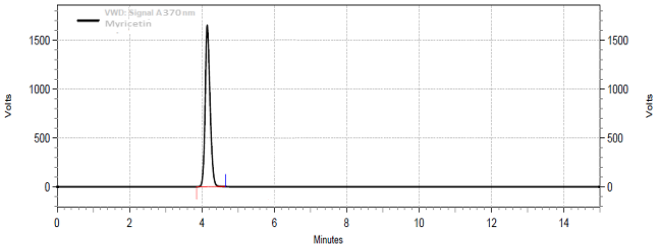

Figure 5: HPLC fingerprinting of myricetin by using SAE in ethanol

Table 2: Total concentration of myricetin obtained from various extraction methods.

Samples	*Retention time*	*Area*	*Mean concentration of myricetin (ng)*
Standard myricetin	4.100	465186	4.200
Myricetin by using hot maceration (Aqueous)	4.172	250789	2.267
Myricetin by using cold maceration (Hydroalcoholic 50:50)	4.139	283102	2.524
Myricetin by using SAE (ethanol)	4.156	226514	2.036

During ethanolic extraction, it was discovered that the overall yield was 10.4%. The concentration of ethanolic extract of myricetin obtained from SAE was 2.036 ng, and the HPLC fingerprint of ethanol extract obtained was shown in Figure 5.

From the HPLC analysis, the total Myricetin concentration obtained using hot and cold maceration, SAE was shown in Table 2 and it was observed that the extract obtained from the hydroalcoholic solvent system shows the highest amount of myricetin.

CONCLUSION

The extraction of myricetin from the leaves of *M. longifolia* was effectively improved using the soxhlet-assisted extraction (SAE), hot maceration, and cold maceration extraction

procedures. HPLC-ESI-MS/MS analysis was used to measure flavonoid and myricetin concentrations of extracts from *M. longifolia* that were produced using hot and cold maceration and SAE techniques. The concentration of standard myricetin was 4.2 ng. The maximum concentration of myricetin (2.524 ng) and extractive yield (19.4%) were found by using a hydroalcoholic solvent system and cold maceration technique. Thus, a solvent solution consisting of equal parts water and ethanol was found to be promising, and cold maceration extraction was shown more effective than hot maceration and SAE for the extraction of myricetin from leaves of *M. longifolia*.

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