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Journal of Current Pharma Research 4 (4), 2014, 1281-1285.

Journal of Current Pharma Research

http://www.jcpronline.in

Original Research Article

Development and Validation of UV Spectrophotometric Method for Estimation of Rutin in Extract of *Millingtonia hortensis Linn.*

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Abstract

The present study aims, to develop analytical method of isolated constituent methanolic extract of leaves of *Millingtonia hortensis linn*. Development of new methods for routine analysis of various chemical constituents present in plants is highly demanded by the industry. The extracts of *Millingtonia hortensis linn* were obtained by continuous heat extraction method by using soxhlet extraction process containing methanol as solvents. Extract was further used for phytochemical study. Phytochemical screening showed presence of flavonoids and triterpenoid. Flavonoid indicates presence of Rutin. Chemical constituents were isolated by using preparative thin layer chromatography by using various mobile phases. TLC, IR, GCMS analysis of methanolic and petroleum ether extracts shown new chemical constituents Rutin. Estimation of Rutin by U.V. spectrophotometer can be used as one of the appropriate analytical method for standardization of certain plants which contains the same marker. A simple and reproducible U.V. spectrophotometric method for determination of Rutin in methanolic extract of *Millingtonia hortensis linn* was developed and validated. Methanol was selected as a suitable solvent. The absorbance maximum was found to be 274 nm. The method obeys Beer's and Lambert law. The method was validated using parameters such as linearity, precision, accuracy, limit of detection, limit of quantification and recovery as per ICH guidelines. The proposed method can be used for the reliable quantification of active marker compound in Methanolic extract of *Millingtonia hortensis linn*.

Keywords: Rutin, *Millingtonia hortensis linn,* UV spectrophotometer, IR, GCMS.

Introduction

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. Cork tree is important medicinal plant in Southern Asia ranging from India, Burma, Thailand and South China. The name Millingtonia comes from Thomas Millington, an English botanist, while hortensis means "grown in gardens". It is also called the Cork Tree, as an inferior cork is processed from its corky bark. The stem bark is used traditionally as mainly lung tonic, anti asthmatic and antimicrobial.

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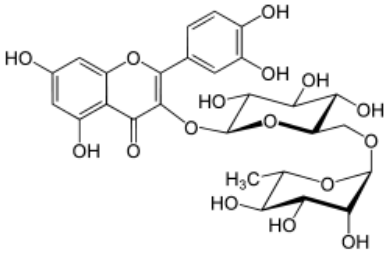
The leaves of *Millingtonia hortensis* are used as antipyretic, sinusitis, cholagogue and tonic in folklore medicine. Phytochemical evaluation is one of the tools for the quality assessment, which includes preliminary phytochemical screening, chemo profiling and marker compound analysis using modern analytical techniques



Structure of Rutin

Rutin is 5, 7, 3, 4, tetrahydroxy flavonol -3-rhamanoglucoside and widely used in

medicine for maintenance of capillary integrity. Both possess antioxidant activity and reduce low density lipoproteins [LDL] oxidation. The literature revealed that no UV-spectrophotometric method is not yet reported for the estimation of rutin in methanolic extract of Millingtonia Hortensis leaves. The method is developed in solvent Methanol. Method validation is done as per ICH guidelines. Thus, this method is more accurate and cost effective. This paper describes simple, rapid, accurate, precise and economical method for determination of rutin in *Millingtonia Hortensis leaves extract.*

Experimental

Materials

Rutin standard purchased from SDFCL, Mumbai. All chemicals and reagents were purchased from Merck, Mumbai.

Plant material

The leaves of Millingtonia Hortensis were collected from Kodoli in Kolhapur district and authentified from Krishna college botany department, Rethare.

Preparation of Extract [3]

The dried leaves were coarsely powdered and subjected to extraction by soxlet. The extraction was done with Methanol.

Phytochemical screening [4,5]

The phytochemical investigations revealed that triterpenoids and Flavonoids in methanolic extract.

Isolation of Rutin by Thin layer chromatography

TLC was performed on TLC plates (20×10cm). The optimized mobile phase was Ethyl acetate: Toluene: Ethanol: Formic acid: Glacial acetic acid.

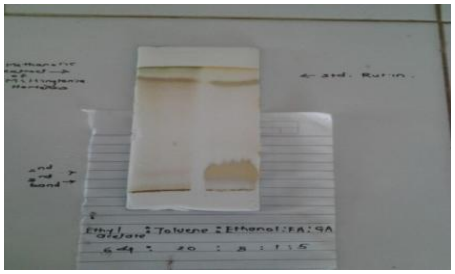


Fig. 1: TLC for isolation of Rutin in Methanolic extract.

Structural Elucidation Of Isolated Constituent [6 -9]

a) FTIR Spectroscopy

Infrared spectrum is an important record which gives sufficient information about the structure of a compound FTIR Spectroscopy was done by KBr pellet technique using Jasco 4100, FTIR spectrophotometer. Isolated constituent was pressed in KBr press at pressure 150 Kg/cm^2. Then it was scanned between 400-4000 cm^{-1}.

b) GCMS analysis of extracts

Isolated compound in extract of methanol were dissolved in respected solvents and GCMS analysis was done by GCMS Spectrometer (Shimadzu,Japan,QP 2010) at Shivaji university, Kolhapur (CFC centre).

Selection of solvent.

After assessing the solubility of drugs in different solvents Methanol has been selected as solvent for developing spectral characteristics

Selection of wavelength:

The stock solution of 1000 µg/ml of Rutin was scanned in the range of 200-400 nm and the λ max of Rutin of was found to 274 by using UV spectrophotometer Jasco V 630.

Validation of the developed methods

a) Linearity

Appropriate dilutions from the above stock solution were taken in 6 different 10mL volumetric flask and the volume was made up to mark with Methanol to get a concentration ranging from 100 - 700µg/mL. The absorbance of the resulting solutions was measured at 274nm against reagent blank. A standard calibration curve of was prepared by plotting absorbance Vs concentration and it was found to be linear over this concentration range to linear over this concentration range.

b) Recovery study

It was carried out by standard addition method at three different levels. Prepared the concentration of drug 50%, 100% and 150% and the concentration of sample solution i.e. methanolic extract 100%. Withdraw one ml from each concentration of drugs and mixed into two ml of sample solution and measured

the absorbance on U.V. spectrophotometer and calculated recovery and % RSD.

c) Precision

From prepared stock solution the intraday precision were determined by estimating the corresponding response 3 times on same day, where as interday precision were determined by estimating the corresponding response on three different days over a period of one week. The results were reported in terms of relative standard deviation (RSD)

d) Limit of Detection (LOD)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The detection limit is usually expressed as the concentration of analyte (percentage parts per million) in the sample.

LOD = 3 × SD / slope of calibration curve, SD = Standard deviation of intercepts

e) Limit of Quantitation (LOQ)

The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. Quantification limit is expressed as the concentration of analyte (e.g. - % ppm) in the sample**.**

LOD = 10 X SD / slope of calibration curve.

Where, (SD = Standard deviation of intercept).

Results and Discussion

Qualitative chemical investigation

The Methanol extract of *Millingtonia Hortensis linn* were subjected to qualitative chemical investigation and it shown presence of Triterpenoid, Flavonoid.

Structural Elucidation of isolated compound by IR & GCMS

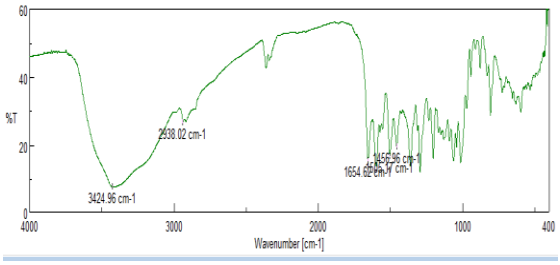


Fig 2: IR Spectra of isolated Rutin in methanolic extract.

Table 1: IR spectra.

Sr. no	Values(cm^{-1})	Description
1	3424.96	(OH) Stretching
2	2938.02	(CH) Stretching
3	1505	(C=C)
4	1456.96	(CH) Bend
5	1654.62	(C=O)

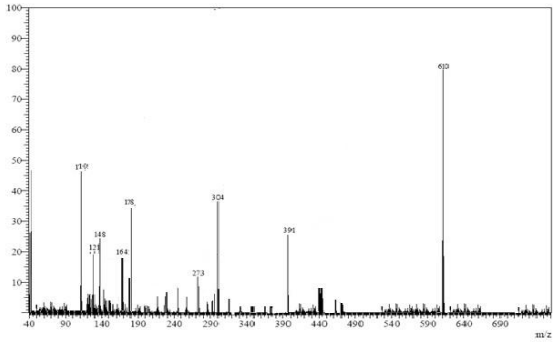


Fig. 3: MS of isolated rutin in Methanolic extract.

Structure of isolated compound

On the results of TLC profile, FTIR studies and GC-MS studies it confirms that isolated compound was “Rutin”

UV method development of Rutin

Selection of Analytical wavelength:

Stock solution of both the marker and isolated constituent was prepared in methanol. Rutin showed maximum absorbance at 274 nm.

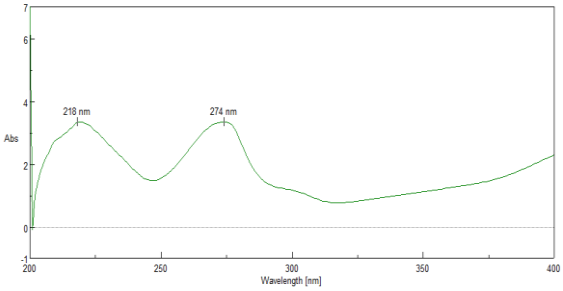


Fig 4: Spectra of Rutin.

Method Validation

a) Linearity study

Linearity was studied by preparing serial dilutions using standard stock solution as shown in dilution scheme .The linearity range for Rutin was found to be 100-700µg/ml

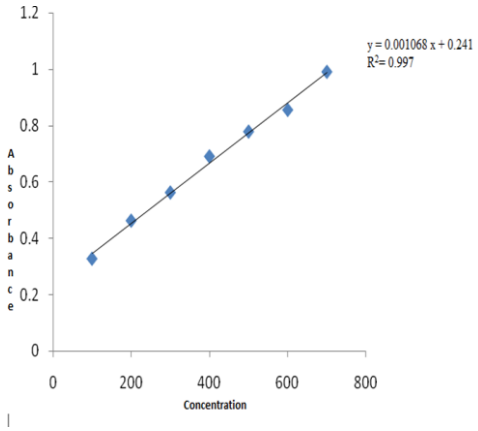


Fig 5: Plot of linearity curve for Rutin.

As graph showed linear curve it obeys Beers-Lamberts law. Rutin indicate good linearity between concentration and absorbance.

Table 3: Linear regression data for calibration curve of Rutin.

Parameters	Methods
Drugs	Rutin
Wavelength range (nm)	274 nm
Slope (m)	0.001068
Intercept (b)	0.241
Correlation coefficients(r^2)	0.997

b) LOD and LOQ

The LOD and LOQ were separately determined which is based on calibration curve.

Table 4: LOD and LOQ for Rutin.

Parameters	Rutin
LOD µg/ml	3.10
LOQ µg/ml	11.11

a) Precision

Precision is determined by studying the interday and intraday precision.

Table 5: Interday and Intraday precision for Rutin.

Interday Precision		Intraday precision	
%Amount	**%RSD**	**% Amount**	**%RSD**
81.20	0.6497±0.5223	82.01±0.6138	0.7475

d) Recovery

Table 6: Recovery for Rutin.

Amount of isolated comp. taken (%)	Amount of Standard drug added (%)	%Mean Recovery ± S.D(n=3)
100	50	84.86±0.191
100	100	86.50±0.278
100	150	84.87±0.504

The validity and reliability of proposed method are assessed by recovery studies. The results were obtained within range by standard addition method

Conclusion

The proposed spectrophotometric method is simple, rapid, accurate, precise, and economic and validated in terms of linearity, accuracy, precision, specificity and reproducibility. This method can be successfully used for estimation of Rutin in methanolic extract of Milllingtonia Hortensis linn..

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