Original Research Article **Analytical Chemistry**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

APPLICATION OF HPLC METHOD FOR SIMULTANEOUS ESTIMATION OF RUTIN AND MYRICETIN FROM MICRO AND NANO POWDERS OF LEAVES OF S*YZYGIUM JAMBOS* (L.) ALSTON.

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ABSTRACT

A simple RP-HPLC method has been used for simultaneous quantitative determination of two flavonoids viz. rutin and myricetin, from the micro and nano powders of leaves of *Syzygium jambos* (L.)Alston.Micro and nano powders of leaves of *Syzygium jambos* (L.)Alston. were prepared by using simple stepwise powdering method. Coarse grinding of the dried leaves of *Syzygium jambos* (L.)Alston.was done using ice jacketed domestic mixer. This powder was sieved through a BSS 85 mesh sieve and was designated as micro powder. Further fine grinding was done by jet milling, followed by ball milling. This powder was designated as nano powder. The amount of rutin and myricetin obtained using the acidified methanol extract of leaf nano powderof *Syzygium jambos* (L.)Alston. was found to be significantly higher ascompared to the amount of rutin and myricetin obtained using the acidified methanol extract of leaf micro powder of *Syzygium jambos* (L.)Alston.

KEYWORDS: Rutin, myricetin, nano powder, HPLC, Syzygium jambos (L.) Alston.

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Received on: 06-02-2017
Revised and Accepted on: 17-03-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.1.p184-190

INTRODUCTION

Syzygium jambos (L.) Alston. is naturalized or cultivated in many parts of the world. It is an evergreen tree from the family Myrtaceae, famous for its edible, juicy fruits. All parts of the plant viz. stem bark, leaves and seeds are reported to have medicinal properties.[1] The leaves are widely used in folk medicine for their digestive properties.[2] They are reported to possess anti-nociceptive, [3] anti-inflammatory,[4] anti-ulcer&anti-oxidant[5] and hepatoprotective activity.[6] During preliminary phytochemical analysis, flavonoids have been shown to be major leaf constituents.[7] Flavonoids are known to possess anti-inflammatory activities.[8] Rutin possesses a wide range of pharmacological properties that have been utilized in medicine and nutrition. It is used as an antimicrobial, antifungal, and anti-allergic agent. [9] It is reported to have antioxidant & anti-hyperglycemic[10]and anti-obesity activity. [11]The potential of rutin in mitigating radiation induced mortality is reported, which may be attributed to the elevation in the antioxidant status.[12] Myricetin has anti-oxidant, anti-depressant, anti-inflammatory and anti-diabetic activities. [13, 14] Studies have shown that myricetin inhibits cellular proliferation and induces apoptosis in tumour cells.It is reported to have potent anti-cancer and chemo preventive effects.[15, 16] Further, it is reported in literature that grinding the plant powder to very fine size enhances the content of phytoconstituents.[17, 18] Methods like jet milling and ball milling have been optimised and reported for grinding plant powders to very fine size.[19, 20] In the present work, a method for grinding dried leaf powder to nano size is developed. It is established that grinding the plant powder to nano size enhances the amount of phytoconstituents. Our group has earlier developed and validated HPLC methodfor simultaneous quantitation of rutin and myricetin from dried leaf powder of *Syzygium jambos* (L.) Alston.[21] In this work, the same method is applied for simultaneous quantitative determination of rutin and myricetin from the dried leaf micro and nano powders of *Syzygium jambos* (L.)Alston.

MATERIALS AND METHODS

Standards, Reagents and Chemicals

The reference standards rutin hydrate (purity ≥94.0% HPLC Grade) and myricetin (purity ≥96.0% HPLC Grade) were purchased from Sigma-Aldrich Chemie GmbH (Aldrich Division, Steinbeim, Germany). All the solvents used in the analysis were of HPLC grade. Methanol (purity- 99.7%),trifluoroacetic acid (purity-99.8%) and distilled water used were procured from LiChrosolv Merck, India. HCl pure (35-38%) was procured from LOBA Chemie, Mumbai, India.

Plant material

Mature leaves of *Syzygium jambos* (L.) Alston. were collected in the month of February, from a domestic garden in Alibaug, District Raigad, Maharashtra, India. The leaves were washed with water to remove soil particles, dried in shade, and finely ground using a specially fabricated domestic mixer with an outer ice bath. The leaf powder was sieved through a BSS 85 mesh sieve. This powder is designated as micro powder.The particle size was monitored using a Malvern Mastersizer 2000 analyser. The leaf powder was further ground to nano size, by passing it thrice through a jet mill, having a 4 inch chamber and two jets of air at pressure of around 8 kg per cm^2. The particle size obtainable in this jet mill was up to one micron. About 400 g jet milled leaf powder was ball milled. About 1600 grams of 3.2 mm chrome balls were used. Ball milling was done at 980 rpm for three cycles of forty five minutes each. Liquid nitrogen was used for cooling. This powder is designated as nano powder. The particle size was monitored using a Malvern Mastersizer 2000 analyser, and then using a Field Emission Scanning Electron Microscope.

Authentication

A herbarium of *Syzygium jambos* (L.) Alston. was prepared and authenticated from Botanical Survey of India, Pune, India. (Certificate No. BSI/WC/Tech/2012/70) Duplicate herbarium was prepared and preserved in Ramnarain Ruia College. The bulk density and tapped density of both the micro powder and nano powder were determined. Both micro powder and nano powder were stored in airtight containers at room temperature (28± 2°C).

Bulk Density measurement in a graduated cylinder

The bulk density of a powder is the ratio of the mass of an untapped powder sample and its volume including the contribution of inter particulate void volume.

Procedure

20 g each of micro and nano powders, weighed with 0.1% accuracy, were gently introduced, without compacting, into dry graduated cylinders of 100 mL. The powder was carefully leveled without compacting, and the unsettled apparent volume (V_0) was read to the nearest graduated unit. The bulk density in (g/mL) was calculated using the formula 20 g /V_0. Three replicate determinations were made.

Tapped Density measurement in a graduated cylinder

The tapped density is an increased bulk density attained after mechanically tapping a container containing the powder sample.

Procedure

About 20 g each of micro and nano powders, weighed with 0.1% accuracy, were gently introduced, without compacting, into dry graduated cylinders of 100 mL. The graduated measuring cylinder containing the powder sample was manually tapped about 20 or more times, until no further volume change was observed. The manual tapping was achieved by raising the cylinder and bringing it down on the working table with a thud. The settled apparent volume (V_f) was read to the nearest graduated unit. The tapped density in (g/mL) was calculated using the formula 20 g /V_f. Three replicate determinations were made.

Untapped volume V_0 mL
Tapped volume V_f mL

Preparation of solutions

Preparation of stock standard solution of rutin (1000.0 µg/mL)

About 51.50 mg of rutin hydrate equivalent to 50.0 mg of rutin was accurately weighed and transferred to 50.0 mL volumetric flask. 20.0 mL of methanol was added and the contents of the flask were sonicated in an ultrasonic bath (Model: TRANS-O-SONIC, Frequency: 50 Hz) for 10 minutes for complete dissolution of rutin. The contents were then diluted up to the mark with methanol to obtain stock solution of rutin with concentration of 1000.0 µg/mL. A series of standard solutions of rutin, in the concentration range of 1.0 µg/mL to 500.0 µg/mL were prepared from this stock standard solution and used for the determination of linear working range of rutin.

Preparation of stock standard solution of myricetin (1000.0µg/mL)

About 50.0 mg of myricetin was accurately weighed and transferred to 50.0 mL volumetric flask. 20.0 mL of methanol was added and the contents of the flask were sonicated for 10 minutes for complete dissolution of myricetin. The contents were then diluted up to the mark with methanol to obtain stock solution of myricetin with concentration of 1000.0 µg/mL. A series of standard solutions of myricetin, in the concentration range of 10.0 µg/mL to 800.0 µg/mL were prepared from this stock standard solution and used for the determination of linear working range of myricetin.

Preparation of sample solutions

About 1.0 g each of dried leaf micro and nano powder of *Syzygium jambos* (L.) Alston. was accurately weighed and transferred to separate 50.0 mL stoppered conical flasks. 9.9 mL of methanol and 0.1 mL of A.R. HCl were added to each flask. The flasks were kept in a hot water bath (50^0C) for 10 minutes and then cooled to room temperature (28 ± 2^0C). Further, sample solutions were filtered through Whatman filter paper no. 41. The filtrates were then finally filtered using 0.45 µm nylon filters (Millipore) before the analysis.

Preparation of mobile phase

The mobile phase used in the present research work for simultaneous quantitation of rutin and myricetin from dried leaf micro and nanopowder of *Syzygium jambos* (L.) Alston. was comprising of 0.3% trifluoroacetic acid in distilled water and methanol (55.0:45.0 v/v). The mobile phase was degassed in an ultra-sonic bath ((Model: TRANS-O-SONIC, Frequency: 50 Hz) for 10 min.

HPLC Conditions

For chromatographic separation a Shimadzu UFLC Prominence chromatograph was used which was equipped with binary gradient pump (LC-20AD), an auto sampler (SIL-20 AC HT), oven (CTO-20 AC) and a PDA detector (SPD-M20A). The chromatograms and data were recorded using LC solutions Software. Analysis was performed on a Spinco C18 G column (250 mm x 4.6 mm, 5 µm) kept at a temperature of 40^0C. The flow rate was adjusted to 1.0 mL/min, 10.0 µL of sample was injected into the chromatographic system and the detection was done at 254 nm. This HPLC method is validated by us earlier[21] and here it is applied for the simultaneous quantitative determination of rutin and myricetin from dried leaf micro powder and nano powder of *Syzygium jambos* (L.) Alston.

RESULTS

Table 1
Comparative results for density of dried micro and nano powder ofleaves of *Syzygium jambos* (L.) Alston.

Parameters	Micro powder Mean± S.D. (n=3)	Nano powder Mean± S.D. (n=3)
Bulk Density	0.302± 0.0025	0.332± 0.0031
Tapped Density	0.536± 0.0081	0.583± 0.010

Table 1 shows the change in density parameters by grinding to finer size.

Figures 1 and 2 represent Field Emission Scanning Electron Microscope images of *Syzygium jambos* (L.) Alston. dried leaf nano powder.

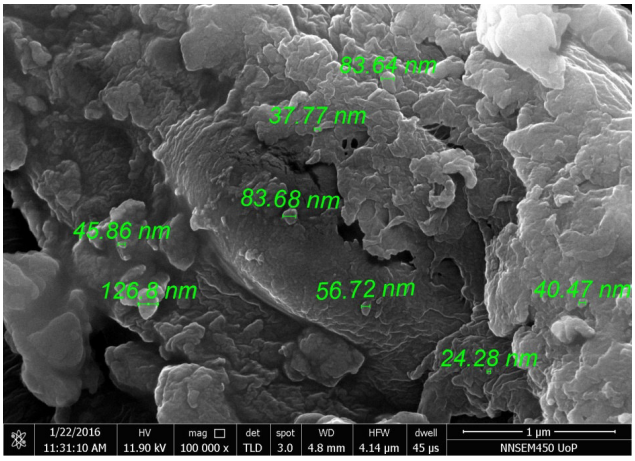

Figure 1
Field Emission Scanning Electron Microscope image

***Syzygium jambos* (L.) Alston. dried leaf nano powder**

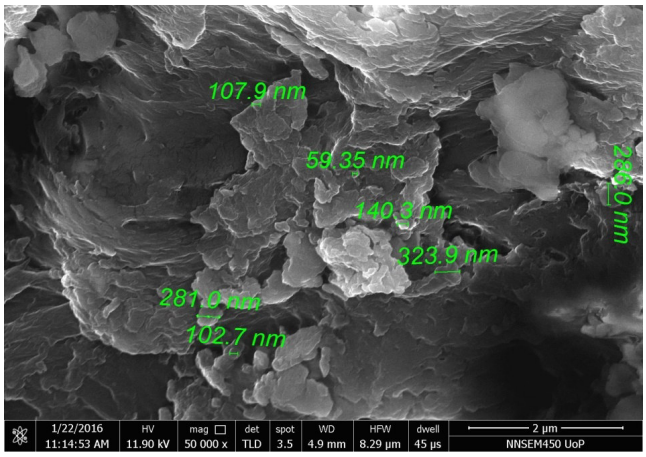

Figure 2
Field Emission Scanning Electron Microscope image
***Syzygium jambos* (L.) Alston. dried leaf nano powder**

HPLC Analysis
Linearity

Linearity of standard rutin was evaluated by injecting different concentrations in the range of 1.0 µg/mL to 500.0 µg/mL of rutin. Each solution was injected three times; the values of peak areas of rutin for each concentration were recorded and mean peak area was calculated. Similarly, linearity of standard myricetin was evaluated by injecting different concentrations in the range of 10.0 µg/mL to 800.0 µg/mL of myricetin. Each solution was injected three times; the values of peak areas of myricetin for each concentration were recorded and mean peak area was calculated. The calibration curves of rutin and myricetin were obtained by plotting graphs of mean peak area against corresponding concentration of both the standards. Figure 3 represents the calibration curve for rutin and Figure 4 represents the calibration curve for myricetin. Table 2 shows a consolidated regression data.

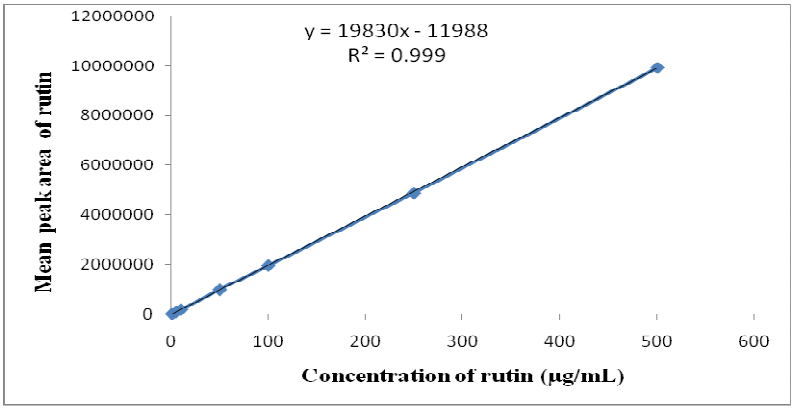

Figure 3
Graph of mean peak area vs. corresponding concentration of rutin

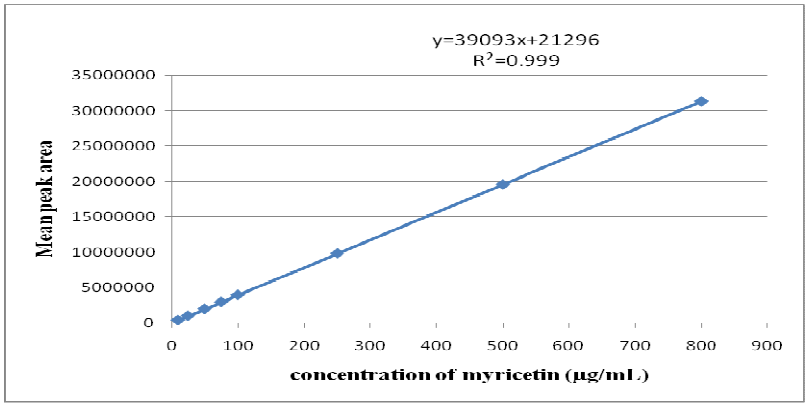

Figure 4
Graph of mean peak area vs. concentration of myricetin

Table 2
Results of the regression data for rutin and myricetin

Standards	Rutin	Myricetin
Slope (m)	19830	39093
Intercept (c)	-11988	21296
Correlation coefficient	0.999	0.999

Assay procedure

The validated HPLC method was used for simultaneous quantitation of rutin and myricetin from the methanolic extract of dried leaf micro and nano powder of *Syzygium jambos* (L.) Alston. 10.0 µL of methanolic extract of the dried leaf micro and nano powder of *Syzygium jambos* (L.) Alston.was injected separately into the chromatographic system under the specified conditions.

Figure 5 represents HPLC chromatogram obtained for a sample solution of the dried leaf micro powder of *Syzygium jambos* (L.) Alston. by injecting 10.0 µL into the chromatographic system under specified conditions.

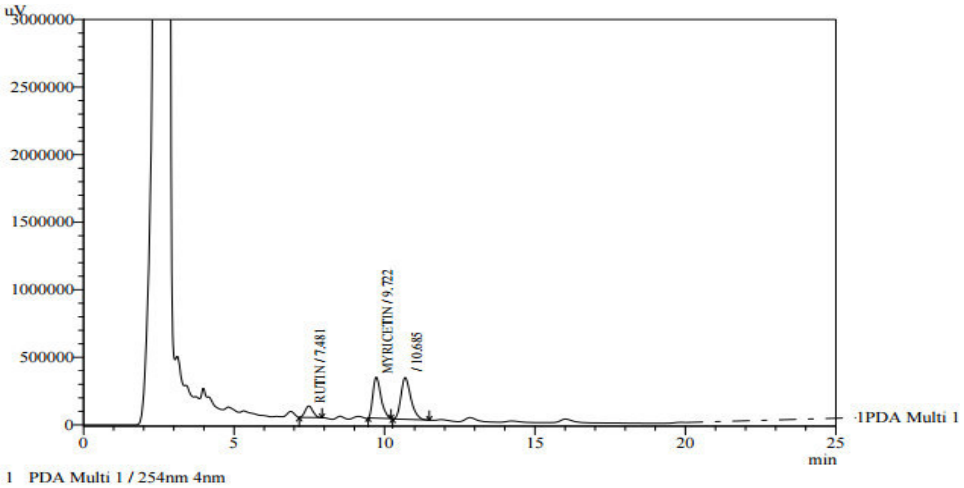

Figure 5
HPLC chromatogram of dried leaf micropowder of *Syzygium jambos* (L.) Alston.

Figure 6 represents HPLC chromatogram obtained for a sample solution of the dried leaf nano powder of *Syzygium jambos* (L.) Alston. by injecting 10.0 µL into the chromatographic system under specified conditions.

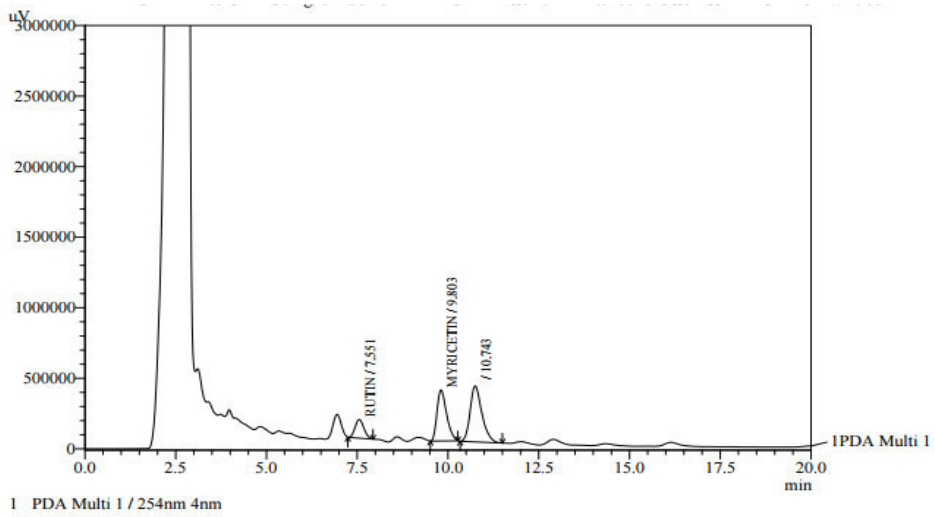

Figure 6
HPLC chromatogram of dried leaf nanopowder of *Syzygium jambos* (L.) Alston.

Amounts of rutin and myricetin present in the sample solutions were determined from the calibration curves, by using the peak area of rutin and myricetin in the sample solution. The results are presented in Table 3.

Table 3
Results for Assay

Sample	Assay (mg/g)	
	rutin	myricetin
Leaf micro powder	0.7648	2.2675
Leaf nano powder	1.1117	3.2268

DISCUSSION

There are some reports available in literature where plant powders were ground to finer size for enhancement in the extraction yields. Nano-powders of *Centella asiatica* (L.) were produced using planetary ball mill in order to investigate the differences of water extraction yield of asiatic acid as compared to micro powders of *Centella asiatica* (L.). Results showed that water extraction yield of asiatic acid using *Centella asiatica* (L.) nano powders was almost 50% higher as compared to the micro powders of *Centella asiatica*. (L.)[18] Effect of Superfine Grinding on physicochemical properties, antioxidant activity and total phenolic content of Red Rice (*Oryza sativa* L.) was studied. The smaller the size for red rice powders, greater was the bulk density. Antioxidant activity and total phenolic content were enhanced by superfine grinding.[19] Rutin and myricetin are important flavonoids present in the leaves of *Syzygium jambos* (L.) Alston. Their available quantities can be enhanced by grinding the leaf powder of *Syzygium jambos* (L.) Alston.to a very fine size. The mean amounts of rutin and myricetin found in the dried leaf micro powder of *Syzygium jambos* (L.) Alston. were 0.7648 mg/g and 2.2675 mg/g respectively.The mean amounts of rutin and myricetin found in the dried leaf nano powder of *Syzygium jambos* (L.) Alston.were1.1117mg/g and 3.2268mg/g respectively.

CONCLUSION

Simultaneous quantitative estimation of rutin and myricetin has been carried out from dried leaf micro powder and nano powder of *Syzygium jambos* (L.) Alston. A significant increase in the quantity of both the components is observed on grinding the powders to a very fine size.

Future Scope: grinding the plant powders to nano size for a significant enhancement in the amount of available phytoconstituents appears to be very useful for those phytoconstituents which are present in very small amounts and this grinding method can be tried for other plants as well.

CONFLICT OF INTEREST

Conflict of Interest declared none.

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We sincerely thank the above reviewers for peer reviewing the manuscript