WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 8, Issue 4, 1743-1750 Research Article ISSN 2278 – 4357

QUANTITATIVE DETERMINATION OF NIACIN IN AQUEOUS EXTRACT OF *OCIMUM SANCTUM* BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

C. T. Sadashiva*, H. M. Firoz Hussain, S. Nanjundaiah, R. Patil, R. Ashok, M. Sudheer, Y.D.Pavithra and P. Arunagiri

Star Hi Herbs Pvt Ltd, Plot No. 50, 3rd Road, 1st Phase, K. I. A. D. B. Industrial Area, Jigani Bengaluru - 560105, Karnataka, India.

Article Received on
15 Feb. 2019,

Revised on 08 March 2019,
Accepted on 29 March 2019

DOI: 10.20959/wjpps20194-13569

***Corresponding Author**
C.T. Sadashiva
Star Hi Herbs Pvt Ltd, Plot No. 50, 3rd Road, 1st Phase, K. I. A. D. B. Industrial Area, JiganiBengaluru - 560105, Karnataka, India.

ABSTRACT

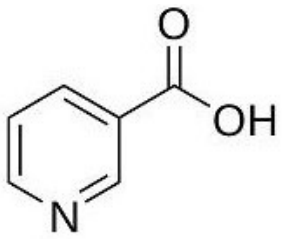
Niacin ($C_6H_5NO_2$), also known as nicotinic acid is one of the precious human nutrient and a form of B3 vitamin group available in herbs. The quantitative estimation of these organic acids in the solvent extract is very crucial and the methodology of the identification and quantification were distinctly different due to its various chemical properties. The present study was designed to evaluate the presence of niacin in the aqueous extract of *Ocimum sanctum*. L by reverse phase high performance liquid chromatography (RP-HPLC). RP-HPLC, a simple, sensitive and precise methodology has been developed, validated and used for quantitative determination of niacin from the aqueous extract of *O. sanctum*. L. The RP-HPLC was carried out followed by the aqueous extraction of *O. sanctum.* The C18 (250mm x 4.6mm, 5 µm) column and a mobile phase comprising of 0.05M Potassium Dihydrogen phosphate acetonitrile (65:35) which was obtained by trial and error method at a flow rate of 0.7 ml /min in an isocratic system. The detection of niacin was carried out at 240 nm. The results were concluded as the higher amount of niacin was present in the aqueous extract of *O. sanctum.* Further studies are warranted to exhibit the various bio-protective effects of *O. sanctum*

KEYWORDS: *Ocimum sanctum*, Niacin, chromatography, RP-HPLC, C18 column.

1. INTRODUCTION

Ocimum sanctum L. (Tulsi) belongs to family Lamiaceae. These plants are an erect, much branched, fragrant and attaining a height of about 30-60 cm when mature. The aromatic leaves of the plant are simple, opposite, elliptic, oblong, obtuse or acute with entire or sub serrate or dentate margins, growing up to 5 cm long. Traditionally, *O. sanctum* is taken in many forms, as herbal tea, dried power or fresh leaf. Many research groups of their recent investigations on these extracts used by various solvents have indicates anti-inflammatory, antioxidant and immune-modulatory and antistress properties.[1-4]

Niacin or nicotinic acid can serve as a wonder drug for correcting lipid metabolic disorders besides it functions as a B group vitamin. Moreover, niacin has been remarkable HDL cholesterol raising capability; lowers the bad cholesterol that is very low density lipoprotein (VLDL) and LDL as well as TG and fatty acids.[5] Preclinical studies has been reported that the niacin has decreased the levels of serum total cholesterol, serum triglycerides, serum low density lipoprotein cholesterol and serum total lipids and it increased the high density lipoprotein cholesterol in both the diabetic as well as non-diabetic rats.[6]



Chemical structure of Niacin

Dietary sources of niacin were much valuable. The availability of niacin is obtained in the diet from a variety of whole and processed foods, with highest contents in fortified packaged foods, tuna, some vegetable and other animal sources. Niacin, a form of vitamin B3, an essential human nutrient. a water-soluble vitamin, is an essential nutrient which is also known as vitamin B3. It exists as nicotinic acid and nicotinamide which have equal biological activity and can be synthesised from tryptophan. Niacin is directly or indirectly involved in many metabolic functions including the digestive system, skin, and nerves. It is also important for converting food to energy. Niacin supplementation has not been found useful for decreasing the risk of cardiovascular disease in those already on a statin, but appears to be effective in those not taking a statin.[7-8] Although niacin and nicotinamide are identical in

their vitamin activity, nicotinamide does not have the same pharmacological effects (lipid-modifying effects) as niacin.

2. MATERIALS AND METHODS

2.1. Plant material collection and processing

Ocimum sanctum leaves were collected and identified from highly equipped and reputed herbal manufacturers (Star Hi Herbs Pvt. Ltd, Jigani, Bangalore, Karnataka, India). Plant material was air dried at room temperature and powdered. The leaf powder (50gm) was extracted With distilled water at 60^{o} C for 6 hours. The resultant extract was filtered, concentrated to dryness under reduced pressure in a rotary evaporator (yield of extract 2.5g) and stored at 4°C until experiments.

2.2. Chemicals

Niacin was procured from Thomas Baker, Mumbai. Methanol (HPLC grade) was Fischer Chem Alerts Guide obtained from Fisher Chemicals Company (USA). All the other organic solvents used in the study were of analytical grade.

2.3. Preparation of standard solution

A stock solution was prepared by accurately weighing 10 mg of Niacin standard in a 25 ml volumetric flask and it was further diluted with HPLC grade methanol up to the mark.

2.4. Preparation of sample solution

250 mg of each aqueous extract was taken in 50 ml volumetric flask and the solution was made in methanol upto 50 ml. This solution was ready for the assay experiment. Same procedure was done for the rest of the sample extract.

2.5. High Performance Liquid Chromatographic profiling of *Ocimum sanctum*

Chromatography is a method of separating a mixture into its various components. The application of high performance liquid chromatography (HPLC) to biochemical samples is now widespread. HPLC is the most popular technique among all the analytical techniques used in quality control of plant products.[9-11] HPLC systems has many advantages like high resolving power, Qualitative and quantitative measurements and isolation of compounds, fast analysis, small amount of samples required, high sensitivity etc.[12-13]

The HPLC system binary gradient Shimadzu LC-2010 VP with a UV detector was used for determination of fingerprints aqueous extracts of *O. sanctum*. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250x4.6 mm internal diameter, particle size 5 µm, Luna 5 µm C-18(2); phenomenex, at 26 °C. Running conditions included: injection volume, 20 µL; mobile phase, 0.05M Potassium Dihydrogen phosphate acetonitrile (65:35); flow rate, 0.7 mL/min and the chromatogram monitored at 240 nm. Samples were filtered through an ultra membrane filter (pore size 0.25 µm; E-Merck, Darmstadt, Germany) and sonicated for 45 min before being used.

3. RESULTS AND DISCUSSION

3.1. HPLC fingerprinting of *Ocimum sanctum* extract

The RP-HPLC chromatographic methodology was successfully developed utilized the parameters as UV detection at 240 nm having methanol of HPLC grade as diluent that could resolve 9 peaks from aqueous extract of *Ocimum sanctum*. Further 0.05M Potassium Dihydrogen phosphate acetonitrile (65:35) in the mobile phase with the similar UV detection at 240 nm could resolve 3 & 2 peaks from aqueous extract of *Ocimum sanctum*. The availability of amount of niacin present in *Ocimum sanctum* as 0.041g/2.5g of extract was analyzed in the plant sample.

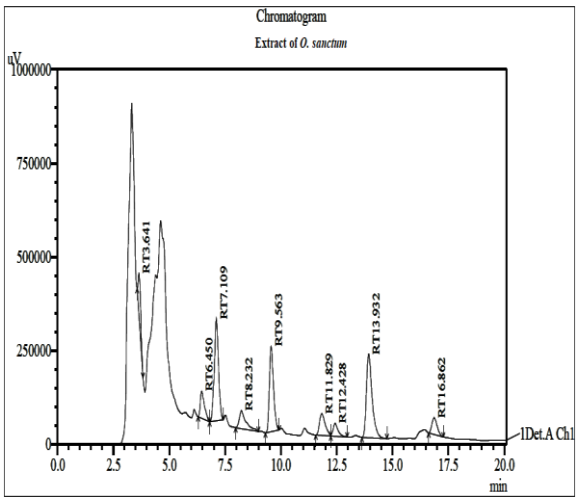


Fig 1. HPLC Chromatogram of *Ocimum sanctum* extract.

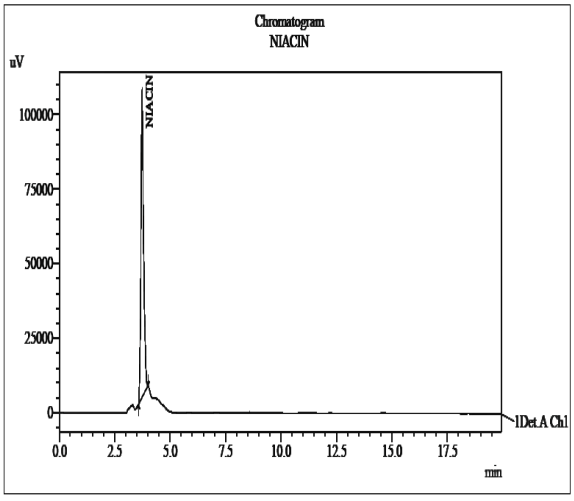
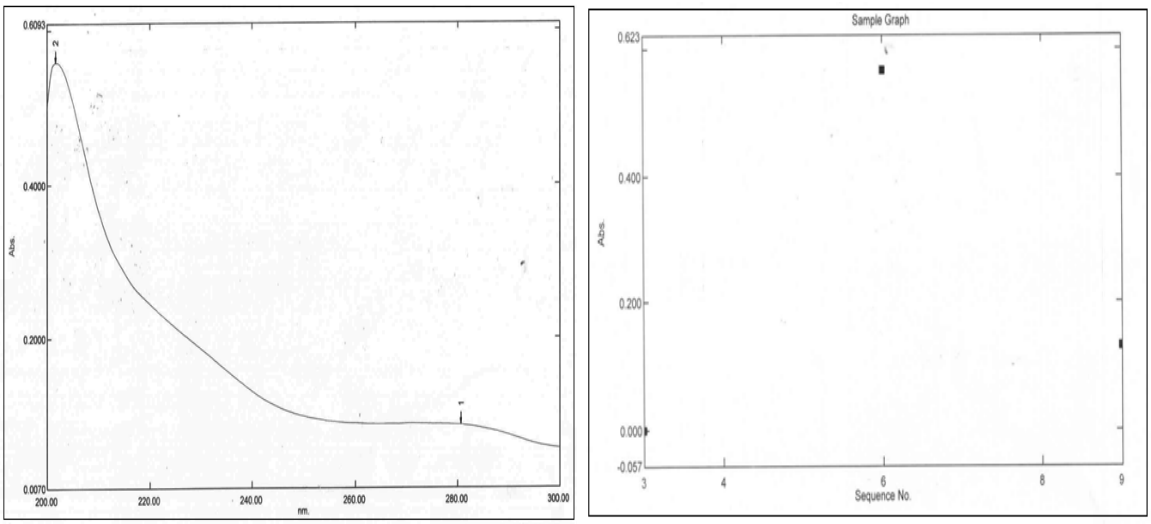
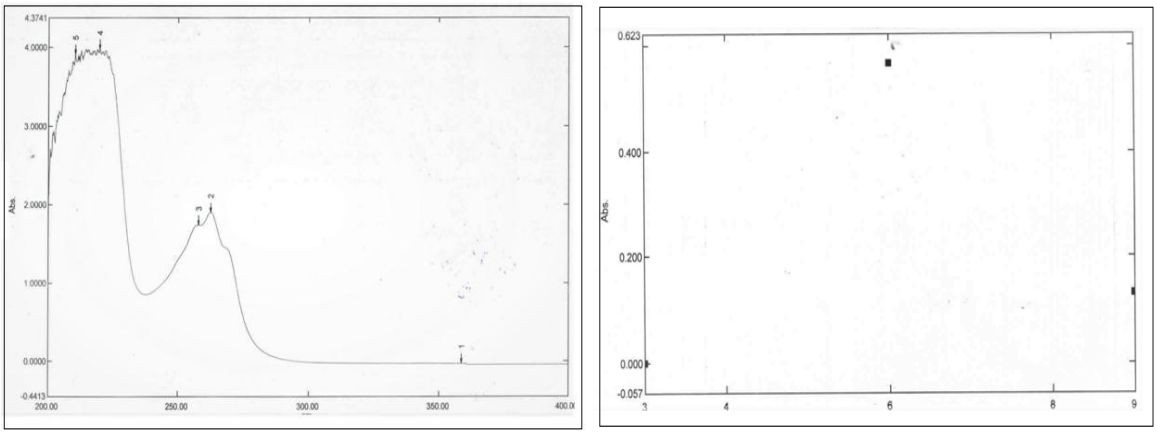


Fig.2. HPLC Chromatogram of Niacin standard.



UV spectrum of *Ocimum sanctum*



UV spectrum of standard Niacin

The medicinal properties of *O. sanctum* have been studied in hundreds of scientific studies including *in vitro*, animal and human experiments. These studies reveal that tulsi has a unique combination of actions that include: colds, headaches, stomach disorders, inflammation, chemopreventive, radioprotective, hepato-protective, neuro-protective, cardio-protective, anti-diabetic, various forms of poisoning and malaria.[14-17] Traditionally, *O. sanctum* L. is taken in many forms, as herbal tea, dried power or fresh leaf. For centuries, the dried leaves of Tulsi have been mixed with stored grains to repel insects.[18]

Previous studies has been reported that the presence of niacin content in the various natural products was negligible. amount of niacin presence in the various plant materials as follows, Portabella Mushrooms -7.6mg, Brown Rice-5.2mg, Peanuts (Dry Roasted)- 4.1mg, Avocados-3.5mg, Green Peas- 3.2mg, Sweet Potatoes-2.4mg, Basil herb Fresh leaves-0.902 mg[19] whereas the presence of niacin in the O. sanctum aqueous extract 0.041g/2.5g of

extract was reported in the present study. The current study was identified that the amount of vitamin B3 in the *O. sanctum* was significantly higher. In addition the mass spectrometry (MS) coupled with liquid chromatography (LC) was developed and quantified in various food materials as the presence of this water soluble vitamin B. However in the present study was accurately improved the RP-HPLC to quantify the exact amount of niacin presence in the *O. sanctum*. Methodology and the presence of the niacin in *O. sanctum*. Tulsi the raw material was taken as 50 g; the total yield of extract -2.5g assay Niacin-1.64% was measured as 1.64 x2.5/100=0.041g; 2.5g of extract contains 0.014g of niacin was analyzed in the present study by RP-HPLC method.

Jostrebova and his research team evaluated that the availability of the total folate content in the beetroot was marked by the method of HPLC[20] (Jostrebova et al., 2003). Nonetheless not being accurately quantified the niacin in the plant material. The development for the identification of niacin was very important due to its instability and complexity of the matrices with more time consuming process[21] Goldschmidt and Wolf 2007).

4. CONCLUSION

On the whole the present study concluded that a simple, precise RP-HPLC method has been developed for quantification of niacin from *O. sanctum*. The availability of niacin in the *O. sanctum* was significantly higher compared to the previous study report form the beetroot sample. Moreover current methodology more important and accurate for the further quantification of niacin in various plants sample. Since the present approach by RP-HPLC showed that good linearity, precision and accuracy to quantify niacin.

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