An Elsevier Indexed Journal ISSN-2230-7346

Journal of Global Trends in Pharmaceutical Sciences

STUDIES ON EXTRACTION OF RUTIN FROM PEEL OF *ALLIUMCEPA*

Anand Kumar Nelapati[1] and Surya Prakash DV[2]

[1]Ph.D Student, Department of Chemical Engineering, NITK, Surathkal, Mangalore-575025

[2]Assistant Professor, Department of Biotechnology, Meerut Institute of Engineering and Technology (MIET), Meerut-250005, UP, India.

[*]**Corresponding author E-mail**: prakashsurya55@gmail.com

ARTICLE INFO

Key Words

Allium cepa, Rutin, Extraction, Purification, Modelling

ABSTRACT

Rutin is mainly in peel and flower of onion. Mostly quercetin is highly contained in root, leaf, peel, flower and tunicated bulb of the onion. Mainly these bioflavonoids are play antioxidant role characters and commonly found in all parts of the onion. These show various pharmacological activities. The rutin extraction was done to study the physicochemical parameters and their optimization. 30.5µg/ml was the maximum optimized concentration. In the soxhlet extraction process, the extraction of rutin concentration was 42.0µg/ml obtained at 4hr. In Column chromatography, there was an increment in the concentration up to 48.0µg/ml. The purity of rutin was improved by column chromatography at 30 min. In the liquid-liquid extraction process, the partition coefficient of rutin was found to be 3.57 and it was carried out using methanol (raffinate phase) and toluene (extract phase) solvents. For this, a mathematical formula was used and verified using different times of extraction. The determined equation at the end is E(t) = 0.134t + 9.766 for rutin.

INTRODUCTION

The onion is a vegetable and is cultivated worldwide. It has different names like bulb onion or common onion. It is the species of the genus *Allium*. *Allium cepa* is the scientific name and belongs to Amaryllidaceae. It contains various phytochemical content, particularly for polyphenols. It has the highest total flavonoid content like quercetin, rutin, kaempferol, myricetin, isorhamnetin and anthocyanin pigments. Rutin is mainly in peel and flower of onion [1]. Mostly these bioflavonoids are plays an antioxidant role characters and commonly found in all parts of the onion. Various activities like anti-inflammatory, anticancer, antimicrobial activities etc has been shown by these. Rutin is a glycoside combining the quercetin flavonoid and phenolic compound. It is commonly found in all varieties of plants. Especially Citrus fruits contain the highest rutin content so it is also called citrus flavonoid. Rutin belongs to the flavonoid's family, and its IUPAC name is 3,3',4',5,7-pentahydroxyflavone-3-rhamnoglucoside.

Rutin supplements have also been promoted for the treatment of a wide spectrum of diseases. The aluminum chloride colorimetric assay method is the common method used to detect the rutin [2]. Determination of rutin and other flavonoids in extracts from plants, foods and beverages HPLC is the most commonly used [3]. In this work, rutin is extracted from the peel of *Allium cepa*. In this project work, we study on extraction of rutin from the peel of *Allium cepa*.

II. MATERIALS AND METHODS

I. Chemicals : Methanol, Ethanol, Chloroform, Toluene, Silica gel, Aluminum chloride, and Potassium acetate were bought from Loba Chemie private limited, Mumbai, India. All other chemicals used were of analytical grade.

II. Collection of plant material: The local market of Meerut was chosen to collect the Onion peel. Then the peel was thoroughly cleaned and dried. After drying the peel, it was powdered and then the airtight container was used to store it as a raw material.

III. Various Extraction Processes:

i. Preparation of Rutin extract from the peel of *Allium cepa*

In a clean flask, 1 gm powder was added in 25 ml ethanol and stirred carefully. The solution was left undisturbed for 1 day for complete soaking. Next day using Whatman No.1 filter paper the soak extract was filtered. The filtrate obtained is heated at 65^{o}C[4]. The solvent in the glass gets evaporated and the extract is stored as a sample.

Determination of Rutin flavonoid: In a clean test tube, 0.5ml methanolic extract was taken and 0.5ml of 2% aluminum chloride($AlCl_3$) was added and solution dissolved in ethanol. The mixture was left undisturbed to stand for 30 minutes at room temperature. The reaction mixture absorption was thereafter measured at 420nm using a spectrophotometer. The concentration of rutin in the sample was calculated using the calibration curve [5].

ii. Extraction of Rutin from the peel of *Allium cepa* by Soxhlet Extractor

8 grams of the peel of onion powder were placed in thimble along with 200ml of ethanol in a round bottom flask and fixed to the condenser. The whole instrument was kept on the heater mantle. The filtrate solution was continuously heated at 65^{o}C for 4 hours using the Soxhlet apparatus. Finally, the solvent was evaporated in the glassware and only the sample extract was left behind. 0.5ml of methanolic extract and 0.5 ml of 2% aluminum chloride ($Alcl_3$) solution was dissolved in ethanol in a test tube. At room temperature, the mixture was left for 30 min [6]. The absorbance at 420 nm using a spectrophotometer was recorded of the reaction mixture. The determination of rutin was done by using calibration curve.

iii. Liquid-Liquid Extraction and Modelling Studies

In this process, a Soxhlet extract sample (50 ml of methanolic concentrate) was filtered with toluene (50 ml) in the ratio of 1:1 using separated funnel. Every 30 min, 0.5ml of extract and raffinate phase sample taken in a test tube and add 2% aluminum chloride solution (0.5ml) dissolved in ethanol. Mixture left undisturbed to stand for 30 minutes at room temperature. The absorbance at 420 nm using a spectrophotometer of the mixture was observed. The rutin was determined by using calibration curve. Rutin transfer from the Raffinate phase (soxhlet ethanolic extract from the peel of onion) to the Extract phase (non-polar solvent – toluene) was explained using the coming theory. The mass transfer coefficient remains constant [7]. The raffinate phase solvents and rutin concentration in the onion extract depending on time. The transfer of rutin is time-independent and a diffusion process. An equation can be formed with the help of this hypothesis.

$$Es = A(t) + B \text{ for Rutin}$$

Here, A&B are constants, Es = Extraction yield (rutin in µg/ml) and t = time per extraction in minutes

IV. Purification of Rutin: Silica gel was used in column chromatography for the stationary phase. A piece of wire cotton was used to add a plug of cotton to the bottom of the column. The whole apparatus was tightly clamped. The silica gel powder was filled until 20cm length from the column neck [8]. Solvent methanol was added in the column, till it was entirely wet with the solvent added. Hand or soft materials were used gently for tapping the column. We can also apply gentle pressure after tapping. Rinse the wall with the sample extract. The samples were collected from the column after 30

minutes and 0.5ml of methanolic extract was transferred to a clean a test tube and added 2% aluminum chloride ($Alcl_3$) solution which was dissolved in ethanol. The mixture was allowed to cool for 30 min at room temperature and absorbance was measured at 420 nm.

III. RESULTS AND DISCUSSION

I. Various Extraction Processes:

a. Rutin Extraction from the peel of *Allium cepa*

i. Effect of Different Solvents

Various organic solvents such as ethanol, methanol, water, and chloroform were used for evaluating its effect on the extraction of rutin optimum yield from peel of *Allium cepa*. Among all the solvents, Methanol [9] was shown the best result for the extraction of rutin and the concentration was 7.5 µg/ml.

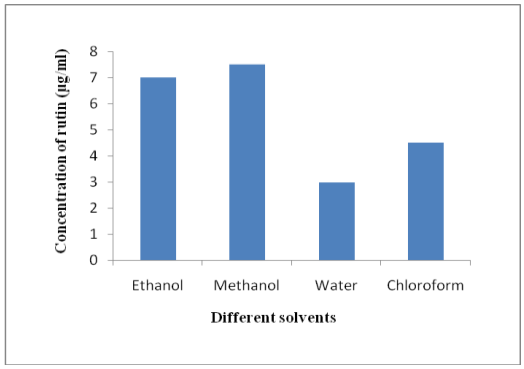

Fig1. Effect of different solvents

ii. Effect of Solvent Different Percentages

Different percentages of solvent Methanol such as 20%, 40%, 50%, 60%, 80%, and 100% were used for checking rutin extraction in this study [9]. After the study, it was found that optimum solvent percentages were 80% methanol for rutin and its concentration was 10.0 µg/ml.

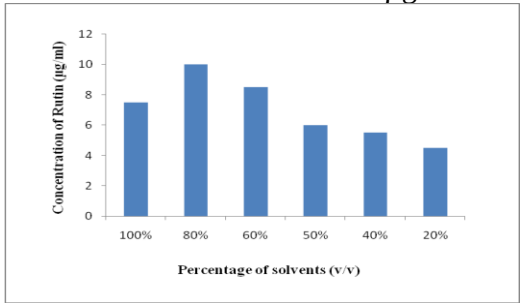

Fig2. Effect of different solvent percentages

iii. Effect of Different Particle sizes

Different particle sizes were studied to check out the best concentrations of rutin. In this process, the optimum particle size and concentration were found to be 125 microns and 18.5µg/ml [10].

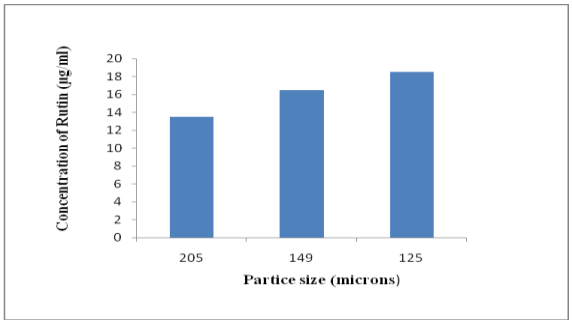

Fig3. Effect of different particle size

iv. Effect of pH: The different pH values were considered such as 4, 5, 6, 7, 8 and 9 to check the optimum pH for the extraction process. The optimum pH and concentration were found to be 5.0 and 24.5µg/ml for rutin extraction.

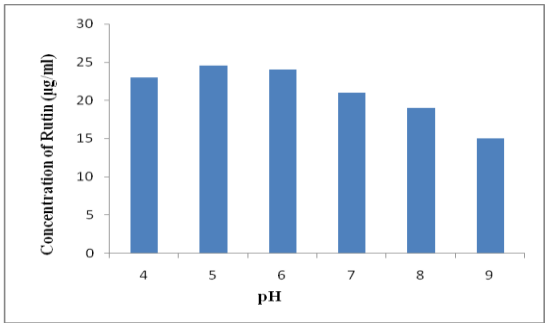

Fig4. Effect of pH

v. Effect of Extraction time: Different time intervals were used to incubate the extract samples for extraction of rutin. It was observed that 24 hr was optimum [11] for the extraction of rutin and its concentration was 30.5 µg/ml.

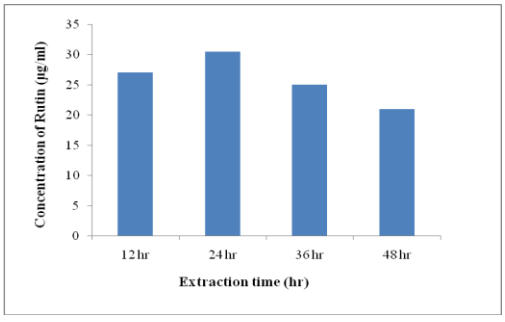

Fig5. Effect of soaking time

b. Extraction of Rutin from the peel of *Allium cepa* by Soxhlet Extractor

In the soxhlet extraction process, 8 gm of the peel of onion powder is placed in a thimble, and 200ml of methanolic solvent is placed in the round bottom flask. The water droplets are coming out from the condenser and fall on the powder. In every cycle, only flavor color is coming out from the powder and falls into the methanolic solvent. At 4 hr it showed a maximum of 42.0µg/ml.

c. Liquid-Liquid Extraction and Modelling Studies

In this process, the Soxhlet extract sample (50 ml of methanolic extract) was purified with toluene (50 ml) in a 1:1 ratio in a separated funnel. After the incubation period, the partition coefficient was found to be 2.81 at 150min.

Partition coefficient = A in extract phase/ A in raffinate phase

= 31.0µg/ml / 11.0µg/ml

= 2.81

Where, A= Amount of rutin component

Modelling of extraction of rutin using Liquid-Liquid extraction : The rutin transfer from Raffinate phase (soxhlet methanolic extract from the peel of onion) to Extract phase (non-polar solvent – toluene) described from the results and satisfied the following linear equation : The determined equation at the end for rutin was

$E(t) = 0.134t + 9.766$ with $R^2 = 0.922$

E_s= Yield extract (rutin in µg/ml) and

t = extraction time (minutes)

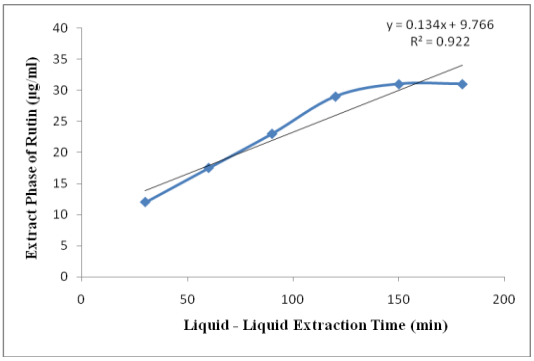

Fig6: Effect of time for liquid-liquid extraction of rutin

II. Purification of Rutin: The rutin concentration of 42.0µg/ml obtained from the methanolic extract of the peel of onion by using the Soxhlet extraction process at 4hrs. Now, this extract was drawn into the chromatography chamber for the purification of rutin [8]. So its concentration was increased to 48.0µg/ml. The purity of rutin was improved by column chromatography at 30 min.

DISCUSSION:

In the present work, various parameters are used for the extraction of rutin from the peel of *Allium cepa*. The physicochemical parameters optimization such as different solvents and its various percentages, particle size, pH and extraction time for the extraction of rutin was studied. The highest rutin concentration for optimized conditions of the peel of *Alliumcepa* was 30.5µg/ml. Polar solvents exhibit the highest amount of bioactive compounds. The characterization of a good solvent depends on the optimal extraction and capacity in maintaining the chemical structure stability of the required compounds. The extraction solvent and its polarity are a significant influence in the level of extracted compounds. Different solvents used for the extraction of rutin and enhancement of phenolic solubility depend on the solvent polarity [9]. So methanol has shown the most efficient solvent and is given optimum yield of rutin from onion peel. Different solvent percentages for extraction, the higher concentration of rutin was extracted with 80% methanol solvent from onion peel due to its higher polarity than remaining solvents [9]. Different particle sizes for extraction, the increasing of rutin was due to increasing contact surface area [10] between material and solvent as well as increasing of diffusivity of material in a solvent. pH for extraction, the increased extraction yield of rutin from under the low pH conditions could be due to the inhibition or arrested of the enzymatic oxidation of phenolics [7]. Rutin is a phenolic compound it's increased with extraction time and the highest values were observed at 24 hrs. The rutin component is increased during the shorter time of extraction [11] and decreases during the longest time of extraction. The

increased extraction time prospectively increases the loss of solvent by evaporation. In the soxhlet extraction process, 8 gm of the peel of onion powder is placed in a thimble, and 200ml of methanolic solvent is placed in the round bottom flask. The water droplets are coming out from the condenser and fall on the powder [6]. In every cycle, only flavor color is coming out from the powder and falls into the methanolic solvent. At 4 hr it showed a maximum of 42.0µg/ml. In the present work, 42.0µg/ml of rutin was obtained from the soxhlet extraction process. Now, this extract sample was used in the column chromatography using silica gel for purification of rutin. Then its concentration was increased to 48.0µg/ml at 30 min. Mainly the crude extract impurities were adsorbed on the surface of silica gel [8]. So, the column gives a clarify sample. In the liquid-liquid extraction process, the extraction solvents methanol (polar solvent) are brought into contact with toluene (non-polar solvent) in order to bring about the transfer of rutin into the first solvent (toluene). Here extract phase is a toluene solvent and the raffinate phase is a methanol solvent [7]. So the rutin concentrations are showing the different values at different time intervals.

V. CONCLUSION

Allium cepa is commonly known as an onion or bulb onion and most widely cultivated and used around the world. It has the highest total flavonoid content like quercetin, rutin, kaempferol, myricetin, isorhamnetin and anthocyanin pigments. Rutin is mainly in peel and flower of onion. Mostly quercetin is highly contained in root, leaf, peel, flower and tunicated bulb of the onion. Mainly these bioflavonoids are plays an antioxidant role characters and commonly found in all parts of the onion. These show various pharmacological activities. The aim of this work was the optimization of different physicochemical parameters such as different extraction solvents and its different percentages, particle size, pH and extraction time for the extraction of rutin was investigated. According to the above results, it was found that extracted solvent methanol and 80 % ethanol, particle size 125 microns, pH 5.0 and time 24 hr are shown the best in the process of rutin extraction. The rutin highest concentration was 30.5µg/ml at optimized conditions. In the process of soxhlet extraction, the extraction of rutin concentration was 42.0 µg/ml obtained at 4hr. This extract was drawn into the Column chromatography chamber and its concentration was increased to 48.0µg/ml. The purity of rutin was improved by column chromatography at 30 min. In the liquid-liquid extraction process, the partition coefficient of rutin was found to be 2.81 and it was carried out using methanol (raffinate phase) and toluene (extract phase) solvents at extraction times to confirm the proposed the mathematical model in this research study. The determined equation of model at the end was $E(t) = 0.123t + 9.766$ for rutin where E_s= Yield extract (rutin in µg/ml) and t = extraction time (minutes).

Acknowledgments: Thankful to the Department of Biotechnology, Meerut Institute of Engineering and Technology (MIET), Meerut, UP.

REFERENCES

1. Aditya G, Ajay K.Saluja (2017). The Pharmacological Potential of Rutin. Saudi Pharmaceutical Journal, 25(2): 149-164.
2. Bystricka J, Musilova J, Tomas J, Noskovic J, Dadákova E, Kavalcova P (2015). *Dynamics of quercetin formation in onion (Allium cepa L.) during vegetation.* Acta Alimentaria, 44 (3):383-389.
3. Jonathan S, María Pilar A, Rosa C (2010). Antimicrobial and antioxidant activity of crude onion (*Allium cepa*, L.) extracts. International Journal of Food Science and Technology, 45(2): 403-409.
4. Koval ski IV, Krasnyuk II, Krasnyuk II, Nikulina O, Belyatskaya AV, Kharitonov YA, Feldman NB Lutsenko SV (2014). Mechanisms of Rutin Pharmacological Action (Review).

Pharmaceutical Chemistry Journal, 48(2): 73-76.

5. Makris DP, Rossiter JT (2001). Domestic processing of onion bulbs (*Allium cepa*) and asparagus spears (Asparagus officinalis): effect on flavonol content and antioxidant status. Journal of Agricultural and Food Chemistry, 49(7):3216-22.

6. Manasa M, Manoj Kumar S and Meena V (2014). A review on medicinal herb : *Allium cepa*. Natural Products an Indian Journal, 10(1):1-6.
7. Manasa M, Manoj Kumar S and Meena V (2013). Optimization of physic-chemical parameters for the extraction of flavonoids and phenolic compounds from the skin of *Allium cepa*, 2(7): 3125-3129.
8. Meena V, Srijana V, Venkata Siva A, Hima Bindu VNV, Nareesh VUB (2015). Experimental Studies and Development of Modeling Equation of Rutin from Pineapple Peel using Soxhlet Extractor. Jour nal of Academia and industrial research, 3(12): 2866-2892
9. Ravi Kant Upadhyay (2016). Nutraceutical, pharmaceutical and therapeutic uses of *Allium cepa*: A review. International Journal of Green Pharmacy 10(1): 46-64.
10. Rodríguez Galdón B, Rodríguez Rodríguez EM, Díaz Romero C (2008). Flavonoids in
11. onion cultivars (*Allium cepa* L). Journal of food science 73(8):599-605.
12. Olanrewaju Sam Olayeriju, Mary Tolulope Olaleye, Olamide Olajusi Crown, Kayode Komolafe, Aline Augusti Boligon, Margareth Linde Athayde, Akintunde Afolabi Akindahunsi (2015). Ethylacetate extract of red onion (*Allium cepa* L.) tunic affects hemodynamic parameters in rats. Food Science and Human Wellness Journal, 4(3):115-122.