

Extraction, Isolation and Quantification of Bioactive Compound (Fisetin) and its Product Formulation

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Abstract— Flavonoids are the class of plant secondary metabolites. Flavonoids are widely distributed into plants, fruits and vegetables. Fisetin is a flavonol, belongs to flavonoid group of polyphenols. Berries contain more amount of fisetin as compared to other fruits and vegetables. Among the berries strawberries contains the highest amount of the fisetin (i.e. 160 µg/ gm. of strawberry). Fisetin has many medicinal properties including anti-cancerous, anti-inflammatory, anti-oxidant, memory stimulator, improve detoxification capacity of the liver etc. so it is necessary to integrate this property into daily consumable form or product. The strawberries were oven dried, then were subjected to extraction with methanol as solvent system. The liquid- liquid extraction technique was used for extraction followed by HPLC for quantification. Then it was formulated in the edible form that is biscuits.

Keywords— Flavonoids; fisetin; medicinal properties; extraction; HPLC.

I. INTRODUCTION

Flavonoids and their conjugates form a very large group of natural products. They are found in many plant tissues, where they are present inside the cells or on the surfaces of different plant organs. The chemical structures of this class of compounds are based on a C6-C3-C6 skeleton [7]. The flavonoids may be modified by hydroxylation, methoxylation, or O-glycosylation of hydroxyl groups as well as C-glycosylation directly to carbon atom of the flavonoid skeleton. Different classes of flavonoids and their conjugates have numerous functions during the interactions of plant with the environment, both in biotic and abiotic stress conditions. Additionally, flavonoid conjugates, because of their common presence in plants, are important components of human and animal diet. Due to the different biological activities of plant secondary metabolites, their regular consumption may have serious consequences for health, both positive and negative. For the mentioned reasons, methods for the efficient and reproducible analysis of flavonoids play a crucial role in research conducted in different fields of the biological and medical sciences.

Fisetin is a flavonol, a structurally distinct chemical substance that belongs to flavonoid group of polyphenols. It can be found in many plants, fruits and vegetables. Berries contain more amount of fisetin as compared to other fruits. Eg. Blueberry, strawberry, raspberry. It is known

to show anti-cancerous, anti-diabetic [8], antioxidant, anti-inflammatory and memory stimulator effects.

Many studies and researches have been done on the physical, chemical and medicinal properties of strawberries and even many literatures have been published on its extraction, but none has been actually incorporated as application bases in food form or any other medicinal or potent form. Fisetin is one amongst them. Strawberry contains more amount of fisetin (160µg/g of strawberry). 37 strawberries are needed to be consumed daily to fulfill dietary needs. So it is necessary to integrate this property into a daily consumable product.

MATERIALS AND METHODS

Sample preparation

411 gms of strawberries were cut in fine slices and were kept for oven drying for 55 hrs at 500°C, the strawberries were grinded in electrical grinder and 30gm fine powder was prepared. This powder was used as sample.

Methanol Extraction

30gm strawberry powder was taken in a petri plate followed by adding hydro-alcoholic mixture (methanol / water 80/20: v/v) for 72 hours, in the dark at room temperature, with renewal of solvent every 24 hours (110 ml x 3).

Solution was filtered by using cheese cloth and evaporated at 50°C. Refrigeration for 48hr after adding 200ml distilled water followed filtration [5].

Liquid-liquid extraction

Chloroform used as a solvent system [5]. The filtrate was poured in separating funnel. 30ml of chloroform was added in the apparatus and left for 2 min. The colourless part of the mixture was taken which was supposed to be containing flavonoids.

Quantification of extract

Fisetin manufactured by Doctor's best, Inc. was used as standard. 10 ppm fisetin standard was prepared by dissolving 0.2 mg of fisetin powder in 20 ml of extra pure methanol. The quantification of samples were done by using HPLC of column C18 (250mm*4.6mm, 5µm), flow rate was 1ml/min, 210 nm and methanol (HPLC grade) was used as mobile phase.

Biscuit preparation

The extract was evaporated at 65°C to remove excess of chloroform. Preheat oven to 230°C. In a large mixing bowl we sift together 150 gm flour, 10 gm baking powder and salt. cut in shortening with fork until mixture resembles coarse crumbs. Then we pour milk into flour mixture while stirring with the fork, mix in milk followed by adding extract until dough is soft, moist and pulls away from the side of bowl. Roll the dough out and cut with a knife. Then we placed biscuit on ungreased baking sheet and baked in preheated oven until golden brown, about 20 min [10].

Biscuit analysis

Prepared biscuit was sent for analysis of polyphenol content, nutritional value and shelf life.

Sodium Hydroxide test

5mg of strawberry powder was added in a 10 ml D/W. The solution was held on a burner till the solution become warm and it was filtered through whatmann filter paper no 41. The filtrate was collected in another test tube. 2 ml of 10% aq. Sodium hydroxide was added to this solution which gives yellow colour. A change in colour from yellow to colourless on addition of dilute HCl occurred which is an indication for the presence of flavonoid.

RESULT AND DISCUSSION

A. For Sodium Hydroxide test

Test tube Shows formation of yellow color due to addition of filtrate and NaOH followed by adding Hcl until the color become colorless which is the indication of presence of flavonoids.

B. For Quantification of extract



Fig. 1 HPLC graph for Flavonoid standard (10ppm)

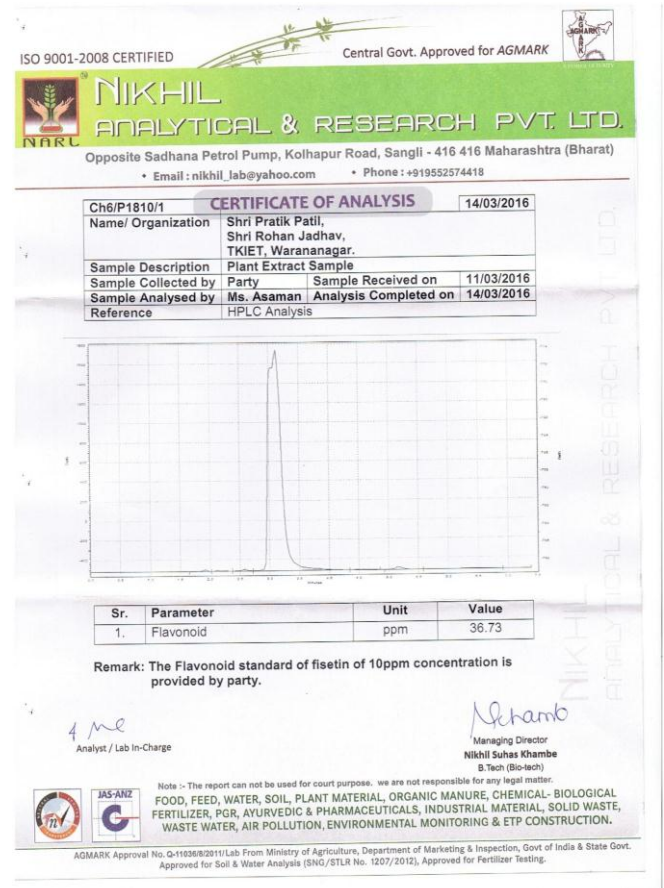


Fig.2 HPLC graph for Fisetin extract.

C. For Biscuit analysis

Sr No.	Parameter	Unit	Value
Nutritional value			
1	Moisture	%	03.04
2	Total minerals	%	04.13
3	Crude Protein	%	09.91
4	Crude Fat	%	19.08
5	Crude Fiber	%	00.12
6	Carbohydrate	%	63.72
7	Energy	Kcal/100g m	466.24
Other Analysis			
1	Shelf Life	Month	1
2	Total polyphenols	Mg/100gm	412.44

Nutritional value

CONCLUSION

Fisetin has many medicinal properties so it is necessary to incorporate these properties in human consumable form. Fisetin is present abundantly in strawberry. The confirmation test of strawberry powder for presence of flavonoid was done and the result was positive. The extraction of fisetin was successfully carried out by using methanol as solvent system. And isolation was done in chloroform by liquid-liquid extraction. The HPLC results of sample was compared with fisetin standard, shows that fisetin was present in sample. The Biscuits that was formulated using fisetin is a functional food since it has the beneficial effect of flavonoid (Fisetin). Biscuit was sent for nutritional analysis and the results came positive. The idea of combining all these ingredients and making a product with the goodness of each of the ingredients gives the synergistic effect in the functional food.

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