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EXTRACTION, EVALUATION AND STANDARDIZATION OF NASUNIN FROM PEELS OF Solanum melongena L. PURPLE PEEL FRUITS

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Abstract: This study has been undertaken to investigate the determinants of stock returns in Karachi Stock Exchange (KSE) using two assets pricing models the classical Capital Asset Pricing Model and Arbitrage Pricing Theory model. To test the CAPM market return is used and macroeconomic variables are used to test the APT. The macroeconomic variables include inflation, oil prices, interest rate and exchange rate. For the very purpose monthly time series data has been arranged from Jan 2010 to Dec 2014. The analytical framework contains**.** This study was planned to characterize main anti-oxidant component Nasunin which is chemically an anthocyanin, from methanolic and aqueous extract of *Solanum melongena* L. purple coloured fruit peels. Peels of *Solanum melongena* L. purple coloured fruit peel were subjected to repeated maceration in acetic acid (10 %) for 24 hours and dried. Filtrate was extracted with 200 ml methanol, filtered and dried. Trifluoroacetic acid (1%) was added to this dried extract and analyzed by HPLC analysis. Peak was obtained at 25.658 and 25.649 minute that corresponds to methanolic and aqueous extract respectively. The peak strength and retention time (R_t) is inline and matches with the standard peak that confirms the presence of Nasunin; an anthocyanin component. From the results it was concluded that that peels of *Solanum melongena* L. contain high amount of anthocyanin component Nasunin which is considered as a strong anti-oxidant. Due to its high quantity, eggplant shows strong anti-oxidant activity which has been proven *in-vitro*. Nasunin is highly soluble in both media and is stable too. So this merits further investigation for anti-oxidant/ hepatoprotective activity *in-vivo*.

Index Terms **- anthocyanin, detector, HPLC, Nasunin, retention time (R_t), *Solanum melongena* L.**

I. INTRODUCTION

Anthocyanins are the class of chemical compounds that are responsible for imparting color to natural resources especially plants. They are thoroughly distributed in the plant kingdom. They decide the colour of any plant product like fruits, vegetables. They occur in two different forms namely: glycosylated form (anthocyanins) and in aglycosylated form (anthocyanidins). These coloring molecules are generally less stable. They have been extracted from plants and used primarily as pigments, dyes or coloring agents as natural color additive, in the color of specific products, such as soft drinks, yogurt, jam, or revive certain types of foods. Mostly extracted anthocyanins that are allowed in food products and food additives are from strawberries, raspberries, blueberries, blackberries, cherries, eggplant, red onions, red cabbage, radish roots. Out of them all, few also carry additional medicinal properties too, to heal downgraded cellular mechanism that is suffering from free radical attack by acting as anti-oxidants (Igarashi et al., 1989; Igarashi et al., 1993; Ishii et al., 1996; Tsuda et al., 1994; Tsuda et all., 1996). Brinjal also called as egg plant or *Solanum melongena* L. is a vegetable that is primarily part of Mediterranean diet. This vegetable also is a power house of some of the strongest anti-oxidant of plant kingdom that are known from ethnobotanical sources to prevent major diseases cardiovascular disease, diabetes and tumours (Sarkar at al., 2019). The peels are purple in colour that have been considered to be the most important contributing factor for its anti-oxidant activity among all phytoconstituents in egg plant. The colouring agent has been isolated and reported as Nasunin (Kuroda et al., 1933). Nasunin (delphinidin 3-(trans-p-coumaloyl-L-rhamnosyl-Dglucoside)-5-glucoside) is the major anthocyanin of eggplant (Ishii et al., 1990; Watanabe et al., 1966). It's also popular for its brilliant dark shiny purple colour too; which is reported as a standard colour in dying industry from ancient times, called as Nasu blue. L. var. (Kuroda et al., 1933). It has very high anti-oxidant activity (Sarkar et al, 2019; Gallo et al., 2016). Peels of purple coloured eggplant fruit also contains chlorogenic acid which is also known to possess considerable free radical scavenging activity. From qualitative tests, it was established that the purple colour pigment Nasunin, is a particular anthocyanin molecule, of flavonoids family, that combines with other anthocyanins and is responsible for its specific Nasu colour. In nature, very few foods and plants contain pigment Nasunin. Among all plants, purple peeled eggplants are among the highest containing ones concentrated in vegetable fruit peels (Gallo et al., 2016). The colour is very sensitive and changeable, so care was taken to protect and preserve the colour from changing or getting lost during extraction from peel and its qualitative evaluation. In this report, we have tried to isolate nasu blue colour anthocyanin pigment Nasunin, the major colouring and anti-oxidant component of purple peeled egg plant.

II. RESEARCH METHODOLOGY

The methodology section outline the plan and method that how the study is conducted. This includes chemical and reagents, sample preparation, equipment used, solvent system and separation conditions

II.1 Chemicals and reagents:

Acetic acid, methanol and trifluoroacetic acid (1% TFA) was purchased from Molychem, India. HPLC by CyberLab (Millibury, USA) system; equipped with 5 µm Capcell Pak C18 column (4.6 mm (ID) × 250 mm, 5 µm) and UV detector system was used for qualitative identification. Labtop magnetic stirrer was used. Millipore water was used as solvent system and also for rinsing of columns. *Solanum melongena* L. or purple peeled eggplant was purchased from local farm in Gurgaon, Haryana, India.

II.2 Sample Preparation:

Fresh eggplants were obtained from market. They were properly rinsed under distilled water and kept for drying in room temperature till trace water got dried up. Then very fine peels were removed from fruit. These fresh peels were weighed, then cut into small pieces (100 g) and immersed in 200 ml of acetic acid (10%; v/v) under constant stirring (on a magnetic stirrer) for a period of 24 hours at room temperature. The mixture was filtered and further 200 ml of more acetic acid were added to these peels. They were macerated again for next 24 hours at room temperature. Macerate was filtered and mixed with previous filtrate. The filtrate was evaporated to dryness using rotary vacuum evaporator. Furthermore, the residue was extracted with 200 ml of methanol, filtered and dried. The remaining part was extracted with water, filtered and dried. Methanol and aqueous dried weights were added with trifluoroacetic acid (1%) and analysed by HPLC analysis.

II.3 Equipment:

CyberLab (Millibury, USA) HPLC system equipped with 5 µm Capcell Pak C18 column (4.6 mm (ID) × 250 mm, 5 µm) and UV detector was employed to carry out the analysis.

II.4 Solvent system and separation conditions:

Solvent A was water with 0.5% trifluoroacetic acid and solvent B was methanol with 0.5% trifluoroacetic acid in a ratio of 75:25 v/v to 30:70. Gradient elution was performed at 20 °C with flow rate 1ml/minute, whereas the detection was carried out at 520 nm with an UV detector.

IV. RESULTS AND DISCUSSION

1V.1 Results

To identify and quantify the anthocyanin (Nasunin) in peels of *S. melongena* L. HPLC analysis was carried out. The concentration of identified compound was calculated as mg/g of extract. The concentration of identified compound was found to be 0.927 and 1.48 mg/g of the methanol and aqueous extracts, respectively. The results so obtained (retention time) were found to be in accordance with Hayashi et al., 1998; and evidenced the presence of Nasunin in *S. melongena* L. purple peel fruit extract. The blank chromatogram and chromatogram of sample extract depicting retention time of identified compound are given in figure 1, 2 and 3, respectively.

Figures

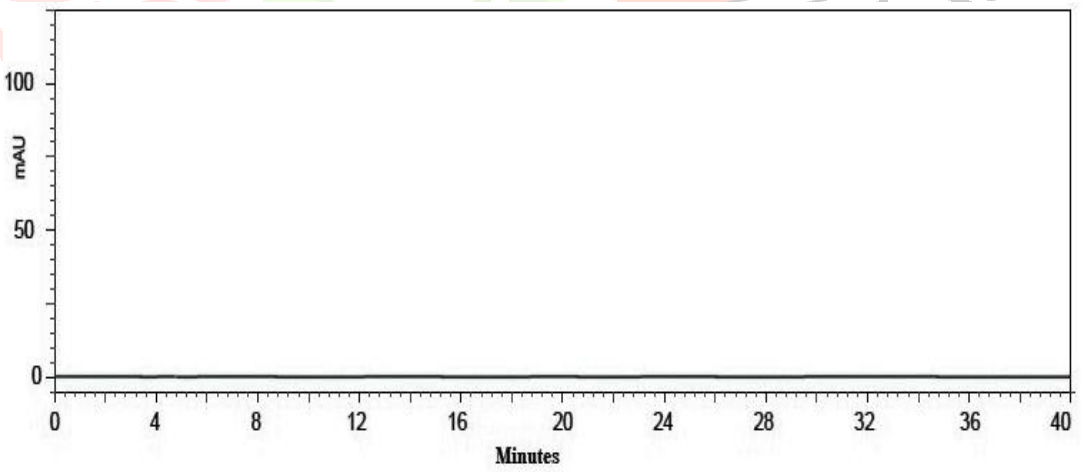

Figure 1. Blank chromatogram

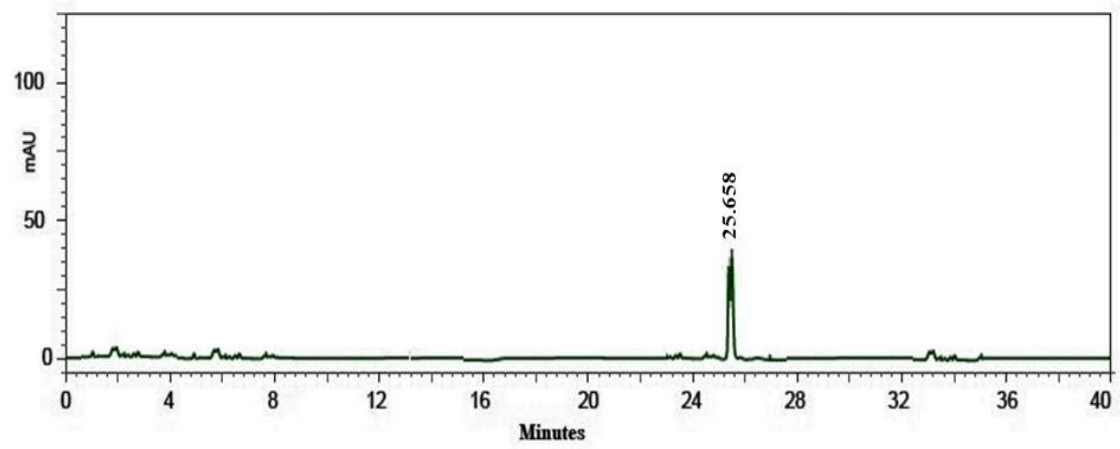

Figure 2. HPLC chromatogram of methanol extract of *S. melongena* L. for identification of Nasunin

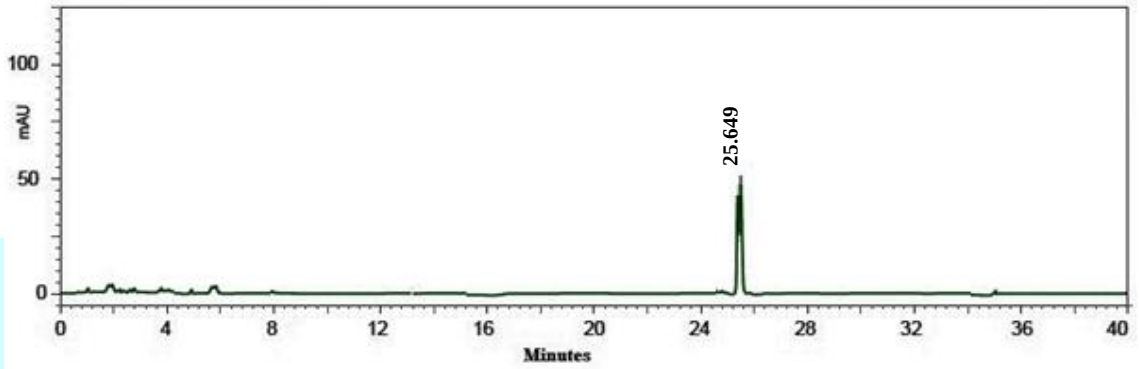

Figure 3. HPLC chromatogram of aqueous extract of *S. melongena* L. for identification of Nasunin

1V.2 Discussion

The purple colour pigment Nasunin is present in abundance in eggplants at concentration of 700 mg/100 g. It is the most prominent anthocyanin in the eggplant peels that was for the first time isolated in 1933 (Kuroda et al., 1933; Kuroda et al., 1935; Sakamura et al, 1963) determined its structure. Chemically it's a stable anthocyanin due to its acylation and exists as a mixture of cis-trans isomers of delphinidin. It has a very high anti-oxidant activity. It complexes with Fe3+ thereby inhibiting chelation formation of hydroxyl free radicals for chelation with the iron. The peak obtained at 25.658 and 25.649 minute corresponds to methanolic and aqueous extract respectively. The peak strength and retention time (Rt) is inline and matches with the peak as obtained [11]. The almost similar Rt of both the peaks in chromatogram signifies that in both extracts, anthocyanin Nasunin is present and elutes at same time in both extracts. But the difference in peak height signifies that Nasunin being an anthocyanin in nature; is more soluble in aqueous media, so signal strength is higher in aqueous than methanolic extract.

1V.3 Conclusion:

This study was designed and conducted to pave way for future studies corresponding to *in-vivo* antioxidant studies and thereby proving hepato-protective action of *Solanum melongena* L. purple peel fruit extract. It lead to characterization and solidifying the views that *S. melongena* L. peel contains Nasunin as anthocyanin responsible for its brilliant purple or nasu colour and also may be responsible for overall strong antioxidant effect of purple peeled eggplant. Hence, this experiment was conducted to prove the same, as result of this activity will be useful to further merit the investigation of hepatoprotective action *in-vivo*. In conclusion, this study served as a proof of concept and helped in standardization of peel extract; to go ahead for future studies both *in-vitro* and *in-vivo*.

III. ACKNOWLEDGMENT

None.

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