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Antiglycemic and Antioxidant Activity of *Terminalia chebula* Fruits Protein

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Abstract:- ***Terminalia chebula*** **fruits proteins antidiabetic activities were studied in** ***in vitro*** **model. Using appropriate standard methods were used to quantify the phytochemicals present in** ***Terminalia chebula*** **fruits extract. The results revealed that, the extract rich with proteins when compared to other phyto chemicals. The DPPH model was used for the analysis of antioxidant activity of the crude proteins. The alpha amylase enzyme, alpha glucosidases enzyme inhibition activity and Glucose uptake in Yeast cells studies were done to check the anti-glycemic activity. These results revealed that, the crude protein showed antidiabetic activity. This potential pharmacological activity of proteins might be due to the presence of proteins.**

Keywords:- *Terminalia chebula, Proteins, Antioxidant, Antidiabetic, Antiglycemic, DPPH.*

I. INTRODUCTION

Diabetes mellitus (DM) is a disorder characterised by way of hyperglycemia and eventual glycosuria by the improper insulin secretion [1, 2]. DM causes not only psychological physical distress but it also causes other life threatening complications like diabetic retinopathy, cardiopathy, neuropathy nephropathy and so on [3, 4]. The International Diabetes Statistics Association reported in a statistic that, about 700 million populations throughout the world will be diagnosed with DM by 2045 [5]. The inhibition activities of alpha-amylase and alpha-glucosidase would slowdown the degradation of carbohydrate, which leads to decrease in the absorption of glucose, resulting in reduction of postprandial blood glucose level [6, 7]. Throughout the world, diabetic patients using or following variety of medicines, diets, physical training and still there is lot of need for new natural and synthetic compounds to overcome diabetic problems [8, 9]. *Terminalia chebula* fruits are widely used for its medicinal properties like antioxidant, anti-diabetic, anti-inflammant etc. [10] The fruits are rich with phytochemicals and are responsible for the above said medicinal properties. Till date no one has reported regarding the importance of proteins present in it. The above results, encouraged us to study the antioxidant and anti-diabeetic activities of the protein part.

II. MATERIALS & METHODS

The required chemicals were purchased from Sigma Aldrich, Hi-Media and all other chemicals used in this study were obtained commercially and were of analytical grade.

- *Extraction*

The *Terminalia chebula* fruits procured locally, cleaned with double distilled water, shade dried, crushed and fine powdered and stored in glass container. 10g of *Terminalia chebula* fruits powder vortexed with 200 ml of double distilled for 4 hours at 20°C. Further centrifuged at 10000 rpm for 20 minutes, the supernatant was separated. The supernatant was subjected to lyophilization. The lyophilized crude extract was subjected to dialysis to remove unwanted contents, using 2.5kDa molecular cut-off biomembrane against water and stored at -10°C for further analysis.

- *Proximate analysis*

The total *Terminalia chebula* fruits proteins (TCFP) was determined by Bradford's [11] method. The total sugar was estimated by the phenol–sulphuric acid method [12], total phenolic content was determined by the Folin– Ciocalteau reagent [13] Ascorbic acid content estimation [14] and the total flavonoid content was determined [15].

- *DPPH radical scavenging activity*

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of TCFP was studied with minor modifications [16, 17]. The crude dialyzed TCFP chosen as 25µg was mixed in freshly prepared 0.5 mM DPPH ethanolic solution (1ml) and 0.1 M acetate buffer (2ml; pH 5.5). Further, incubated at 37°C for 30 min and read at 517 nm using UV-Visible Spectrophotometer. Ascorbic acid and Alpha-tocopherol were taken at a dosage of 15µg each used as control in same dosage.

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➢ *In vitro methods employed in anti-diabetic studies*

- *Inhibition of alpha amylase enzyme*

The TCFP samples (500 µl) and standard drug (100 to 1000µg/ml) were added to of 0.20 mM phosphate buffer of pH 6.9 (500 µl) containing α-amylase solution (0.5mg/ml) and incubated at 25°C for 10 min. 1% starch solution (500 µl) in 0.02 M sodium phosphate buffer of pH 6.9 was added to each tube and then incubated at 25°C for 10 min. The reaction was arrested by adding 1.0 ml of 3, 5 dinitrosalicylic acid and incubated in boiling water bath for 5 min and cooled. 10 ml distilled water added to reaction mixture and absorbance was measured at 540 nm. Control indicates 100% enzyme activity [18, 19].

- *Inhibition of alpha glucosidases enzyme*

A starch substrate (2 % w/v maltose or sucrose) was incubated with 0.2 M Tris buffer pH 8.0 (1ml) to determine the TCFP inhibitory activity, where various concentration of crude proteins used for 5 min at 37°C. Further 1ml of α-glucosidase enzyme was added to initiate the reaction and kept for a duration of 10min at 37°C. Again, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction. The amount of liberated glucose is measured by glucose oxidase peroxidase method [20, 21].

- *Glucose uptake in Yeast cells*

Commercially available baker's yeast was procured and by repeated centrifugation it was washed in water till the supernatant clear. 10% (v/v) suspension was prepared in distilled water. A maximum dose of crude proteins (15 mg) were added to 1mLof glucose solution of dosese 5, 10 and 25mM and incubated together for 10 min at 37°C. By adding 100 µl of yeast suspension and the reaction was initiated, vortexed and incubated at 37°C for 60 min. Later, the glucose was estimated in the supernatant. Metformin drug was taken as standard. The percentage increase in glucose uptake by yeast cells was calculated. [22, 23].

III. STATISTICAL ANALYSIS

Statistical analysis was done in SPSS (Windows Version 10.0.1 Software Inc., New York) using a one-sided student's t-test. All results refer to means ± SD. $P < 0.05$ was considered as statistically significant when compared to relevant controls.

IV. RESULTS

Table-1: Proximate analysis of dialyzed *Terminalia chebula* fruit proteins (TCFP)

Phytochemicals	g%
Proteins	4.12
Carbohydrates	0.21
Polyphenols	0.02
Flavonoids	0.01
Ascorbic acid	0.01

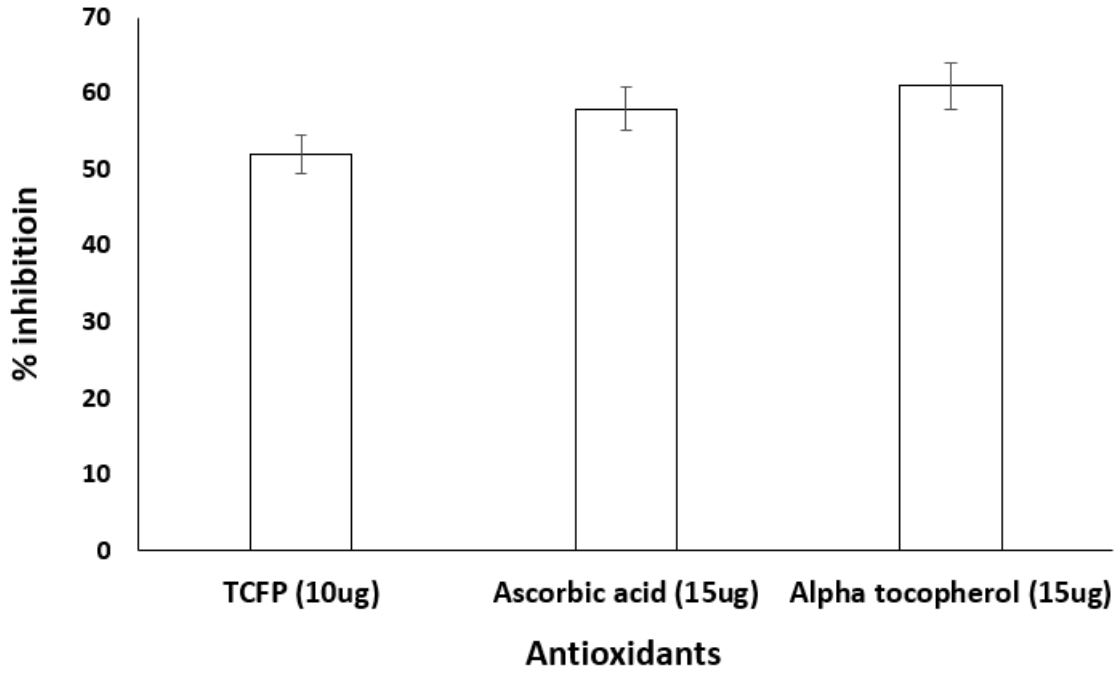

Fig 1: DPPH radical scavenging activity of TCFP

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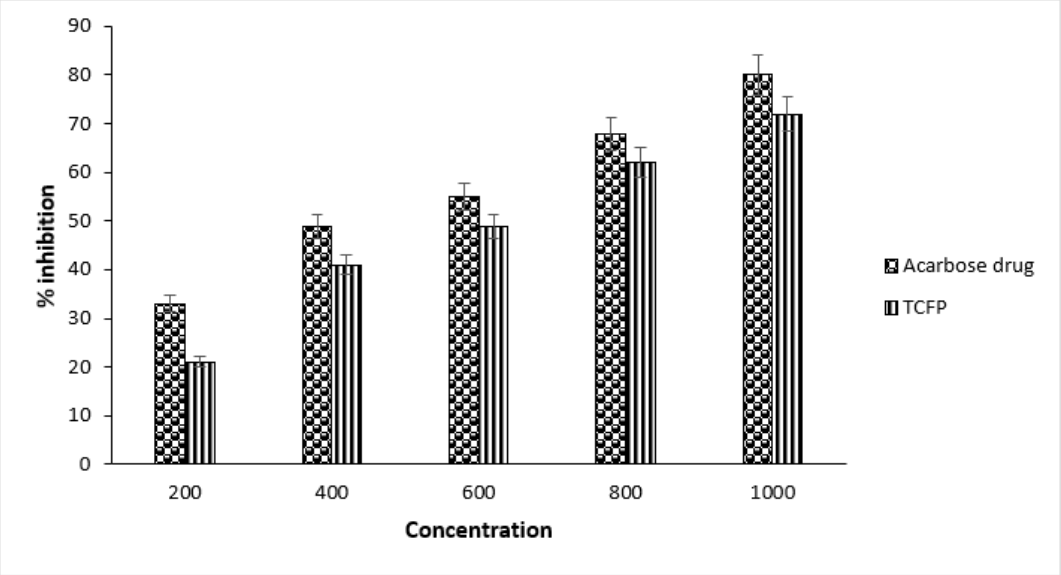

Fig 2: Alpha amylase inhibitory activity

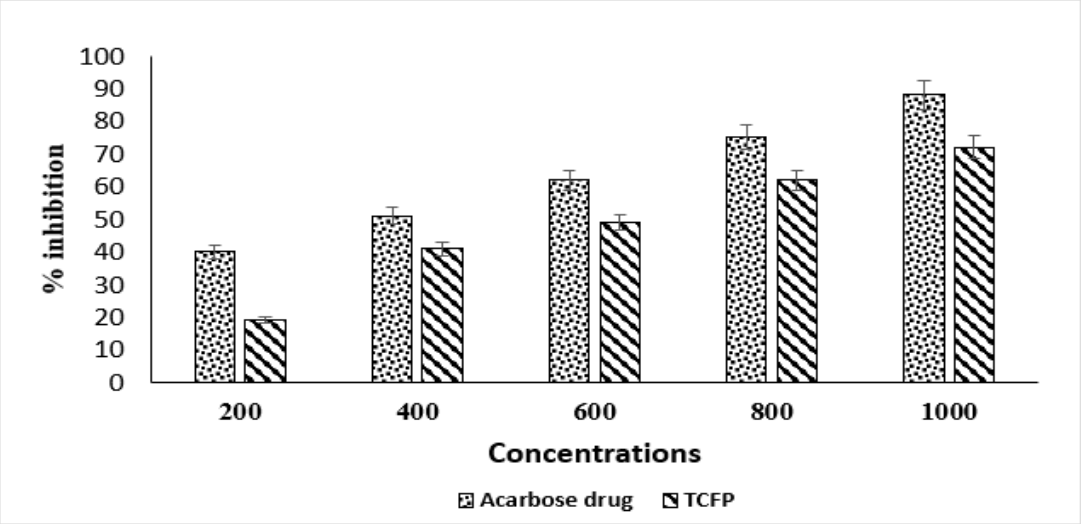

Fig 3: The *in vitro* α-glucosidase inhibitory activity of TCFP

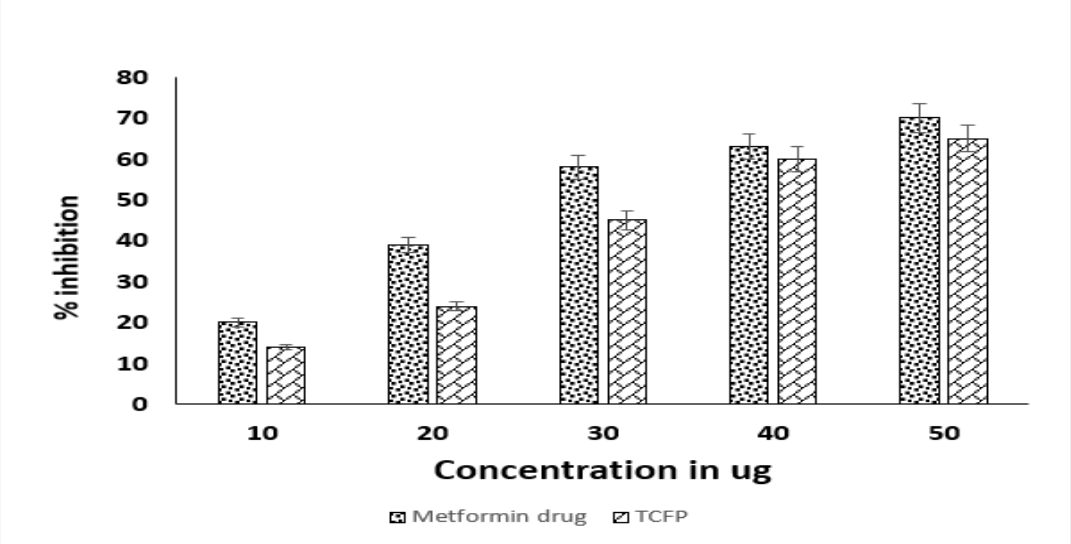

Fig 4: Glucose uptake in yeast cells

V. DISCUSSION

Vegetables and fruits are rich source of antioxidants and having medicinal values. Spices are also well known for their medicinal values. Spices like Turmeric, Ginger, Piper longum, Curry leaves, methi leaves are containing useful phytochemicals and widely used as antioxidants, anti-inflammants, DNA protectants, antimicrobial and so on [24, 25, 26, 27]. The crude proteins were isolated from *Terminalia chebula* fruits as explained in methods. The proximate analysis studies were done for dialyzed proteins to find amount of the phytochemicals presents. The results are as shown in Table-1 that, the dialyzed crue extract contains more proteins. The obtained crude proteins were studied for their antioxidant activity by DPPH radical scavenging activity model where α-tocopherol and Ascorbic acid were used as standard antioxidants at a maximum dosage of 15µg each whereas TCFP taken at a dosage of 10µg. The inhibition of DPPH radicals by TCFP and others as shown in Figure No 1. The dose-dependent *in vitro* α-amylase inhibitory activity of TCFP was done as explained in methods. It was noticed that, there is an increase in percentage inhibitory activity with the increase in dosage against α-amylase enzyme, Acarbose a standard drug was used with same dosage to compare inhibitory capacity of the TCFP. Figure-2 indicates that, the % inhibitory activity of TCFP ranges a minimum of 21.21±0.03 (at 100µg/ml) to a maximum of 72.01±0.04 (at 1000µg/ml) where as the standard drug Acarbose showed % inhibitory activity ranges from 33.03±0.01 (at 100µg/ml) to a maximum of 80.11±0.01 (at 1000µg/ml). The *in vitro* α-glucosidase inhibitory activity of TCFP was done as explained in methods. It was noticed that, there is an increase in percentage inhibitory activity with the increase in dosage against α-glucosidase and as a standard drug Acarbose was used as positive control. Figure-3 showed that, the % inhibitory activity of TCFP ranges a minimum of 20.10±0.11 (at 100µg/ml) to a maximum of 72.13±0.11 (at 1000µg/ml) where as the standard drug Acarbose showed % inhibitory activity ranges from 40.12±0.01 (at 100µg/ml) to a maximum of 88.21 (at 1000µg/ml). The rate of glucose transport across cell membrane in yeast cells system is as presented in Figure-4. In Yeast (*Saccharomyces cerevisiae*) glucose transport takes place via diffusion. After the treatment of the yeast cells with these TCFP in a dose dependent manner, the glucose uptake was found to increase and the % increase in glucose uptake by the yeast cell at different glucose concentrations i.e., 25mM, 10mM and 5mM respectively was found. The TCFP exhibited significant activity at all glucose concentrations in comparison with standard drug Metformin.

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REFERENCES

[1]. Vieira, R.; Souto, S.B.; Sánchez-López, E.; López Machado, A.; Severino, P.; Jose, S.; Santini, A.; Fortuna, A.; García, M.L.; Silva, A.M.; Souto, E.B. Sugar-lowering drugs for type 2 diabetes mellitus and metabolic syndrome—Review of classical and new compounds: Part-I. Pharmaceuticals. 2019:12(4);152.

[2]. Chikkanna. D.; Dinesha Ramadas.**;** Subha Chandrappa, M.; Santosh Kumar, N.; Shwetha, K.L.; Ranjitha, P. Antidiabetic activity of Ginger tuber proteins: *In vitro* studies. Asian Journal of Pharmaceutical and Health Sciences, 2016**;**6(2):1472-1476.

[3]. Mezil, S.A.; Abed, B.A. Complication of diabetes mellitus. Annals of the Romanian Society for Cell Biology, 2021;1546-1556.

[4]. Harding, J.L.; Pavkov, M.E.; Magliano, D.J.; Shaw, J.E.; Gregg, E.W. 2019. Global trends in diabetes complications: a review of current evidence. Diabetologia. 2019;62:3-16.

[5]. Stöhr, J.; Barbaresko, J.; Neuenschwander, M.; Schlesinger, S. Bidirectional association between periodontal disease and diabetes mellitus: a systematic review and meta-analysis of cohort studies. Scientific Reports, 2021;11(1):1-9.

[6]. Zhao, L.; Wen, L.; Lu, Q.; Liu, R. Interaction mechanism between α-glucosidase and A-type trimer procyanidin revealed by integrated spectroscopic analysis techniques. International journal of biological macromolecules. 2020;143:173-180.

[7]. Padhi, S.; Nayak, A.K.; Behera, A. Type II diabetes mellitus: a review on recent drug based therapeutics. Biomedicine & Pharmacotherapy. 2020;131:110708.

[8]. Rahman, M.M.; Islam, M.R.; Shohag, S.; Hossain, M.E.; Rahaman, M.S.; Islam, F.; Ahmed, M.; Mitra, S.; Khandaker, M.U.; Idris, A.M.; Chidambaram, K. The multifunctional role of herbal products in the management of diabetes and obesity: a comprehensive review. *Molecules.* 2022;*27*(5):1713.

[9]. Cox-Georgian, D.; Ramadoss, N.; Dona, C.; Basu, C. Therapeutic and medicinal uses of terpenes. *Medicinal plants: from farm to pharmacy*. 2019;333-359.

[10]. Nigam, M.; Mishra, A.P.; Adhikari-Devkota, A.; Dirar, A.I.; Hassan, M.M.; Adhikari, A.; Belwal, T.; Devkota, H.P. Fruits of Terminalia chebula Retz.: A review on traditional uses, bioactive chemical constituents and pharmacological activities. Phytotherapy Research. 2020;34(10):2518-2533.

[11]. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein, Analytical Biochemistry. 1976;72:248-54.

[12]. Dubois, M.; Gilles, K.A.; Hamilton, J.K.; Rebers, P.A.; Smith, F. Colorimetric method for determination of sugar and related substances, Analytical Chemistry. 1956;28:350-356.

ISSN No:-2456-2165

[13]. Kujala, T.S.; loponen, J.M.; Klika, K.D.; Pihaja, K. Phenolics and betacyanins in red beetroot (Beta Vulgaris) root: description and effect of cold storage on the content of total phenolics and three individual compounds. Journal of Agricultural Food Chemistry. 2000;48:5338-5342.
[14]. Dinesha Ramadas.; Leela Srinivas. Antioxidant effects of 28kda Protein from Turmeric (*Curcuma Longa L*). Asian Journal of Pharmaceutical and Clinical Research, 2011;4(1):75-79.
[15]. Dinesha, R.; Thammanna Gowda, S.S.; Harsha, R.; Leela Srinivas. Antioxidant and antimicrobial activity of partially purified proteins from hot water extract of Turmeric (*Curcuma longa L).* Pharmacologyonline. 2010;1:996-1004.
[16]. Dinesha R, Santhosh Kumar N, Chikkanna D and Vedamurthy Joshi. Metal ion chelation and anti-glycation properties of polysaccharides of *Phyllanthus amarus* plant. GSC Biological and Pharmaceutical Sciences. 2021;15(03):349–353.
[17]. Dinesha Ramadas, Santhosh Kumar Nune, Chikkanna D, Maheshwara KV and Vedamurthy Joshi. Anti-glycation and antioxidant properties of *Abutilon indicum* plant leaves. International Journal of Science and Research Archive. 2021;02(01):269–273.
[18]. Gupta, A.; Kumar, R.; Pandey, A.K. 2020. Antioxidant and antidiabetic activities of *Terminalia bellirica* fruit in alloxan induced diabetic rats. South African Journal of Botany. 2020;130:308-315.
[19]. Smina, C.S.; Lalitha, P.; Nagabhushana, H.; Sharma, S.C. *Terminalia bellirica* dried fruit and seed extract offers alpha-amylase inhibitory potential in tackling diabetes. Applied Nanoscience. 2020;10:4325-4339.
[20]. Li, Y.; Li, Y.; Chen, J. Screening and identification of acetylcholinesterase inhibitors from *Terminalia chebula* fruits based on ultrafiltration and ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry. Microchemical Journal. 2021;168:106438.
[21]. Zhang, X.R.; Qiao, Y.J.; Zhu, H.T.; Kong, Q.H.; Wang, D.; Yang, C.R.; Zhang, Y.J. Multiple in vitro biological effects of phenolic compounds from *Terminalia chebula* var. tomentella. Journal of Ethnopharmacology, 2021;275:114135.
[22]. Kumar, N.; Khurana, S.M. Phytochemistry and medicinal potential of the Terminalia bellirica Roxb.(Bahera). Indian Journal of Natural Products and Resources (IJNPR) [Formerly Natural Product Radiance (NPR)]. 2018;9(2):97-107.
[23]. Bulbul, M.R.H.; Chowdhury, M.N.U.; Naima, T.A.; Sami, S.A.; Imtiaj, M.S.; Huda, N.; Uddin, M.G. A comprehensive review on the diverse pharmacological perspectives of *Terminalia chebula* Retz. Heliyon, 2022:10220.
[24]. Dinesha Ramadas.; Vedamurthy Joshi.; Ramesh, B.; Santhosh Kumar Nune.; Rajesh Kowti. DNA protection against UV Rays: A study on polyphenols of Curry leaves (*Murraya koenigii*). International Journal of Innovative Science and Research Technology 2020;5(10):1278-1280.
[25]. Dinesha Ramadas.; Shobith Rangappa.; Santhosh Kumar, N.; Chikkanna, D.; Manjula, R.V.; Tejaswini, B.M.; Divya, Y. Protease inhibitory and thermal stability properties of Turmerin protein from Turmeric (*Curcuma longa Linn*) rhizome, World Journal of Pharmacy and Pharmaceutical Sciences. 2019;8(2):756-763
[26]. Dinesha Ramadas.;Shobith Rangappa.; Santhosh Kumar, N.; Chikkanna, D.; Manjula, R.V.; Tejaswini, B.M., Divya, Y. Heat stability and protease inhibitory properties of 28kDa glycoprotein from Turmeric (*Curcuma longa*). European Journal of Pharmaceutical and Medical Research. 2019;6(2):393-99.
[27]. Dinesha Ramadas.; Harsha R Kashyap.; Chikkanna, D.; Santosh Kumar, S. UV rays induced DNA damage: Protection by *Curcuma aromatica*, SAS Journal of Medicine. 2017;3(11):323-327.