 http://doi.org/ 10.18231/j.ijcbr.2019.040

Iron chelating, reducing and anti-glycation activities of *Coleus aromaticus* leaves protein

Santhosh Kumar N[1], Kalaivanam K.N[2*], Aliya Nusrath[3], Dinesha Ramadas[4]

[1]Research Scholar, [2]Associate Professor, [3]Professor and Head, [4]Scientific Officer, [1-3]Dept. of Biochemistry, [4]AIMS-Central Research Laboratory, [1-3]Adichunchanagiri Institute of Medical Sciences, Mandya, Karnataka, [2]Shridevi Institute of Medical Sciences and Research Hospital, Tumkur, Karnataka, [4]Adichunchanagiri Institute for Molecular Medicine, Mandya, Karnataka, India

***Corresponding Author: Kalaivanam K.N**
Email: kalaivanam@yahoo.com

Received: 26th December, 2018 **Accepted:** 8th March, 2019

Abstract

Introduction: *Coleus aromaticus* a medicinal plant is grown as weeds in the tropical countries like India. It has a wide range of active constituents, which has potent medicinal properties. The present study, the authors investigated the Iron chelating, reducing and anti-glycation properties of *Coleus aromaticus* leaves protein (CALP) in *invitro* model system.

Materials and Methods: Proteins from the extract is isolated by Ammonium sulphate protein precipitation method. *In vitro* antioxidant studies were carried out by iron chelating activity and iron reducing power methods. The alpha amylase inhibitory activity, alpha glucosidase inhibitory activity and glucose uptake by yeast cells invitro studies was done.

Results: The iron chelating activity and iron reducing power of the crude protein of *Coleus aromaticus* was compared with standard antioxidants like EDTA and Ascorbic acid. In anti-glycation studies, standard drugs Acarbose and Metformin was used. Results showed the percentage inhibition of ferrous ion chelating activity, increased with increase in concentrations of crude protein of Coleus aromaticus extract is comparable with standard antioxidants. Anti-glycation property and the inhibitory activity of CALP was compared with standard drugs.

Conclusion: This study concluded that the *Coleus aromaticus* proteins possess significant anti-glycation activity and antioxidant activity when compared to standard antioxidants. Antioxidant activities increased with increasing concentrations in concentration dependent manner. It was found to be significant and valuable.

Keywords: Antioxidant, *Coleus aromaticus* leaves protein (CALP), Ferric ion reducing and ferrous ion chelating activity.

Introduction

Oxidative stress leads to many diseases due to an imbalance between formation of ROS/RNS and their neutralization when endogenous antioxidant mechanisms are unable to reduce the free radicals.[1] The synthetic antioxidants are used to scavenge the free radicals but these have side effects, thus the natural antioxidants are safer. [2, 3]

Iron is a trace element, found in hemoglobin, myoglobin, the cytochromes; enzymes.[4] Iron has a capacity to alter its oxidation and reduction in response to liganding and changes the various cellular processes.[5] In several health conditions induced due to oxidative stress there is associated iron overload.[6, 7]

In biological systems, the reactive oxygen species (ROS) originate from the interaction of iron with enzymatically / non enzymatically generated superoxide (Haber-Weiss reaction) or hydrogen peroxide (H_2O_2, Fenton reaction).[8]

A non-enzymatic reaction glycation takes place between free amino groups of proteins and reducing sugars.[9] This is associated with the pathogenesis of age and diabetes related complications. If the oxidation is involved in glycation process, is called as glyco-oxidation. Glyco-oxidations generates a heterogeneous group of substances called advanced glycation end products (AGEs).[10] AGEs act, either by modifying structural intra- and extracellular proteins or by binding to their receptors that belong to the immunoglobulin family.[11,12]

Apart from natural defense system, synthetic drugs are also available which improve the capacity of the body to counter oxidative stress. Due to the harmful side effects of synthetic drugs, research on natural products has taken a leap in recent years.[13]

Karpurvalli (*Coleus aromaticus*) is a household herb in Asian countries, grown in kitchen gardens and used for culinary purpose.[14] On revising the reviews and the studies of the medicinal plant *Coleus aromaticus* till the date, a majority of studies has been worked on its phytochemical profile of different active principles rich in alkaloids, polyphenols, flavonoids, and pharmacognostic studies, pharmacological activities such as antidiabetic, anti-inflammatory; antioxidant, antibacterial, anti-microbiological, hepatoprotective, anticarcinogenic & anti-mutagenic etc,.[14-21]

However studies on *invitro* antioxidant activities in the different antioxidant parameters have not been done in terms of ferrous ion chelating and ferric ion reducing activity. Thus in the present study, an effort has been made to overview the iron chelating activity, ferric ion reducing activity and anti-glycation activities of *Coleus aromaticus* leaves protein.

Materials and Methods

All the chemicals and reagents used were of Analytical grade were purchased from Chetana chemicals, Mysore and the Merck Co, and S.d. fine chem., Mumbai, India.

Extraction of Protein from *Coleus aromaticus* Leaves

Coleus aromaticus leaves collected from authentic source,

cleaned with 0.1% $KMnO_4$ solution, followed with double distilled water, crushed, shade dried and powdered (British Pharmacopoeia 100 mesh) and stored in glass bottle. The 5gm of *Coleus aromaticus* leaves powder mixed with 100 ml of double distilled water and vortexed for 4 hours. The vortexed mixture is centrifuged at 8000 rpm for 20 minutes at -4°C, the supernatant was separated. The supernatant was subjected to 65% ammonium sulphate precipitation and vortexed over night. The mixture was centrifuged at 8000 rpm for 20 minutes at -4°C. The precipitated proteins was collected and subjected to dialysis using 2.5kDa molecular cutoff bio-membrane against double distilled water for 76 hours with an interval of 6 hours. The dialyzed *Coleus aromaticus* leaves protein (CALP) was separated and stored at -10°C for further analysis.

Phytochemical Analysis

The dialyzed *Coleus aromaticus* leaves protein (CALP) obtained was analyzed for phytochemical components using following standard protocols.

The proteins estimation was carried according to Bradford's method using BSA as standard and absorbance was read at 535nm.[22] Total phenols were determined according to the method of Folin-Ciocalteu reaction using Gallic acid as a standard and absorbance was read at 750 nm[23]. Flavonoids estimation was done using Quercetin as a standard; absorbance was measured at 415 nm.[24] Total Sugars estimation was done according to Dubois method and the absorbance was read at 520 nm.[25] The concentrations were calculated accordingly using standard graph.

The protein content in dialyzed CALP is considerably higher than the same in other solvent extracts. This work already published and continuous work of Santhosh et.al, 2018.[26] The high concentrated CALP selected for ferrous ion chelating and Ferric ion reducing power assays and Anti-glycation activity assays.

Ferrous Ion Chelating Ability of CALP

Ferrous ion chelating ability of CALP was measured according to the method of Suter and Richter (2000) with minor modifications.[27] The reaction solution containing 100 µl of 200µM ferrous chloride ($FeCl_2$) and 200 µl of 400µM potassium ferricyanide with or without CALP at various concentrations (20 to 120µl) and EDTA as standard was added and the volume of the mixture was finally adjusted to 2 ml with distilled water. The reaction mixture was incubated at 20°C for 10 minutes. Formation of the potassium hexacyanoferrate complex was measured at 700 nm. The control was without any chelating compounds. The ability of the extract to chelate ferrous ion was calculated using the following formula.

$$\% \text{ Ferrous ion chelating ability} = \frac{(\text{Abs of Control} - \text{Abs of Sample})}{\text{Abs of Control}} \times 100$$

Ferric ion reducing power of CALP

The reducing power of the extracts was evaluated by the method described of *Oyaizu* (*1986*) with minor modifications of P. Jayanthi et.al, 2011.[28,29] Ascorbic acid used as standard and extract of CALP in different concentrations (20µl to 100µl) were mixed with 2.5ml of 0.2 mM phosphate buffer, (pH 6.6) and add 2.5 ml of 1% potassium ferricyanide then incubate at 50°C for 10 min. 2.5 ml of 10% trichloroacetic acid was added to this mixture, followed by centrifugation at 3000 rpm for 10 min. Subsequently, 2 ml of upper layer of mixture was added to 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride, and then incubated at ambient temperature for 10 min. The absorbance was measured at 700 nm. Increase in absorbance reflects increased reducing power.

***In vitro* Methods Employed in Anti-glycation Tudies**

Inhibition of Alpha Amylase Wnzyme

The CALP and standard drug Acarbose (100-500µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min. After this, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.[30-32]

Inhibition of Alpha Glucosidase Enzyme

The inhibitory activity was determined in a dose dependent manner along with standard drug Acarbose (100 to 500ug) by incubating a solution of starch substrate (2% w/v maltose or sucrose) 1ml with 0.2 M Tris buffer (pH 8.0) and various concentration of CALP for 5 min at 37°C. The reaction was initiated by adding 1ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37°C. Then, 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.[33-35]

Glucose Uptake in Yeast Cells

Yeast cells were prepared according to the method of Gupta et al., 2013 with minor modifications. Yeast cells briefly, commercial baker's yeast was washed in distilled water by repeated centrifugation till the supernatant clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of CALP (10–50 µg) were added to 1mL of glucose solution (5, 10 and 25 mM) and incubated together for 10 min at 37°C. Reaction was started by adding 100 µl of yeast suspension, vortexed and further incubated at 37°C for 60 min. After 60 min, the tubes were centrifuged and glucose was estimated in the supernatant. Metformin was taken as standard drug. The percentage increase in glucose uptake by yeast cells was calculated. All the tests were performed in triplicate.[36-38]

Statistical Analysis

All data are expressed as mean ± standard deviation of triplet (n=3). The significance of the experimental observation was checked by student's t-test and $P < 0.05$ was considered as statistically significant when compared to relevant controls.

Table 1: % Inhibition iron chelating activity of *Coleus aromaticus* protein and standards

Concentration (µg/ml)	% Inhibition of Iron Chelating Activity	
	EDTA	*Coleus aromaticus* Protein
20	15.73 ± 0.54	5.237 ± 0.986
40	39.39 ± 0.497	18.02 ± 3.041
60	49.68 ± 1.194	38.74 ± 0.551
80	71.6 ± 0.231	50.54 ± 1.067
100	90.26 ± 0.931	62.01±0.422
120	89.34 ± 0.435	61.12±0.243

All data are expressed as mean ± standard deviation of triplet (n=3).

Results

Iron chelating ability of crude protein of *Coleus aromaticus* at different concentrations was determined and result showed (Table 1). The color complex formed by the interaction of potassium ferricyanide with ferrous ions is decreased by the action of metal chelator compounds that exist in the reaction mixtures. In this study, the *Coleus aromaticus* protein shows maximum chelating activity of 62.01±0.422 significantly, when compared to standard chelator of EDTA is 90.26 ± 0.931, indicates strong the ferrous ion binding strength at 700nm.

Table 2: Ferric ion reducing activity of *Coleus* ***aromaticus*** Protein & standards

Concentration (µg/ml)	Absorbance at 700nm	
	Ascorbic acid	*Coleus aromaticus* Protein
20	0.601 ± 0.008	0.028 ± 0.002
40	0.746 ± 0.048	0.134 ± 0.011
60	0.834 ± 0.046	0.201 ± 0.011
80	0.837 ± 0.016	0.313 ± 0.015
100	0.882 ± 0.001	0.456 ± 0.029
120	0.853 ± 0.023	0.412 ± 0.031

All data are expressed as mean ± standard deviation of triplet (n = 3).

The *Coleus aromaticus* protein shows maximum reduction potential is 0.456±0.029 compared to standard ascorbic acid is 0.882±0.001 at 700nm shown in Table 2. High absorbance reflects more reducing ability of the samples. The extract showed good reducing power ability in a concentration dependent manner, which was comparable to that of ascorbic acid. Reducing power of the extracts increases with the increase in concentration.

The dose-dependent *in vitro* α-amylase inhibitory activity of CALP was done as explained in methods. It was found that, there is increase in percentage inhibitory activity with the increase in dosage against α-amylase enzyme. As a standard drug, Acarbose was used with similar dosage to compare inhibitory capacity of CALP.

Fig. 1 showed that, the % inhibitory activity of CALP ranges a minimum of 34.21±0.12 (at 100µg/ml) to a maximum of 69.13±0.23 (at 500µg/ml) where as the standard drug Acarbose showed % inhibitory activity ranges from 25.14±0.11 (at 100µg/ml) to a maximum of 62.12 (at 500µg/ml).

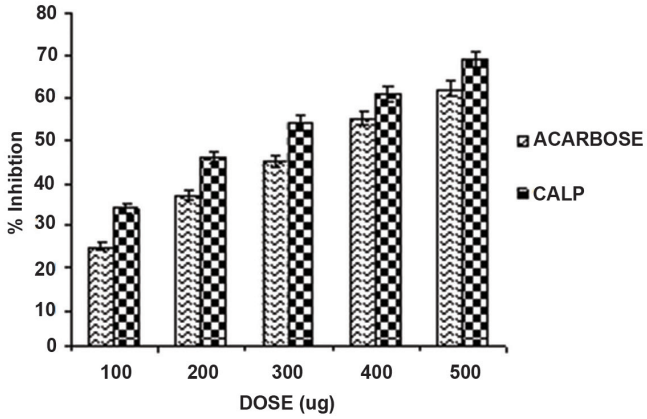

Fig.1: α-amylase inhibitory activity by CALP

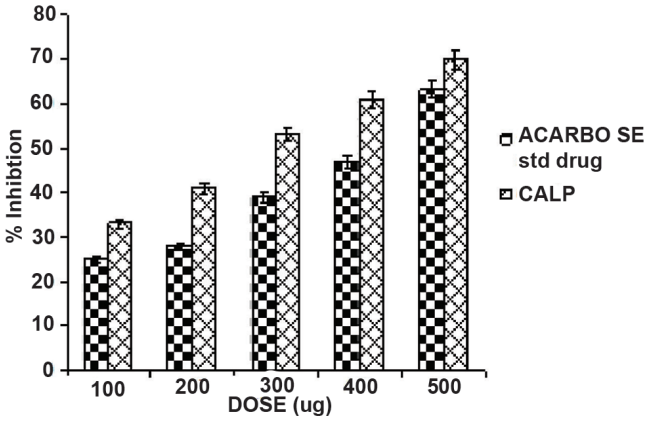

Fig. 2: The *in vitro* alpha-glucosidase inhibitory activity **of** CALP

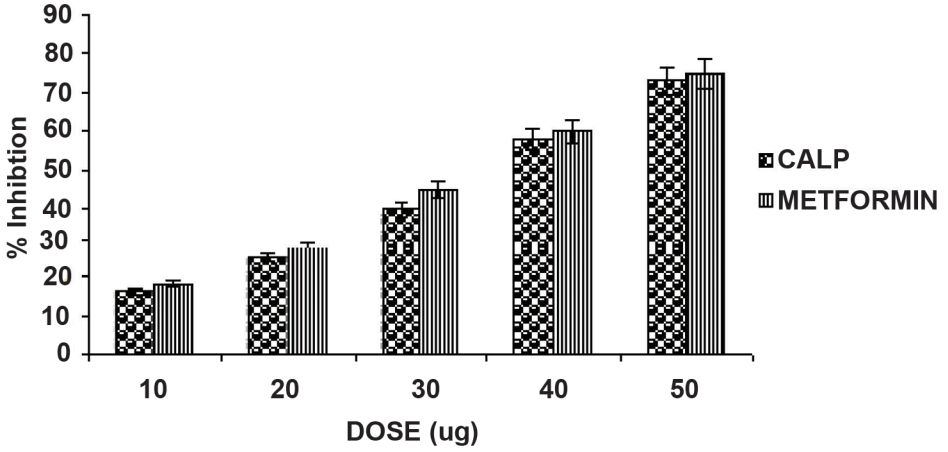

Fig. 3: Glucose uptake in yeast cells by CALP

The *invitro* α-glucosidase inhibitory activity of CALP was studied as explained in methods. It was found that, there is increase in percentage inhibitory activity with the increase in dosage against α-glucosidase. As a standard drug, Acarbose was used with similar dosage to compare inhibitory capacity of the *Coleus aromaticus Protein.* Fig. 2 showed that, the % inhibitory activity of CALP ranges a minimum of 33.13±0.22 (at 100µg/ml) to a maximum of 70.31±0.21 (at 500µg/ml) where as the standard drug Acarbose showed % inhibitory activity ranges from 25.11±0.13 (at 100µg/ml) to a maximum of 63.21 (at 500µg/ml).

The rate of glucose transport across cell membrane in yeast cells system is as presented in Fig. 3**.** In Yeast (*Saccharomyces cerevisiae*) glucose transport takes place via diffusion. Diabetes like type -II is described as the deficiency of insulin resulting in increased amount of glucose in blood. After the treatment of the yeast cells with these CALP in a dose dependent manner, the glucose uptake was found to increase. The % increase in glucose uptake by the yeast cell at different glucose concentrations i.e. 25mM, 10mM and 5mM respectively. The CALP exhibited significant activity at all glucose concentrations in comparison with standard drug Metformin.

Discussion

Natural antioxidants are alternative to synthetic antioxidants in counteracting oxidative stress associated diseases. The antioxidant activity is demonstrated in various in vitro methods. In this study, CALP at different concentrations was assessed for their ferrous ion chelating and ferric ion reducing activity in an *in vitro* model.[30]

Chelating activity of *Coleus aromaticus* protein at different concentrations are determined by the color complex formed by the interaction of potassium ferricyanide with ferrous ions concentrations is decreased by the action of metal chelator compounds that exist in the reaction mixtures. In the present study, the *Coleus aromaticus* protein shows chelating activity is 62.01±0.938 significantly, when compare to standard chelator of EDTA is 90.26 ± 0.938, indicates strong the ferrous ion W strength at 700nm.

Iron is required for oxygen transport and activity of many enzymes. Reducing property is based on the ability of extract to reduce concentration of ferric ions to ferrous ions. The compounds with antioxidant capacity react with potassium ferricyanide to form potassium ferrocyanide, which then reacts with ferric chloride, to form ferric-ferrous complex. In this study, reducing property of *Coleus aromaticus* protein was significant when compared with ascorbic acid as standard.

Therefore, search for antiglycative and antioxidant agents from various plant sources is gaining lot of importance. There are several reports, which mentions about the identification of antiglycative and antioxidant agents from different medicinal plant species. Bains Y and Gugliucci A, 2017, reported that, presence of chlorogenic acid, caffeic acid in the mate tea (*Ilex paraguaiensis*) are responsible for the anti-glycation effect,[39] and Adel Yarizade et,al 2017, reported that, leaves of Custard apple leaves showing promising antiglycation and antioxidant properties.[40] In the present study the protein of *Coleus aromaticus* leaves when compared with standard drugs Acarbose and Metformin, showed significant anti-glycation activity.

Conclusion

The C*oleus aromaticus* leaves protein has shown a significant iron chelating ability, reducing property and anti-glycation activity. The C*oleus aromaticus* protein might contribute a certain level of health protection against oxidative damages. There are very limited in vivo studies reporting therapeutic potentials, which needs to be taken to further heights.

Conflict of Interest: None.

References

1. Fadzai Boora, Elaine Chirisa, and Stanley Mukanganyama. Evaluation of Nitrite Radical Scavenging Properties of Selected Zimbabwean Plant Extracts and Their Phytoconstituents. *J Food Processing* 2014;7-14.
2. Ebrahimzadeh M. A., Nabavi S. M., Nabavi S. F, Bahramian F and Bekhradnia A. R. "Antioxidant and free radical scavenging

activity of H. officinalis L. var. angustifolius, V. odorata, B. hyrcana and C. speciosum," *Pak J Pharm Sci* 2010;23(1):29–34.

3. Rao A. S. V. C, Reddy S. G., Babu P. P. and Reddy A. R. "The antioxidant and anti-proliferative activities of methanolic extracts from Njavara rice bran," *BMC Complement Altern Med* 2010;10(4).
4. Rhitajit Sarkar, Bibhabasu Hazra, and Nripendranath Mandal. Reducing power and iron chelating property of Terminalia chebula (Retz.) alleviates iron induced liver toxicity in mice. *BMC Complement Altern Med* 2012;12:144.
5. Kruszewski M. Labile iron pool: the main determinant of cellular response to oxidative stress. *Mutat Res* 2003;531:81–92.
6. Liu ZD, Hider RC. Design of iron chelators with therapeutic application. *Coord Chem Rev* 2002;232:151–71.
7. Birch N, Wang X, Chong HS. Iron chelators as therapeutic iron depletion agents. *Expert Opin Ther Patents* 2006;16:1533–56.
8. Patel R. M.. Ferrous ion chelating activity (FICA): a comparative antioxidant activity evaluation of extracts of eleven naturally growing plants of Gujarat, India," *Int J Sci Res* 2013;2(8).
9. Plaza M, Amigo-Benavent M, del Castillo M.D, Ibáñez, E. and Herrero M. Neoformation of antioxidants in glycation model systems treated under subcritical water extraction conditions. *Food Res Int* 2010;43(4):1123-9.
10. Hashim Z and Zarina S. Advanced glycation end products in diabetic and non-diabetic human subjects suffering from cataract. *Age* 2011,1;33(3):377-84.
11. Ott C, Jacobs K, Haucke E, Santos AN, Grune T and Simm A. Role of advanced glycation end products in cellular signaling. *Redox Biol* 2014;1(2):411-29.
12. Kumaran. C, Omkar. N G, Dinesha. R, Santhosh Kumar. N, Tejaswini BM. Anti-glycation and antioxidant activities of Polysaccharides from Muntingia Calabura roots. *International Journal of Medical Science and Current Research* (*IJMSCR*) 2018;1(2):224-9.
13. Kumar U. A., Manjunath C, Thaminzhmani T, Kiran Y. R. and Brahmaiah, Y. A review on immunomodulatory activity plants. *Indian J Novel Drug Deliv* (*IJNDD*)2012;4(2):93–103.
14. Rashmi Sahay Khare, Shanta Banerjee and Kanika Kundu. *Coleus aromaticus* benth – A nutritive medicinal plant of potential therapeutic value. *Int J Pharma Bio Sci* 2011;2(3):488-500.
15. Subhas Candrappa M, Shivakumar Hugar Itgappa M, Nagarajappa K. Antidiabetic and Antioxidant Potential of *Coleus aromaticus* leaf extracts in alloxan induced diabetic rats. *Pharmacol* 2009;3:1054-61.
16. Neelam Begum, C. Mayuren, K. Sateesh, K. L. Sowjanya, Raviteja N and. Rajesh Kumar N. V. N. Screening of anti-inflammatory activity of *Coleus Aromaticus*. *Int J Chem Sci* 2009;7(4):2384-8.
17. Subhas chandrappa M, Harsha R., Dinesha R., Thammanna Gowda SS. Antibacterial activity of *Coleus aromaticus* leaves. *Int J Pharm Pharm Sci,* 2010; 2(3):63-6.
18. Annie Pritima R, Selvaraj Pandian R. Antimicrobial activity of coleus aromaticus (benth) against microbes of reproductive tract infections among women. *Afr J Infect Dis* 2011;1(1):18–24.
19. Revathi A, Thangabalan B, Vengal Rao P, Vadivel K. Anti microbiological Activity of Essential Oil Extracted from Coleus aromaticus Linn. Leaves. *RJPBCS*, 2014;2(1):12-5.
20. Vijayavel K, Anbuselvam C, and Ashok Kumar B. Protective effect of *Coleus aromaticus* Benth (Lamiaceae) against naphthalene-induced hepatotoxicity. *Biomed Environ Sci* 2013;26(4):295-302.
21. Ramalakshmi, P., Subramanian, N., Saravanan, R, Mohana krishnan, H and Muthu, M. Anticancer effect of *Coleus Ambionicus* (karpooravalli) on human lung cancer cell line (A549). *Int J Dev Res* 2014;4(11):2442-9.
22. Bradford M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein –dye binding anal. *biochem* 1976;72:248-54.
23. Singleton V, Rossi A. Colorimetry of total phenolic with phosphomolybdic/ phospho tungstic acid reagent. *Am J Enol Vitic* 1965;16:144-58.
24. Chang C, Yang M, Wen H, Chern J. Estimation of Flavonoid content in Propolis by Two Complementary methods. *J Food Drug Anal* 2002;10:178-82.
25. Dubois M, Giles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for the determination of sugars and related substances. *Anal Chem* 1956;28:350-6.
26. Santhosh Kumar N, Aliya Nusrath, Dinesha Ramadas. Quantitative Analysis of chemical constituents in medicinal plant *Coleus aromaticus* extracts. *J Res Med Sci* 2018;6(3):1002-5.
27. Suter M, Richter C. Anti- and pro-oxidative properties of PADMA 28, a Tibetan herbal formulation. *Redox Rep* 2000;5(1):17-22.
28. Oyaizu M. Studies on products of browning reaction: Antioxidant activities of products of browning reaction prepared from glucosamine. *Jpn J Nutr* 1986;44:307-15.
29. Jayanthi P and Lalitha P. Reducing power of the solvent extracts of Eichhornia Crassipes (MART.) Solms. *Int J Pharm Pharm Sci* 2011;3(3):126-8.
30. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity. assays. *J Agric Food Chem* 2005;53:1841-56.
31. Thalapaneni N.R., Chidambaram K.A., Ellappan T., Sabapati M.L., Mandal S.C.. Inhibition of of carbohydrate digestive enzymes by Talinum portulacifolium (Forssk) leaf extract. *J Complement Integr Med* 2008;5(1):1-10.
32. Heidari, R., Zareae S., and Heidarizadeh M.. Extraction, purification, and inhibitory effect of alpha-amylase inhibitor from wheat (Triticum aestivum Var. Zarrin). *Pak J Nutr* 2005;4:101-5.
33. Narkhede, M.B., Ajimire P,V., Wagh A.E., Manoj Mohan, Shivashanmugam A.T. In vitro antidiabetic activity of Caesalpina digyna (R.) methanol root extract," *Asian J Plant Sci Res* 2011;1(2):101-6.
34. Andrade-Cetto., Adolfo., Jaime Becerra-Jiménez., René Cárdenas-Vázquez. Alfa-glucosidase-inhibiting activity of some Mexican plants used in the treatment of type 2 diabetes." *J Ethnopharmacol*, 2008;116(1):27-32.
35. Matsuura H., Asakawa C., Kurimoto M., and Mizutani., α-Glucosidase inhibitor from the seeds of balsam pear (Momordica charantia) and the fruit bodies of Grifola frondosa." Bioscience, biotechnology, and biochemistry., 2002;1576-78.
36. Chikkanna D, Dinesha Ramadas & Santhosh Kumar N. Anti diabetic activity of ginger tuber proteins Invitro studies." *Asian J Pharm Health Sci* 2016;6(2):1472-6.
37. Suganya G., Sampath kumar P., Dheeba B, Sivakumar R. In Vitro Antidiabetic, Antioxidant And Anti-Inflammatory Activity Of Clitoria Ternatea L." *Int J Pharm Pharm Sci* 2014;6(7):342-7.
38. Rajesh P., Dinesh Kumar Damiki Laloo and Siva Hemalatha.. Evaluation of phytochemical and antioxidant activities of the

different fractions of Hybanthus enneaspermus Linn F Muell Violaceae. *Asian Pac J Trop Med* 2009;391-6.

39. Bains Y and Gugliucci A. Ilex paraguariensis and its main component chlorogenic acid inhibit fructose formation of advanced glycation endproducts with amino acids at conditions compatible with those in the digestive system. *Fitoterapia* 2017;1:117:6-10.
40. Adel Yarizade, Ali Niazi, Hassan Hassani Kumleh. Investigation of antiglycation and antioxidant, potential of some antidiabetic medicinal plants, *J Pharm Sci Res* 2017;9(12):2382-7.

How to cite this article: Santhosh Kumar N, Kalaivanam KN, Nusrath A, Ramadas D. Iron chelating, reducing and anti-glycation activities of *Coleus aromaticus* leaves protein. *Int J Clin Biochem Res* 2019;6(2):*170-5*.