

Anti-glycation and antioxidant properties of *Abutilon indicum* plant leaves

Dinesha Ramadas ^{1,5}, Santhosh Kumar Nune ^{2,3}, Chikkanna D ³, Maheshwara KV ⁴ and Vedamurthy Joshi ^{5,*}

¹ Adichunchanagiri Institute for Molecular Medicine, Adichunchanagiri Institute for Medical Sciences, Adichunchanagiri University, BG Nagara-571448, Karnataka, India.

² Department of Medical Biochemistry, Shridevi Institute of Medical Sciences & Research Hospital, Tumakur, Karnataka, India.

³ Department of Medical Biochemistry, Adichunchanagiri Institute for Medical Sciences, Adichunchanagiri University, BG Nagara-571448, Karnataka, India.

⁴ GIPS, Nijaganahally, KR Nagara Tq, Mysore Dist, Karnataka, India.

⁵ Department of Pharmaceutics, CORMIL & CMPAT, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B.G. Nagara-571 448, Karnataka, India.

Abstract

The study was done to investigate the *in vitro* anti-diabetic activity of Ethanol-water extract of *Abutilon indicum* plant leaves. The phytochemicals present in the extract was analyzed by using standard methods. *In vitro* antioxidant study was done using DPPH radical scavenging activity. The *In vitro* anti-diabetic studies were done by alpha amylase enzyme, alpha glucosidases enzyme inhibition studies and Glucose uptake in Yeast cells studies. The phytochemical analysis showed that the extract rich with proteins, polyphenols, flavonoids, alkaloids, tannins, polysaccharides. The *in vitro* antiglycation potential of extract was confirmed through alpha amylase enzyme, alpha glucosidases enzyme inhibition studies and Glucose uptake in Yeast cells studies. The results of the present study showed the significant antioxidant and antidiabetic activity. The potential pharmacological activity of *Abutilon indicum* might be the presence of phytochemicals.

Keywords: Anti-diabetic; Antioxidant; *Abutilon indicum*; Phytochemicals

1. Introduction

Many chronic diseases like Cancer, Alzheimer disease, Parkinson disease, Diabetes, Cardio vascular diseases, Inflammation, Viral infections, Autoimmune pathologies, etc are due to vital role of reactive oxygen species (ROS) [1-3]. Lipid peroxidation can induce by ROS and hence, promotes glycation of protein and also in immune deficient diseases [4-6].

A non-enzymatic reaction like Glycation takes place in amino groups of proteins and reducing sugars [7-8]. This process is associated with the pathogenesis of age and diabetes related complications. In living organisms, it represents post translational status of proteins, which can impair their functions. In the above glycation process, if the oxidative step is involved then it is called glycoxidation. During which, advanced glycation end (AGE) products like free radicals, products of the autooxidation of the glycating sugar, and a heterogeneous group of substances are formed [9-10].

* Corresponding author: Vedamurthy Joshi

Department of Pharmaceutics, CORMIL & CMPAT, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B.G. Nagara-571 448, Karnataka.

In India, *Abutilon indicum* plant commonly called as “Thuthi” in hindi, “Atibala” in Kannada, is a native plant of South Asia. *Abutilon indicum* (Linn.) is a perennial plant and it grows very well in even in dry and poor soils [11]. Natural sources or herbs or spices or medicines are effective in action without (sometimes considerably very less) side effects. From Vedic period, India is one of the most medico- culturally diverse countries in the world where the medicinal plant sector is a part of time- honored tradition had value even today. The usage of the plant highly cited in Ayurveda, Unani, Chinese medicine and Siddha practices. The various parts of the plant *Abutilon* such as roots, leaves, bark, seeds and even flowers are documented to possess various medicinal properties in ethnobotanical surveys conducted by ethnobotanists and in traditional systems of medicine such as Ayurveda [12]. The extracts of the whole plant *Abutilon* were scientifically evaluated for tonic and oja, vardhaka, augment and ojas, the subtle essence of all vital fluids, responsible for health, harmony and spiritual growth [13]. Since then large variety of compounds have been isolated from genus *Abutilon* and majorities of them are flavonoids, steroids, terpenoids and phenolics [14].

2. Material and methods

All required plant materials, chemicals and reagents used were of analytical grade were procured from authentic source, Merck Co, and S.d. fine chem., Mumbai, India.

2.1. Extraction of *Abutilon indicum* plant leaves (AIPL)

Abutilon indicum plant was identified by authorized Botanist. The plant leaves were collected and washed with double distilled water, crushed into paste form and stored in glass bottle. The 5gm of *Abutilon indicum* plant leaves paste mixed with 100 ml of 50:50 Ethanol and double distilled water and vortexed for 4 hours. The vortexed mixture was allowed to settle and filtered using glass wool. The supernatant volume reduced to required volume by using vacuum concentrator.

2.2. Phytochemical Analysis

The extraction of *Abutilon indicum* plant leaves (AIPL) 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. Total phenols were determined according to the method of Folin-Ciocalteu reaction using Gallic acid as a standard and absorbance was read at 750 nm². Flavonoids estimation was done using Quercetin as a standard; absorbance was measured at 415 nm. Total Sugars estimation was done according to Dubois method and the absorbance was read at 520 nm. The alkaloid estimation and the tannin estimation were done as per the standard protocols [15-21]. The concentration was calculated accordingly using standard graph.

2.3. In vitro antioxidant and anti-glycation studies

2.3.1. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity of AIPL

DPPH radical scavenging activity study was done in a dose dependent concentration of 0 to 10 µg each was mixed in 1 ml of freshly prepared 0.5 mM DPPH ethanolic solution and 2 ml of 0.1 M acetate buffer pH 5.5. The resulting solutions were then incubated at 37°C for 30 min and measured spectrophotometrically at 517 nm. Ascorbic acid and α-tocopherol was used as positive control under the same assay conditions. Negative control was without any inhibitor or AIPL. Lower absorbance at 517 nm represents higher DPPH scavenging activity. The % DPPH radical scavenging activity of extracts of AIPL was calculated from the decrease in absorbance at 517 nm in comparison with negative control [22].

2.4. Inhibition of Alpha Amylase Enzyme

The AIPL 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 represents 100% enzyme activity and were conducted in similar way by replacing extract with vehicle [23].

2.5. 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 AIPL 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 [24-26].

2.6. Glucose Uptake in Yeast Cells

Yeast cells were prepared according to the method of Gupta et al., 2013 with minor modifications. Commercially procured 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 AIPL (10–50 μ g) 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 μ L 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 [27-29].

2.7. Statistical Analysis

All data are expressed as mean $\pm$ 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.

3. Results and discussion

The ethanol and water extract of *Abutilon indicum* plant leaves found rich Polyphenols, polysaccharides and moderate amount of alkaloids, proteins and flavonoids.

Table 1 "DPPH radical scavenging & Anti-glycation activity of ethanol and water extract of *Abutilon indicum* plant leaves

	% Inhibition of DPPH radicals scavenging activity	% Inhibition of glycation activity
<i>Abutilon indicum</i> plant leaves extract (25 μ g/ml)	62	60
Ascorbic acid (10 μ g/ml)	56	78
Vitamin – E (10 μ g/ml)	68	72

3.1. Evaluation of DPPH radical scavenging potential of AIPL

A dose dependent DPPH radical scavenging activity of AIPL was done where well known antioxidants Ascorbic acid and α -tocopherol are used as positive control. AIPL (25 μ g) showed 62%, Ascorbic acid (56%) and α -tocopherol (68%) at a highest dose of 10 μ g concentration (Table 1). The above results showed AIPL is a potential DPPH scavenger in comparison with Ascorbic acid and α -tocopherol. The antioxidant property of AIPL could be due to the supply of hydrogen, which combined with radicals and thus forming a stable radical.

3.2. Evaluation of anti-glycation activity

The inhibition study for the production of AGEs was carried out for AIPL. The extract was able to inhibit the production AGEs by 60% at a maximum dosage of 25 μ g in comparison to pure Ascorbic acid (78%) and Vitamin - E (72%) at much lower dose (Table 1). The ethanol – water crude extract of AIPL acted as a glycation inhibitor because of its free radical scavenging property. The effectiveness of AIPL in inhibiting DPPH radical formation and AGEs formation can speak about its uses for diabetic patients.

The effect of AIPL on the formation of fructosamine was studied by the reduction of NBT from the 1st day of incubation to the 3rd day. AIPL inhibited fructosamine formation by 71% after 3 days of incubation. The possible explanation for the less formation of fructosamine in AIPL treated sample was that might have the ability to modify the amino or carbonyl groups in the Millard reaction that resulted in the inhibition of fructosamine formation.

The above two studies suggested that the inhibition of glycation exhibited by AIPL was not only due to its DPPH radical scavenging property but also due to the modification in the amino or carbonyl groups in the Millard reaction, which resulted in the inhibition of fructosamine formation.

4. Conclusion

The results of the present work indicated that the phytochemicals isolated from *Abutilon indicum* plant leaves extract (AIPL) possessed antioxidant, and anti-glycation properties. However, the *in vivo* antioxidant activity and the mechanism of action need to be further studied.

Compliance with ethical standards

Acknowledgments

The authors gratefully acknowledge the financial support of Adichunchanagiri Institute for Molecular Medicine, Adichunchanagiri Institute of Medical Sciences, Adichunchanagiri University, BG. Nagara.

Disclosure of conflict of interest

All authors declared no conflicts of interest.

References

- [1] Mylarappa B, Ningappa, Ramadas Dinesha, Leela Srinivas. Antioxidant and free radical scavenging activities of polyphenol-enriched curry leaf extract (*Murraya koenigii* L.) Food Chemistry. 2008; 106: 720-728.
- [2] Smitha S, Dhananjaya BL, Dinesha R, Leela Srinivas. Purification and characterization of a ~34 KDa antioxidant protein (beta-turmerin) from Turmeric (*Curcuma longa*) waste grits, Biochemie. 2009; 91: 1156-1162.
- [3] Kopp W. How western diet and lifestyle drive the pandemic of obesity and civilization diseases. Diabetes, metabolic syndrome and obesity: targets and therapy. 2019; 12: 2221.
- [4] Dinesha R, Leela Srinivas, BGS-Haridrin – A new antioxidant glycoprotein of Turmeric (*Curcuma longa* L), Indian Journal of Nutrition and Dietetics. 2010; 47: 118-121.
- [5] Fournet M, Bonté F, Desmoulière A. Glycation damage: A possible hub for major pathophysiological disorders and aging. Aging and disease. 2018; 9(5): 880.
- [6] Dinesh KN, Dinesha R, Leela Srinivas. Antioxidant activity of Indian medicinal plants, Indian Journal of Nutrition and Dietetics. 2014; 51: 39.
- [7] Sarmah S, Pahari S, Das S, Belwal VK, Jana M, Singha Roy A. Non-enzymatic glycation of human serum albumin modulates its binding efficacy towards bioactive flavonoid chrysin: A detailed study using multi-spectroscopic and computational methods. Journal of Biomolecular Structure and Dynamics. 2021; 39(2): 476-92.
- [8] Hemmler D, Roullier-Gall C, Marshall JW, Rychlik M, Taylor AJ, Schmitt-Kopplin P. Insights into the chemistry of non-enzymatic browning reactions in different ribose-amino acid model systems. Scientific reports. 2018; 8(1): 01-10.
- [9] Sobhy R, Zhan F, Mekawi E, Khalifa I, Liang H, Li B. The non-covalent conjugations of bovine serum albumin with three structurally different phytosterols exerted antiglycation effects: A study with AGEs-inhibition, multispectral, and docking investigations. Bioorganic chemistry. 2020; 94: 103478.
- [10] Mohamed Azmathulla Khan Y, Dinesha Ramadas, Subhas Chandrappa Mundasada, Santosh Kumar N, Harsha R Kashyap, Chikkanna D. *In vitro* anti-diabetic activity of Muntingia Calabura root proteins, World journal of pharmacy and pharmaceutical sciences. 2015; 4(10): 1526-1534.
- [11] Akbar S. Handbook of 200 Medicinal Plants: A Comprehensive Review of Their Traditional Medical Uses and Scientific Justifications. 2020.
- [12] Ganesan K, Xu B. Ethnobotanical studies on folkloric medicinal plants in Nainamalai, Namakkal District, Tamil Nadu, India. Trends in Phytochemical Research. 2017; 1(3): 153-68.
- [13] Rajeshwari S, Sevakodiyone SP. Medicinal properties of *Abutilon Indicum*. Open Journal of Plant Science. 2018; 3(1): 022-025.
- [14] Surywanshi VS, Umate SR. A Review on Phytochemical Constituents of *Abutilon indicum* (Link) Sweet-An Important Medicinal Plant in Ayurveda. Plantae Scientia. 2020; 3(3): 15-9.

- [15] Bradford MM. 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.
- [16] Singleton V, Rossi A. Colorimetry of total phenolic with phosphomolybdic/ phospho tungstic acid reagent. Am J Enol Vitic. 1965; 16: 144-58.
- [17] 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.
- [18] 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.
- [19] Shamsa F, Monsef H, Ghamooshi R, Verdian-rizi M. Spectrophotometric determination of total alkaloids in some Iranian medicinal plants. Thai J Pharm. Sci. 2008; 32: 17–20.
- [20] Waterman PG, Mole S. Analysis of phenolic plant metabolites. Blackwell Scientific Publications, Oxford, UK. 1994.
- [21] Karamać M. Chelation of Cu (II), Zn(II), and Fe(II) by tannin constituents of selected edible nuts" Int J Mol Sci. 2009; 10(12): 5485–5497.
- [22] Kalaivanam KN, Santhosh Kumar N, Dinesha R. *In vitro* Anti-oxidant activity of Crude protein of Muntingia Calabura leaves extract. International Journal of Biotechnology and Biochemistry. 2018; 14(1): 71-76.
- [23] Heidari R, Zareae S, Heidarizadeh M. Extraction, purification, and inhibitory effect of alpha-amylase inhibitor from wheat (Triticum aestivum Var. Zarrin). Pak J Nutr. 2005; 4: 101-5.
- [24] Narkhede, MB, Ajimire PV, Wagh AE, Manoj Mohan, Shivashanmugam AT. *In vitro* antidiabetic activity of Caesalpina digyna (R.) methanol root extract," Asian J Plant Sci Res. 2011; 1(2): 101-6.
- [25] 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.
- [26] Matsuura H, Asakawa C, Kurimoto M, 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.
- [27] Chikkanna D, Dinesha Ramadas, Santhosh Kumar N. Anti diabetic activity of ginger tuber proteins *In vitro* studies. Asian J Pharm Health Sci. 2016; 6(2): 1472-6.
- [28] 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.
- [29] Rajesh P, Dinesh Kumar Damiki Laloo, 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.