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Antiglycation potential of Indigoferin a, Indigoferin B and Indigoferin C natural products from *Indigofera heterantha* Brandis

Ayesha Khan¹, Ajmal Khan^{2*}, Manzoor Ahmad³, Mumtaz Ali³, Umar Farooq^{1*}, Farhan Ahmad Khan¹ and Syed Majid Bukhari¹

Abstract

Background: Diabetes is a long-lasting and serious disease that effect in worldwide individual lives, families, and societies. Hyperglycemia of diabetes mellitus produced Advance Glycation End Products that are associated with diabetic complications like neuropathy, nephropathy, retinopathy, and cardiovascular diseases.

Methods: In this study, the natural products isolated from of *Indigofera heterantha* Brandis, Indigoferin A (S1), Indigoferin B (S2) and Indigoferin C (S3) were evaluated for their in vitro antiglycation activity.

Results: The compounds exhibited a significant inhibitory activity against the formation of Advanced Glycation End-Products with IC₅₀ values of 674.25 ± 3.2 μM, 407.03 ± 4.7 μM and 726.41 ± 2.1 μM, respectively. Here, important structure-activity relationship was observed, when the intramolecular hydrogen bonding interactions suppressed the antiglycation activity of compound S3. Thus, the study clearly demonstrates that the number and the position of substituents act as an assisting factor and directly influence the inhibitory activity of the natural product by altering the sugar or protein binding affinity.

Conclusions: This study explain first time the antiglycation inhibitory ability of chemical constituents isolated from *I. heterantha* and can be used for above late diabetic complications.

Keywords: *Indigofera heterantha* Brandis, Advanced Glycation end-products, Antiglycation activity

* Correspondence: ajmalchemist@yahoo.com; umarf@cuiatd.edu.pk

²Natural and Medical Sciences Research Center, University of Nizwa, 616 Birkat Al Mauz, PO Box 33 Nizwa, Oman

¹Department of Chemistry, COMSATS University Islamabad Abbottabad Campus, Abbottabad, Pakistan

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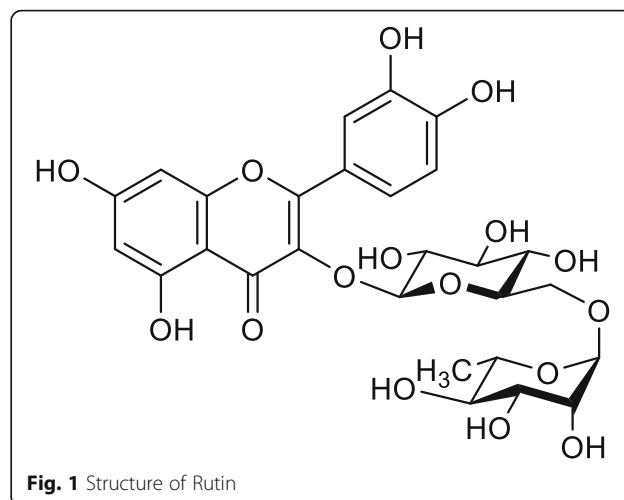
Introduction

Indigofera heterantha Brandis (*I. heterantha*) belong to family Fabaceae, is a small tree or shrub widely distributed in tropical and subtropical regions with pinnate leaves. The name *Indigofera* is due to the presence of indigo flowers in most of the species of this family. The plants of this genus have many important medicinal properties, thus are widely used as folk medicine for the treatment of whooping cough, hepatitis [1], and tooth ache [2]. Previous studies showed that compounds isolated from of this genus act as an excellent anti-inflammatory agent specially for snake bite or insect sting, possess good antimicrobial, antifungal [3], antibacterial [4], and urease inhibitory activities [5]. Due to several important medicinal properties exhibited by the compounds isolated from the *I. heterantha*, it was envisioned to investigate the natural products isolated from this specie for their in vitro antiglycation potential. The antiglycation activity of natural products from *I. heterantha* have not been explored previously, which make them interesting candidates for investigation into their diverse biological properties.

Diabetes mellitus causes high blood sugar level leading to a condition commonly termed as hyperglycemia [6]. Hyperglycemia when persist for longer period of time facilitates the synthesis of special non-enzymatic glycated products called Advance Glycation End Products (AGEs) [7]. Previous studies have revealed a positive association between tissue AGEs and microvascular as well as macrovascular complications related to diabetes [8], with glucose acting as a long-term fuel for diabetic complications [9]. Several factors contribute towards the development of AGEs including the duration and the degree of hyperglycemia, tissues permeability to free blood glucose, and protein's half-life [10]. AGEs through crosslinking cellular matrix of long-lived proteins alter the tissue function and mechanical properties [11] resulting in onset of late diabetic complications like neuropathy, nephropathy, retinopathy, and cardiovascular diseases.

Currently various pharmacological and natural antiglycating agents have been under investigation to prevent the formation of AGEs. These include aminoguanidine, rutin (Fig. 1), pyridoxamine, antioxidants, aspirin, and RAGE blockers. Several synthetic compounds and plant extracts have also shown significant antiglycation activity [12]. Aged garlic extract exhibited excellent antiglycation activity in vitro [13]. Polysaccharide fractions extracted from pumpkin and *Punica granatum* have also been reported as good inhibitors for glycation [14, 15].

The current study describes the antiglycation activities of three natural compounds indigoferin A (S1), indigoferin B (S2) and indigoferin C (S3, Fig. 2) isolated from *I. heterantha* [5]. The detailed extraction and isolation has been published, while the compounds had been characterized based on 1D and 2D NMR data. This is the first report on the antiglycating activity of these compounds.



Materials and method

Chemicals and reagents

Precoated aluminum sheets and silica gel 60F-254 were purchased from E. Merck. All solvents, such as methanol (HPLC grade), n-hexane (HPLC grade), chloroform (HPLC grade), ethyl acetate (HPLC grade), n-butanol (HPLC grade) and ceric sulphate reagent (laboratory grade) were purchased from Sigma-Aldrich. Chemicals including dimethylsulfoxide (DMSO, analytical grade), glucose anhydrous (laboratory grade), disodium hydrogen phosphate (Na_2HPO_4 , laboratory grade), sodium azide (NaN_3 , laboratory grade) and sodium dihydrogen phosphate (NaH_2PO_4 , laboratory grade), methylglyoxal (MG, laboratory grade) were purchased from Sigma-Aldrich, while bovine serum albumin (BSA, laboratory grade) was purchased from Research Organics, Cleveland (USA).

Plant material

I. heterantha Wall were collected from upper Dir, Khyber Pakhtunkhwa (Pakistan), during the month of April 2005. The plant was identified by Prof. Dr. Jahandar Shah, plant taxonomist, University of Malakand, Chakdara. The voucher specimen number GI-014 was placed in the herbarium of botany department, University of Malakand, Chakdara, Dir (L), Pakistan.

Extraction and isolation

The detail extraction and isolation of these compounds Indigoferin A (S1), Indigoferin B (S2) and Indigoferin C (S3) from *I. heterantha* were published in our previous article [5]. The collected *I. heterantha* Wall (10 kg) was shade dried for 3 weeks, followed by pulverization into fine powder, then soaked in MeOH (80% v/v) with rare stirring at room temperature. After 2 weeks, the material was filtered 3 times and the filtrate obtained was concentrated *in vacuo* at 40 °C. The MeOH extract (463.5 g) obtained was then suspended in distilled water and extracted

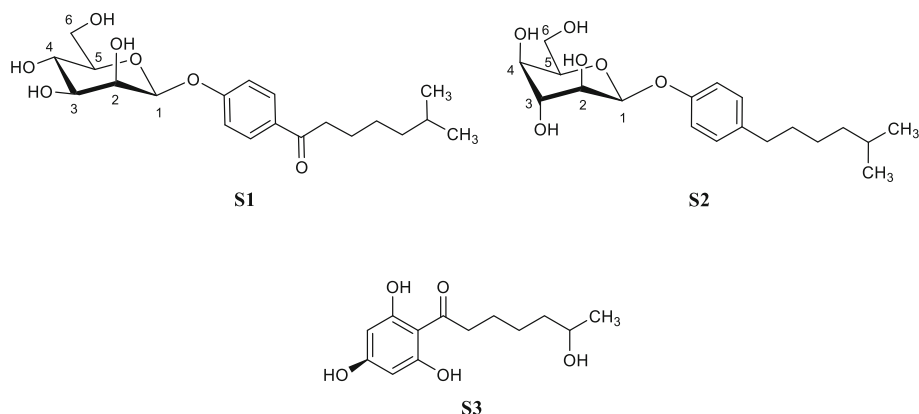


Fig. 2 Structures of compounds isolated from *I. heterantha* [5]

with *n*-hexane (20.71% w/w), chloroform (15.96% w/w), ethyl acetate (12.94% w/w), and *n*-butanol (19.41% w/w), and finally the aqueous (30.96% w/w) fraction was obtained. Each organic extract was then evaporated to dryness.

In vitro Antiglycation assay

Buffer was prepared by mixing a calculated amount of Na_2HPO_4 and NaH_2PO_4 along with NaN_3 , for preventing the bacterial interactions and pH was maintained at 7.4 with concentrations 67 mM and 3 mM respectively. BSA (10 mg/mL) and MG (50 mg/mL) solutions were prepared in buffer while test samples (1 mM/mL) were prepared in DMSO. In vitro antiglycation activity was performed according to the reported method [16] with few modifications. The samples (S1, S2 and S3) were prepared in DMSO at 1 mM concentration. For IC_{50} serial dilutions were used. Triplicate samples, in a 96-well plate assay each well having a reaction mixture of 200 μL , a glycosylated control containing 20 μL of test compound solution, 50 μL of BSA, 50 μL of MG, and 80 μL of phosphate buffer, while blank control containing 20 μL of DMSO was incubated for 9 days maintaining the temperature at 37 °C. After incubation assessment of fluorescence (excitation at 330 nm and emission at 440 nm) for the change in fluorescence intensity was evaluated using microplate ELISA reader, Spectra Max Plus³⁸⁴ (Molecular Devices, CA, USA) at 37 °C. The percentage inhibition for each compound was calculated by using formula.

$$\% \text{inhibition} = \left(1 - \frac{\text{fluorescence}_{\text{test compound}}}{\text{fluorescence}_{\text{control}}} \right) \times 100$$

Rutin (Fig. 1) is used as a positive control with IC_{50} value of $294.5 \pm 1.5 \mu\text{M}$.

Statistical analysis

S.E.M = IC_{50} value was presented as mean \pm S.E.M (standard error of the mean) calculated by using the formula

$$S.E.M = \frac{s}{\sqrt{N}}$$

Where *s* = sample standard deviation

$$s = \sqrt{\frac{1}{N-1} \sum_{i=1}^N (x_i - \bar{x})^2}$$

$x_i - x_n$ = sample data set

\bar{x} = mean value of sample data

Results and discussion

Extraction and isolation

Compounds Indigoferin A (S1) (89 mg), Indigoferin B (S2) (105 mg) and Indigoferin C (S3) (145 mg) were isolated as black gummy solid, brown powder and yellow amorphous powder, respectively from the ethyl acetate fraction of the methanolic extracts of *I. heterantha*. The detail spectroscopic data of all these compounds were published in our previous article [5]. On the basis of spectroscopic data, the structures of all compounds were determined as Indigoferin-A [(6-methyl-1-(4-((2S,3S,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yloxy)phenyl)heptan-1-one)], Indigoferin-B, (2R,3R,4R,5R,6S)-2-(hydroxymethyl)-6-(4-(5-methylhexyl)phenoxy)tetrahydro-2H-pyran-3,4,5-triol and Indigoferin-C 6-hydroxy-1-(2-(2,4,6-trihydroxyphenyl)heptan-1-one).

In vitro Antiglycation activity

To explore the medicinal importance of compounds from *I. heterantha*, the compounds Indigoferin A (S1), Indigoferin B (S2) and Indigoferin C (S3) were evaluated for their in vitro antiglycation potential. Here, all three natural products were found to be significantly active with IC_{50} values of $674.25 \pm 3.2 \mu\text{M}$, $407.03 \pm 4.7 \mu\text{M}$ and $726.41 \pm 2.1 \mu\text{M}$, respectively albeit lower in potency than the standard rutin (Table. 1). Although the potency of S1, S2, and S3

Table 1 In-vitro antiglycation potential of compounds isolated from *I. heterantha* ($N = 3$)

Compounds	%Inhibition	IC ₅₀ ± S.E.M ^a (μM)
S1	52.10	674.25 ± 3.2
S2	75.17	407.03 ± 4.7
S3	59.08	726.41 ± 2.1
Rutin ^b	83.23	294.5 ± 1.5

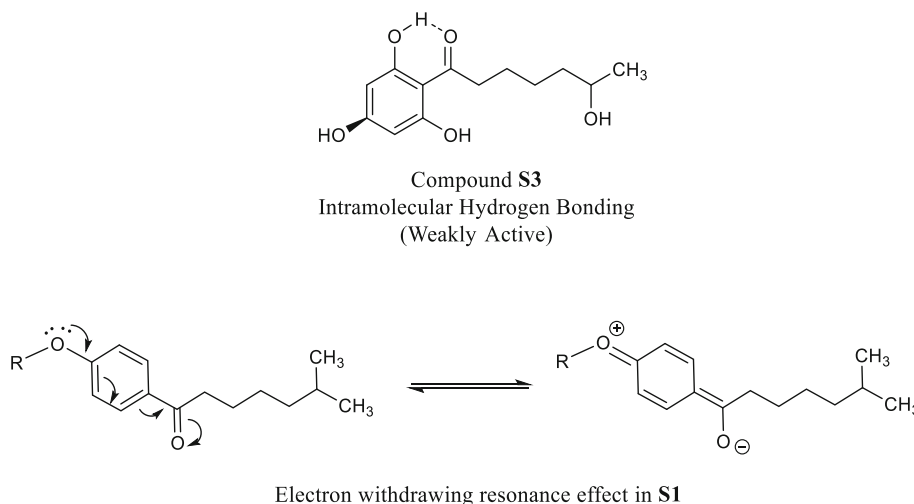
^aS.E.M Standard error of mean^bRutin = standard inhibitor for antiglycation assay

are lower than the standard but are potent to show inhibitory effect. The difference potential is attributed to the structural differences in compounds and the standard rutin, whereas rutin is a disaccharide with a α -substituted chromen-4-one (with hydroxyl and phenyl substituents), while **S1** and **S2** are monosaccharides with β -phenyl substituents, while **S3** lacks the sugar moiety. The compound **S2** ($407.03 \pm 4.7 \mu\text{M}$) was identified to possess better inhibitory potential compared to the other two natural products **S1** and **S3**. While the compound **S3** ($726.41 \pm 2.1 \mu\text{M}$) was found to be the least active among the isolated natural products, which could be attributed to the intramolecular hydrogen bonding between the *ortho* substituted hydroxyl group and the carbonyl oxygen (Fig. 3). The unavailability of hydroxyl group to interact via intermolecular hydrogen bonding with the protein can be the contributing factor to the lower potency of the natural product **S3**. The suppressing influence of intramolecular hydrogen bonding on activity of synthetic compounds was previously published [17]

for synthetic compounds but the phenomenon was first time discussed for natural compounds.

The comparison of inhibitory potential of **S1** ($674.25 \pm 3.2 \mu\text{M}$) and **S2** ($407.03 \pm 4.7 \mu\text{M}$) shows a significant difference (Fig. 4), which can be due to the structural differences between the two compounds. Here, the lower inhibitory potential of **S1** could be due to the presence of carbonyl group, which extends resonance creating a dominant electron withdrawing effect (Fig. 3) [17, 18]. However, **S2** has significant antiglycation activity due to absence of carbonyl group and multiple hydroxyl groups [18, 19]. Thus, the substituent and its position remarkably effect the protein or sugar binding activity of each analogue.

Additionally, it was observed that the presence of sugar moiety and its stereochemistry also influences the antiglycation potential of compounds. The absence of sugar moiety in **S3** could be another contributing factors towards the lower inhibitory potential of **S3** compared to the other two compounds with sugar moiety. While the comparison of **S1** and **S2** clearly exhibit the influence of the stereochemistry of sugar moiety upon antiglycation activity of these compounds. Here, the **S1** with mannose sugar moiety has lower inhibitory potential than **S2**, while the change of stereochemistry at position 3 and 4 of sugar moiety in **S2** led to the better inhibitory potential against glycation (Fig. 2). The hydroxyl at position 3 and 4 of the sugar moiety could be involved in facilitating the hydrogen bonding interactions of hydroxyls with the binding site [17–19]. Overall, this study provides a useful insight into the structure-activity relationship and antiglycation potential of natural products from *I. heterantha*.

**Fig. 3** Intramolecular hydrogen bonding interaction of *I. heterantha* (S3)

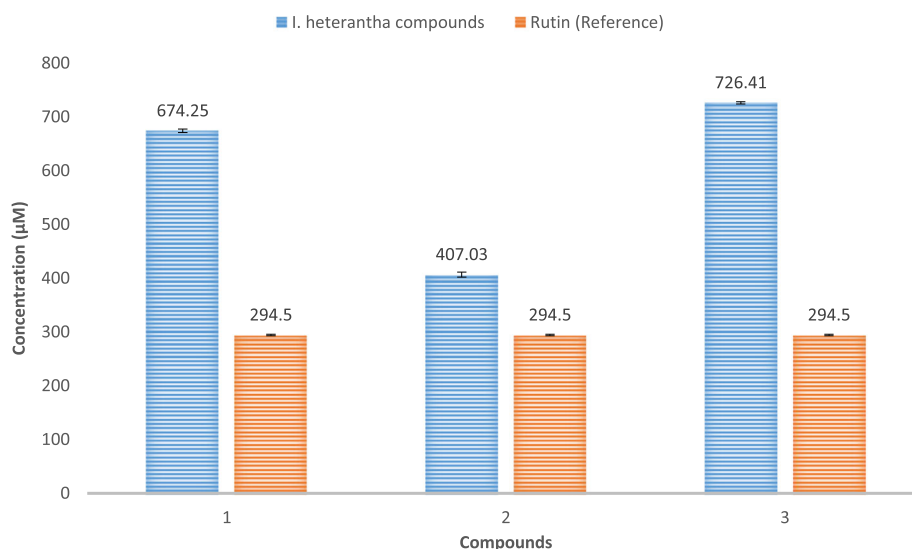


Fig. 4 Comparison of in-vitro antiglycation activity of *I. heterantha* compounds with reference compound (rutin). Number of sample treatment (N) = 3. The results were expressed as mean \pm SEM

Conclusion

Here, we report for the first time the antiglycation inhibitory ability of natural products isolated from *I. heterantha*. The study explains the influence of the stereochemistry of sugar moiety, the availability of hydroxyl groups for hydrogen bonding interactions with the binding site, and the influence of electron withdrawing resonance effect upon antiglycation potential of the natural products. The compounds exhibit different extent of potential influenced by the number and the position, and the stereochemistry of hydroxyl substituents and influenced their inhibitory activity. Additionally, compound **S2** could be utilized as the starting point for structure-activity relationship studies aimed at designing potent antiglycating agent.

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Authors' contributions

AK and UF conceived and designed the study. Ayesha K. performed antiglycation activity. MA and M. Ali performed isolation, and FAH and SMB analyzed the data. AK and Ayesha K. wrote the manuscript with inputs and comments from all co-authors. All authors have read and approved the final version of the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not Applicable.

Consent for publication

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Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Chemistry, COMSATS University Islamabad Abbottabad Campus, Abbottabad, Pakistan. ²Natural and Medical Sciences Research Center, University of Nizwa, 616 Birkat Al Mauz, PO Box 33 Nizwa, Oman. ³Department of Chemistry, University of Malakand, Chakdara, Dir (L), Pakistan.

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