Overview on natural dyes and their IR-spectra – Part II: Indigo containing plant dyes

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ABSTRACT
This review paper is part of a paper series reporting on natural dyestuffs and their infrared spectra (IR spectra). The current part II is related to the dye indigo (vat blue 1). Indigo is probably the most used and the most important dye. It is related to the typical clothing product – the blue jeans. In current study, the IR spectra of natural indigos from different origin are determined and compared to the IR spectrum of synthetic indigo. Further discussed materials are a powder of indigo leaves, a pigment named indigo green, the dye indigotine blue and the natural indigoid dye tyrian purple. The recorded spectra are discussed with respect to the chemical structure of the dye and with view to the presence of possible byproducts. It is possible to estimate the origin of natural indigo by infrared spectroscopic measurements of a certain plant source. The presented overview can be used as helpful tool for persons working in the field of dyeing or quality control to support and help them to identify products by IR-spectroscopic methods.

Keywords
indigo, natural dyes, plant based dyes, infrared spectroscopy, indican, indigo green, luteolin, indigotine blue, tyrian purple

1 Introduction
Due to the world wide distributed and most famous fashion product – the blue jeans – the dye indigo is one of the most used and most important dyestuffs [1-4]. The chemical structure of indigo is determined by the indigoid chromophore containing an aromatic ring system with keto groups and amino groups (compare the chemical structure in Figure 1) [2]. The sum formula of indigo is C_16H_10N_2O_2, corresponding to a molecular weight of 262 g/mol. Indigo is applied as vat dye on cellulosic natural fibers as cotton or linen. It is also named as vat blue 1 and indicated with the number C.I. 73000 [5-8]. Indigo ring dyeing
and dye recipes for cotton yarns dedicated for denim fabrics are reported [9]. Nowadays, indigo dye is mainly produced by chemical synthesis using the Heumann-Pfleger process [10,11]. Beside the conventional chemical synthesis of the indigo dye, in recent years different approaches for the biotechnological production of indigo are developed which may offer the chance for a sustainable future of this important dyestuff [12,13]. Indigo dye can be produced by fermentation with bacteria or by using enzymes [12,14-16]. However, before invention of chemical synthesis of indigo, the dye was gained as natural product from different plants. The indigo plant (true indigo) with scientific name Indigofera tinctorial can occur in different varieties containing different yield and dye content. From 100 g leaves from indigo plant around 0.1 g of dye can be gained [17].

![Chemical structure of the dye Indigo.](image)

Fig. 1 Chemical structure of the dye Indigo.

Even if in most industrial dyeing processes synthetic indigo is used, the interest in using natural based indigo is still strong and reflected by many literature and publications in this field [18-20]. The popular trend of sustainability may enhance this interest in natural indigo in near future [21-23]. With this background, the currently presented overview like to give a summary on several natural based indigo products and support a comparison to synthetic indigo. The current overview is the second article in a series on natural dyestuffs and their infrared spectra. The first article in this series is published recently on the field of naphthoquinone and anthraquinone containing natural dyes [24]. The IR spectra of natural indigos from different origin are determined and compared to the IR spectrum of synthetic indigo. Further discussed materials are a powder of indigo leaves, a pigment named indigo green, the dye indigotine blue and the natural indigoid dye tyrian purple. Infrared spectra (IR spectra) are recorded from these dye products, with the aim to support spectral data for material identification and discussion of chemical structure. Further, the recorded spectra are discussed with respect to the chemical structure of the dye and with view to the presence of possible byproducts. The infrared spectroscopy is an established analytic method in the organic and polymer chemistry [25-27]. It can be used for identification and quantification of higher plants and plant substances [28,29]. This spectroscopic method is also very suitable for identification of synthetic and natural fiber materials and leather alternatives [30-34]. One aim of the current overview is to support IR spectra of indigo products for analytical purposes and for quality control.

2 Materials and Methods

2.1 Investigated indigo containing materials

In the current overview different indigo containing products from natural origin are considered. These materials are supplied by three different companies – Kremer Pigmente GmbH (Aichstetten, Germany), Pflanzenfärbershop (Hückelhoven-Baal, Germany) and AMA Herbal (India). A comparison is made with synthetic indigo supplied as pure chemical by the company Carl Roth GmbH (Karlsruhe, Germany). The investigated materials are summarized in Table 1 together with the related product web pages of the suppliers to support additionally detailed product information and information on work safety.
Table 1. Investigated indigo dyestuffs and further materials.

<table>
<thead>
<tr>
<th>No.</th>
<th>Content</th>
<th>Supplier</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>I1</td>
<td>Synthetic indigo / C.I. 73000</td>
<td>Carl Roth</td>
<td>[35]</td>
</tr>
<tr>
<td>I2</td>
<td>Indigo, genuine</td>
<td>Kremer</td>
<td>[36]</td>
</tr>
<tr>
<td>I3</td>
<td>Indigo, genuine</td>
<td>Pflanzenfärbershop</td>
<td>[37]</td>
</tr>
<tr>
<td>I4</td>
<td>Indigo, Japanese, Polygonum</td>
<td>Kremer</td>
<td>[38]</td>
</tr>
<tr>
<td>I5</td>
<td>Indigo from Woad</td>
<td>Kremer</td>
<td>[39]</td>
</tr>
<tr>
<td>I6</td>
<td>Indigo from Woad</td>
<td>Pflanzenfärbershop</td>
<td>[40]</td>
</tr>
<tr>
<td>I7</td>
<td>Indigo from Dyer’s Knotweed / France</td>
<td>Pflanzenfärbershop</td>
<td>[41]</td>
</tr>
<tr>
<td>I8</td>
<td>Indigo, genuine / Guatemala</td>
<td>Pflanzenfärbershop</td>
<td>[42]</td>
</tr>
<tr>
<td>I9</td>
<td>Indigo, bio indigo product</td>
<td>Ama Herbal</td>
<td>[43]</td>
</tr>
<tr>
<td>I10</td>
<td>Indigo leaves, powder</td>
<td>Kremer</td>
<td>[44]</td>
</tr>
<tr>
<td>I11</td>
<td>Indigo green pigment</td>
<td>Kremer</td>
<td>[45]</td>
</tr>
<tr>
<td>I12</td>
<td>Indigotine Blue</td>
<td>Kremer</td>
<td>[46]</td>
</tr>
<tr>
<td>I13</td>
<td>Tyrian Purple, genuine</td>
<td>Kremer</td>
<td>[47]</td>
</tr>
</tbody>
</table>

2.2 Infrared spectroscopy

The infrared spectra (IR spectra) of investigated dyes are recorded with a FT-IR spectrometer IRTracer-100 from Shimadzu (Japan) using a Specac Golden Gate ATR unit. For spectroscopic measurements the dyes are taken as powders as received from the suppliers without further purification.

3 Results and Discussion

3.1 Indigo

The infrared spectrum of pure synthetic indigo (C.I.73000) is shown in Figure 2. According to supplier information this product exhibits a purity of >95%. The IR spectrum of this synthetic indigo shows a broad signal at 3254 cm\(^{-1}\) related to the N-H stretching vibration in the indigo molecule. The weak signal at 3059 cm\(^{-1}\) is assigned to C-H stretching vibration of the hydrogen atoms bonded to the aromatic ring system of indigo [25,26]. The strong peak at 1599 cm\(^{-1}\) is assigned to the C=O stretching vibration of the keto group. Compared to the peak position for C=O vibration of keto groups in other compounds, the peak for the keto group from indigo appears at lower wavenumber. A probable reason for this shift to lower wavenumber can be the weakening of the keto group by formation of intramolecular hydrogen bridge bonds to the N-H unit next by in the indigo molecule [48]. The strongest signal in the IR spectrum is determined at 1061 cm\(^{-1}\) and can be assigned to a rocking vibration of the C=O bond. However, this signal is also assigned to the vibration of an aromatic ring with five carbon atoms neighbored to an aromatic ring with six carbon atoms. A further signal related to stretching vibration of ring systems is the signal at 1458 cm\(^{-1}\), which is assigned to a combined vibration of a C-C stretching vibration in the aromatic ring with an out-of-plane C-H vibration. The signal at 694 cm\(^{-1}\) is related to the wagging vibration of the C=O bond [49]. The signals at 1389 cm\(^{-1}\) and 1121 cm\(^{-1}\) can be attributed to a combination of rocking vibration of the N-H bond with a stretching vibration of the C-N bond [49].
Fig. 2 Infrared spectrum of synthetic dye Indigo (product I1 listed in Table 1).

The IR spectra of genuine indigo (C.I.75780) from natural source – indigo plant – are presented in Figure 3. In actual investigation, two different products from two different suppliers are compared. Both indigo products are gained from the plant Indigofera tinctorial grown in India [36, 37]. For both samples the same strong signals in the fingerprint area are identified. An excellent overview and discussion of infrared and raman spectra of natural indigo and indirubin is given by Ju et al. [50]. However, Ju et al. discuss these spectra mainly as pure substance and not under the respect that a natural dye product also contains several by-products. The IR spectra of the two investigated products exhibit mainly 11 strong signals in the fingerprint area at around following positions – 1611 cm\(^{-1}\), 1460 cm\(^{-1}\), 1392 cm\(^{-1}\), 1298 cm\(^{-1}\), 1171 cm\(^{-1}\), 1124 cm\(^{-1}\), 1066 cm\(^{-1}\), 1009 cm\(^{-1}\), 878 cm\(^{-1}\), 752 cm\(^{-1}\) and 696 cm\(^{-1}\) (Figure 3). These signals can be roughly also identified in the IR spectrum of the synthetic indigo (compare Figure 2). For this, a comparison of the synthetic and natural products by infrared spectroscopy is possible. However, several differences in the spectra can be identified. For the natural product I2, weak signals at 2928 cm\(^{-1}\) and 2855 cm\(^{-1}\) are determined which are related to C-H stretching vibration of aliphatic components containing -CH\(_2\)- units [25]. This natural product probably contains additional to indigo also byproducts containing units with aliphatic chemical structure. The product I3 exhibits a clear peak at 1685 cm\(^{-1}\), which appears for product I2 only as a weak shoulder. This peak is probably related to a C=O stretching vibration which is not caused by the keto group of the indigo molecule. This is the second hint for possible byproduct in these natural indigo materials. The signals at 1611 cm\(^{-1}\), 1066 cm\(^{-1}\) and 697 cm\(^{-1}\) can by assigned to different vibrations of the C=O unit (stretching, rocking and wagging) [49].
The indigo containing product I4 is gained according to supplier information from the plant Japanese Polygonum. This plant is also named as Polygonum tinctorium [5,38]. Growth conditions and indigo yield from this subtropical plant are excellently reported by Angelini et al. [51]. Recently an interesting approach shows that by illumination with blue light the indigo yield from Polygonum tinctorium can be increased [52]. Other studies report on the antibacterial or antiviral activity of products from this plant [53,54]. An infrared spectrum recorded from indigo gained from the plant Japanese Polygonum is shown in Figure 4. The main nine peaks in the fingerprint area of the IR spectrum appear at 1612 cm\(^{-1}\), 1460 cm\(^{-1}\), 1394 cm\(^{-1}\), 1317 cm\(^{-1}\), 1171 cm\(^{-1}\), 1069 cm\(^{-1}\), 872 cm\(^{-1}\), 752 cm\(^{-1}\) and 698 cm\(^{-1}\). These peaks are in certain accordance to the spectra of genuine indigo products. The indigo content in this product I4 can be therefore identified. Similar to the spectrum of product I2, also the IR spectrum of product I4 exhibits a weak signal at around 2930 cm\(^{-1}\) assigned to a C-H stretching vibration of an aliphatic component. Probably also in the natural product I4 aliphatic byproducts are present.
In Europe historically the blue dye indigo is gained from the leaves of woad plant - Isatis tinctoria [5,55-57]. The IR spectra of two different indigo products gained from woad plant are presented in Figure 5. Both samples exhibit mainly similar signals in the fingerprint area of the spectrum at around 1626 cm⁻¹, 1460 cm⁻¹, 1317 cm⁻¹, 1066 cm⁻¹, 1008 cm⁻¹ and 872 cm⁻¹. By those signals the presence of indigo from Woad can be identified. However, for product I6 additional signals in the fingerprint area are determined, so the composition of both natural products is not exactly similar. Both products, exhibits a broad signal at around 3260 cm⁻¹ assigned to O-H and N-H stretching vibrations. For product I5, two weak signals at 3065 cm⁻¹ and 2930 cm⁻¹ are determined. These signals are related to C-H stretching vibrations on aromatic and aliphatic units. For product I6 only a very weak signal at 2944 cm⁻¹ assigned to C-H stretching vibration of aliphatic -CH₂- unit is determined. Probably both samples contain aliphatic by-products.

![Fig. 5 Comparison of IR spectra of powders from Indigo from Woad. Shown are the products I5 and I6 (compare Table 1).](image)

To complete the view on commercially available indigo products, the infrared spectra of another three indigo products are recorded and presented in Figures 6 and 7. The product I7 is from the plant dyer’s knotweed (Polgonum tinctorium) planted in France [41]. The product I8 is gained from the plant Indigofera suffruticosa grown in Central America. This product I8 is sold under the name Guatemala indigo [42]. The infrared spectra of both indigo products are compared in Figure 6. In both IR spectra the main signals in the fingerprint area can be detected, so the indigo is clearly identified. The IR spectrum of product I7 does not exhibit a signal around 2940 cm⁻¹, which would be related to the C-H stretching vibration of an aliphatic unit. For this, for the indigo product I7 a lower content of aliphatic by-products compared to the other natural indigo products can be estimated.

The indigo product I9 is named by the supplier as Bio indigo product and especially the advantages for sustainability compared to synthetic indigo products are claimed by the supplier [43]. This product is gained from the plant Indigofera tinctorial grown in India. The infrared spectrum of product I9 is presented in Figure 7. The main signals in the fingerprint area can be detected, so basically the indigo content can be identified.

The main signals from infrared spectra gained from evaluation of all nine indigo products, their assignments and related components are summarized in Table 2. Here selected main signals are listed with their spectral range, where they are occurring. It is also stated, if the signal is related to indigo by itself or a possible by-product in the natural product.
Fig. 6 Comparison of IR spectra of powders from Indigo from different origin (from Dyer’s Knotweed / France and genuine Indigo / Guatemala). Shown are the products I7 and I8 (compare Table 1).

Fig. 7 Infrared spectrum of Indigo, bio indigo product (product I9 listed in Table 1).

Table 2. Overview on some main signals in IR spectra of indigo products, their assignments and related component.

<table>
<thead>
<tr>
<th>Signal range (cm⁻¹)</th>
<th>assignment</th>
<th>Related component</th>
</tr>
</thead>
<tbody>
<tr>
<td>3270 – 3240 (broad / medium)</td>
<td>N-H and O-H stretching vibration</td>
<td>Indigo for N-H vibration; also for by-products with OH groups / O-H vibration</td>
</tr>
<tr>
<td>Around 3060 (weak)</td>
<td>C-H stretching, aromatic unit</td>
<td>Indigo</td>
</tr>
<tr>
<td>2930 – 2850 (weak)</td>
<td>C-H stretching, aliphatic unit</td>
<td>By-product with aliphatic units</td>
</tr>
<tr>
<td>1685 (weak or shoulder)</td>
<td>C=O stretching vibration</td>
<td>By-product</td>
</tr>
<tr>
<td>Around 1610 (strong)</td>
<td>C=O stretching, keto unit</td>
<td>Indigo</td>
</tr>
<tr>
<td>Around 1460 (strong)</td>
<td>Combined vibration of C-C stretching from C6 ring and C-H out-of-plane</td>
<td>Indigo</td>
</tr>
<tr>
<td>Around 1065 (strong)</td>
<td>C=O rocking vibration / combined different C-C stretching from ring systems</td>
<td>Indigo</td>
</tr>
<tr>
<td>Around 695 (strong)</td>
<td>C=O wagging vibration / combined</td>
<td>Indigo</td>
</tr>
</tbody>
</table>
3.2 Indigo leaves

The product I10 is a green colored powder from indigo leaves which is offered by the company Kremer Pigmente for dyeing purposes [44]. Indigo leaves contain the compound indicant from which by further processing blue indigo is formed [58]. The chemical structure of indican is shown in Figure 8. From chemical point of view here an indoxyl unit is bond to a glucose unit. Under acidic conditions or enzymatically these both units can be split. Following the Indoxyl can be oxidize by oxygen from air to the indigo molecules [11,30].

![Chemical structure of the compound indicant containing the indoxyl unit and the glucose unit.](image)

The infrared spectrum of the powder from indigo leaves is presented in Figure 9. This spectrum exhibits lower number of signals in the fingerprint area compared to the spectra of indigo dye products. Also, the determined signals are broader. The broad peak at 3292 cm\(^{-1}\) is related to N-H and O-H stretching vibration. The medium peaks at 2919 cm\(^{-1}\) and 2851 cm\(^{-1}\) are assigned to C-H stretching vibration in aliphatic units. The strong peak at 1636 cm\(^{-1}\) is probably related to C=O stretching vibration. Because the molecule of indican does not contain C=O units, this signal is caused by other components in the powder of the indigo leaves. The strongest signal at 1020 cm\(^{-1}\) can be assigned to C-O stretching vibrations. No signal around 1460 cm\(^{-1}\) can be identified, which is typical for all indigo products I1 to I9 discussed above.

![Infrared spectrum of indigo leaves, powder (product I10 listed in Table 1).](image)
3.3 Indigo green pigment

The supplier Kremer Pigmente offers a product named Indigo Green (product I11). According to supplier information, this green pigment is gained by joined precipitation of indigo and reseda [45]. The indigo green pigment is therefore a combined product of two different natural dyes. An indigo-green pigment is also reported to be detected on an ancient map [60]. One main color component of the reseda plant is the luteolin, which chemical structure is presented in Figure 10 [5,61,62].

![Chemical structure of Luteolin as a main color component in Reseda plant.](image)

The IR spectrum of the pigment indigo green (product I11) is presented in Figure 11. This IR-spectrum exhibits only few significant signals. A broad signal is determined at 3255 cm$^{-1}$ containing two shoulders at 3077 cm$^{-1}$ and 2936 cm$^{-1}$. This broad signal is assigned to the O-H and N-H stretching vibrations. The shoulders are assigned to C-H stretching vibrations of aromatic and aliphatic components. In the fingerprint area only three dominant peaks can be identified at 1626 cm$^{-1}$, 1412 cm$^{-1}$ and 1067 cm$^{-1}$. Here, the peak at 1626 cm$^{-1}$ can be assigned to C=O stretching vibrations. The signal at 1067 cm$^{-1}$ can be assigned to C-O stretching vibrations. The indigo typical peak around 1460 cm$^{-1}$ cannot be identified.

![Infrared spectrum of the pigment Indigo Green (product I11 listed in Table 1).](image)
3.4 Indigotine blue

Indigotine Blue is an acid dye (Acid Blue 74, CI 73015) which is also named as water-soluble indigo with the chemical formula \( \text{C}_{16}\text{H}_{8}\text{N}_{2}\text{Na}_{2}\text{O}_{8}\text{S}_{2} \). The chemical structure of indigotine blue is shown in Figure 12. This dye is also named as Indigocarmine and as food colorant under E132 [63]. Other names are Indigotin-5,5'-disulfonic acid or CI Natural Blue 2 [5]. Indigotine Blue is produced by heating indigo in presence of sulfuric acid. By this, the indigo molecules are sulfonated and by addition of two sulfonate functional groups a water solubility is gained. The optical spectra for visible light of indigo and indigotine blue are reported to be nearly similar, so the auxochromic effect of the attached sulfonic groups is small [64]. Due to lower lightfastness indigotine blue is usually used as an indicator dye and for food coloration. In case of application onto textile fabrics indigotine is dedicated for the dyeing of polyamide, silk or wool materials, because of its negatively charged sulfonic groups. Further, the dyeing of cotton is possible and the dyeing results can be enhanced by cationization of cotton [65]. The application onto cationized acrylic fibers is as well reported [66].

![Chemical structure of the compound Indigotin-5,5'-disulfonic acid (Indigotine Blue).](image)

The infrared spectrum of Indigotin blue (product I12) is presented in Figure 13. The broad signal with the clear maximum at 3361 cm\(^{-1}\) can be assigned to a N-H stretching vibration as it is expected from the chemical structure of the dye. The weak signal at 3084 cm\(^{-1}\) is related to C-H stretching vibration of hydrogen atoms bond to the aromatic ring system of the chromophore. The strong signals at 1610 cm\(^{-1}\) and 1635 cm\(^{-1}\) are caused by C=O stretching vibration of the keto group of the dye. The occurrence of two signals may be explained by different bonds and arrangement to hydrogen bridges (intra- and intermolecular). The very weak signal at 1703 cm\(^{-1}\) can be assigned to a C=O stretching vibration in an ester group. This could be a hint on small amounts of a by-product in this dye sample. The signal at 1469 cm\(^{-1}\) can be set in accordance to the typical indigo signal, detected as well for the products I1 to I9. The other signals in the fingerprint area are hardly to correlate with the infrared spectra recorded from the indigo products. Indigotine blue exhibits two sulfonate groups and according to literature such groups causes asymmetric stretch vibration in range of 1140 cm\(^{-1}\) to 1250 cm\(^{-1}\) and symmetric stretch vibration in the spectral range of 1030 cm\(^{-1}\) to 1070 cm\(^{-1}\) [19]. These stretch vibrations probably overlap with several vibrations caused by the chromophore of indigotine blue, so a clear assignment of the signals in the fingerprint area is not possible.
3.5 Tyrian purple

The infrared spectrum of natural tyrian purple is presented in Figure 14. Tyrian purple is also named as C.I. Natural Violet 1 (C.I. 75800) [5]. The main color component in tyrian purple is 6,6’-dibromindigo with the sum formula C_{16}H_{8}Br_{2}N_{2}O_{2} corresponding to a molecular weight of 420 g/mol (compare chemical structure in Figure 10). The chromophore of tyrian purple is similar to the indigo molecule but two bromine atoms are attached to the aromatic system as auxochromic groups. Tyrian purple is probably one of the oldest and most expensive pigments [67-69]. Same as indigo, tyrian purple is applied as vat dye. Remarkable is that tyrian purple is a brominated organic compound, which is quite unusual for natural organic compounds. A broad range of different brominated and chlorinated compounds can be generated by reaction of indigo with agents like bromine. These industrially produced brominated dyes can have a quite high durability in the environment [70].

![Chemical structure of the compound 6,6'-Dibromindigo (Tyrian purple).](image)

The infrared spectrum of tyrian purple is presented in figure 15. The broad signal at 3269 cm\(^{-1}\) is assigned to N-H and O-H stretching vibration. The shoulder at 3074 cm\(^{-1}\) is assigned to C-H stretching vibration of aromatic components. The three signals of different intensity at 2957 cm\(^{-1}\), 2921 cm\(^{-1}\) and 2852 cm\(^{-1}\) are related to C-H stretching vibration of aliphatic components. Because tyrian purple does not exhibit aliphatic units, these signals are caused by aliphatic by-products. In the fingerprint area several prominent signals are determined. The strongest peak at 1635 cm\(^{-1}\) is assigned to the C=O stretching vibration, which can be caused by tyrian purple but also by possible byproducts. The stretching vibration of the C-Br bond cannot be identified by the current investigation, because the signal
related to this vibration appears at wavenumbers below 700 cm\(^{-1}\) and is by this out of the actually determined measurement range [26].

![Infrared spectrum of the dye Tyrian Purple (product I13 listed in Table 1).](image)

**Fig. 15** Infrared spectrum of the dye Tyrian Purple (product I13 listed in Table 1).

### 4 Conclusions

Several indigo products from different natural sources are evaluated and compared with synthetic indigo. Main typical signals are identified in the fingerprint area of the IR spectra of these indigo materials. Typical for all indigo based products is a strong peak around 1460 cm\(^{-1}\). By these signals, indigo containing materials can be identified. Main differences in the spectra are related firstly to the occurring signals around 2900 cm\(^{-1}\) according to C-H stretching vibrations of aliphatic components. Second difference is the signal for C=O stretching vibration not caused by the keto group of the indigo molecule. These differences are probably caused by additional components in the natural originated indigo products. It is remarkable that all investigated indigo products I1 to I9 exhibit a clear peak around 1460 cm\(^{-1}\), which can be used for identification of indigo dye products. Nevertheless, indigo based products can be identified by IR-spectroscopy, so this spectroscopic method can be a helpful tool for identification of indigo based natural and synthetic products.

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All product and company names mentioned in this article may be trademarks of their respected owners, even without labeling. There is no conflict of interest and no funding for the presented research.

### Conflicts of Interest

The author declares no conflict of interest.

### References


