Influence of blanching and guar gum pretreatments on total phenolic content and antioxidant activity of cabinet-dried white bitter gourd *Momordica charantia* L.

Sonali Vatsyayan*
Amity Institute of Horticulture Studies and Research, Amity University Uttar Pradesh, Sector-125, Noida - 201313 (Uttar Pradesh), India

Ravinder Raina
Food and Agriculture Foundation, Amity University Uttar Pradesh, Sector-125, Noida - 201313 (Uttar Pradesh), India

Mukesh Kumar
School of Agriculture, IGNOU, New Delhi.

*Corresponding author. Email: sonalivatsyayn@rediffmail.com

**How to Cite**

**Abstract**
Currently, the global food industry is experiencing a shift in consumer preference towards natural sources of antioxidants derived from edible fruits and vegetables. In view of this, white skinned bitter gourd with bioactive properties has great potential. Additionally, guar gum has the characteristic ability to form strong hydrogen bonds with water molecules, thereby reducing shrinkage during dehydration. Thus, the present investigation was carried out to study the influence of blanching and guar gum pretreatment on the retention of the total phenolic content (TPC) stability and antioxidant activity (AA) of white *Momordica charantia* L. when cabinet dried at 60±5 °C. The results are indicative of a significant (P values of < 0.05) impact of using guar gum along with blanching before dehydration of white *Momordica charantia*. The findings obtained clearly show the positive impact of guar gum on the stability and retention of TPC (Folin Ciocalteau) and AA (percentage of 2,2-diphenyl-1-picrylhydrazyl DPPH scavenging activity) in the dried product in comparison to the untreated control. Water Blanching + 1% Guar gum (T5) and 2% Salt Blanching + 0.2% Potassium meta bisulphite + 1% Guar gum (T16) showed higher AA [20.29% (T5) and 40.13% (T16)] and TPC [30.8 (T5) and 39.5 (T16) GAE per 100 g of dried weight (DW)]. Therefore, the application of guar gum as a pretreatment with blanching turns out to be beneficial for higher retention of TPC and AA, thereby maintaining product quality as a whole.

**Keywords**: Antioxidant activity; Blanching; Guar gum; *Momordica charantia*; Total phenolic content

**INTRODUCTION**
Globally, with an increase in the population index, there has emerged an increased need for providing a continuous supply of food to all sections of society (Comunian et al., 2021). Unfortunately, this is not yet achievable in developing countries such as India due to small landholdings, poor waste management, and the miserable infrastructure of cold chains. With the exponential growth of the food industry in recent years, the focus of this growing industry has shifted towards developing functional products from natural sources to accomplish this. The health-conscious consumer base has increased manifold, making it necessary to develop new products with nutritional as well as phytochemical properties to combat lifestyle diseases such as diabetes, hypertension, obesity, and heart problems (Rodriguez-Jimenez et al., 2018). It has been reported that the regular consumption of antioxidants plays a crucial role in the prevention and treatment of ailments due to lifestyle and oxidative stress (Munekata et al., 2021). The food industry either relies on synthetic sources or natural sources for antioxidants, with the latter having a probable risk of toxicity or carcinogenesis. Bitter gourd, commonly known as "Karela", belongs to the family Cucurbitaceae, a tropical and subtropical...
crop (Tiwari et al., 2021). According to Sonifa (2018), India long green, India long white, and Hybrid India baby types of bitter gourds are commercially grown in India. Bitter gourd fruits have a peculiar bitter taste, and this bitterness of fruits shows variation according to the colour of the fruits. The fruits of green skin-coloured bitter gourd have a more bitter taste than white skin-coloured bitter gourd fruits (Islam and Jalaluddin, 2019). Bitter gourd is an important vegetable crop and is highly valued for its phytochemical and bioactive properties, particularly its polyphenols and antioxidant capacity (Kusat et al., 2021). Bitter gourd naturally consists of many phytometabolites with therapeutic potential (Gao et al., 2021). The poor shelf life of bitter gourd fruit is attributed to its climacteric type of respiration, although it is harvested in an immature stage for market purposes (Bhattacharjee and Dhua, 2017). Hence, Indian bitter gourd growers suffer huge losses every year due to a lack of postharvest technology, processing, and cold chain infrastructure (Kusat et al., 2021). To combat this situation, the process of dehydration of fresh bitter gourd fruits can come into hand.

According to Baradey et al. (2016), drying involves heat and mass transfer, which are mainly affected by the temperature of the air stream and the properties of the raw material. Drying ultimately results in the reduction of the moisture content of the product, thereby having a deliberate effect on enzymes, yeasts, molds, and bacteria (Srinivasan and Balusamy, 2015). The use of pretreatments before dehydration has shown an impact on the overall quality of the dried product (Sra et al., 2011). The raw material is subjected to pretreatments before dehydration to improve the quality of the dried product and reduce the drying losses. The pretreatment step helps in the loss of moisture from the cells, enzyme deactivation, improving permeability, and hastening the drying rate, thereby assisting in the retention of physicochemical properties and subsequently improving the shelf life of the dried product. The process of blanching also serves as a pretreatment for the deactivation of browning enzymes and minimises the microbial load of the product (Tayade et al., 2021). Guar gum is obtained from the endosperm of the Indian cluster bean (Cyamopsis tetragonoloba L.) plant and has diverse applications in the food industry owing to its molecular weight, long polymeric chain, high water solubility, ample supply, low cost, and green nature (Rastegar and Atrash, 2017). Therefore, combining blanching and pretreatment with the dehydration process helps to safeguard the total phenolic content and antioxidant activity along with brilliant product quality and preservation (Elangovan and Natarajan, 2021).

To the best of our knowledge, pretreated white bitter gourd drying is investigated for the first time, focusing on the retention of total phenolic content (TPC) and antioxidant activity (AA) in the final dried product. Therefore, the objective of this study was to understand the influence of blanching and guar gum pretreatments on the TPC and AA of cabinet dried white bitter gourd.

**MATERIALS AND METHODS**

**Raw material and chemicals**

Fresh, raw & disease-free fruits of white bitter gourd were selected for experimentation. All the chemicals and reagents used in the study were of analytical grade and obtained from the market.

**Sample preparation**

The selected bitter gourd fruits were properly washed to remove any dust and foreign matter adhered to their surface. With the help of a sharp stainless-steel knife, the fruits were cut into rings. For each treatment, 500 g of freshly cut white bitter gourd rings were taken, and each treatment was replicated thrice.

** Blanching methods**

Blanching in hot water: To ensure full coverage of the rings, the white bitter gourd rings were dipped in hot water at 90 °C for 3 minutes (Wang et al., 2018).

Blanching in salt solution: For uniform coverage of the rings, the white bitter gourd rings were dipped in a hot water (at 90 °C) container containing 2% salt solution for approximately 3 minutes (Abano et al., 2013).

** Pretreatment methods**

The bitter gourd rings after blanching were soaked in different steeping solution viz., T1 (WB + 2% SS), T2 (WB + 0.2% KMS), T3 (WB + 1% SC), T4 (WB + 1% GG), T5 (WB + 2% SS + 0.2% KMS), T6 (WB + 2% SS + 1% SC), T7 (WB + 2% SS + 2% SC + 1% GG), T8 (WB + 2% SS + 1% SC), T9 (WB + 0.2% KMS + 1% SC), T10 (WB + 0.2% KMS + 1% GG), T11 (WB + 1% SC + 1% GG), T12 (2%SB + 2% SC), T13 (2%SB + 1% GG + 1% SC), T14 (2%SB + 0.2% KMS + 1% SC), T15 (2%SB + 0.2% KMS + 1% GG) and T16 (2%SB + 0.2% KMS + 1% SC + 1% GG), where WB – Water Blanching; SB – Salt Blanching; SS – Salt Solution; KMS – Potassium meta bisulphite; SC- Sodium carbonate; GG – Guar gum. The respective blanched sample was dipped in the pretreatment steeping solution for approximately 5 minutes. After the samples were removed from the pretreatment solution, they were spread uniformly on a perforated tray and allowed to shade-dry for 1 hour to remove the excess solution (Nyangena et al., 2019).

**Drying method**

The pretreated samples were spread uniformly over perforated aluminum trays and subjected to cabinet drying in a cabinet drier at 60 ± 5 °C until the final moisture content of the sample was reduced to 5-6% (Tiwari et al., 2019).
Preparation of control sample
For the control sample T0 (WB), the white bitter gourd rings were not subjected to any pretreatment solution but only blanched in hot water at 90 °C for 3 minutes and subsequently shade dried for 1 hour, followed by drying in a cabinet drier at 60 ± 5 °C until the final moisture content of the control sample was reduced to 5-6% (Tiwari et al., 2021).

Antioxidant activity (TAA) estimation
The estimation of AA of dried white bitter gourd samples was conducted using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay method (Tan et al., 2013 and Musa et al., 2011). Approximately 40 mg of DPPH was dissolved in 100 ml of methanol to prepare the stock solution and stored at -20 °C for subsequent use. A spectrophotometer was used to obtain an absorbance of 0.70±0.01 units at 516 nm wavelength by mixing 350 ml stock solution with 350 ml methanol. Approximately 100 μL dried white bitter gourd sample extracts were prepared with 1.5 ml methanolic DPPH solution and kept overnight for the scavenging reaction to take place in the dark. An aliquot (250 μL) of samples (white bitter gourd sample extracts with methanolic DPPH solution) and blank were then monitored at 516 nm wavelength on the next day with the help of a spectrophotometer. The percentage of AA (in terms of DPPH scavenging activity) was expressed as a percentage:

\[
\text{Antioxidant activity} (\%) = \frac{\text{Sample A}}{\text{Blank A}} \times 100
\]

where A = absorbance

Total phenolic content (TPC) estimation
The estimation of TPC of dried white bitter gourd samples was conducted using Folin–Ciocalteau (FC) assays (Tan et al., 2013 and Musa et al., 2011). To prepare the stock solution, add approximately 50 μL of dried bitter gourd extracts with 0.2 mL distilled water and 0.25 mL diluted Folin-Ciocalteau reagent. The sample (dried white bitter gourd extracts with Folin-Ciocalteau reagent) was left for 5 minutes before adding 0.5 mL 7.5% sodium carbonate (w/v) to it. After 2 hours, with the help of a spectrophotometer, the absorbances were recorded at a wavelength of 765 nm. The activity of the samples was estimated by setting up the calibration curve of gallic acid. The result was expressed as mg of gallic acid equivalents (GAE) per 100 g of dried sample (mg GAE/100 g of DW).

Statistical analysis
The data collected were statistically analysed using OPSTAT software. The significant differences among the treatments were determined by using Duncan's multiple range test (DMRT) at P values of < 0.05.

RESULTS AND DISCUSSION
Antioxidant activity of dried white bitter gourd
In the present study, samples (T1-T16) were tested for their antioxidant activities by the DPPH radical scavenging method. All antioxidant assays showed significant variation among the tested samples compared to the control, as shown in Table 1. The percentage of AA (in terms of DPPH scavenging activity) was in the range of 18.16% to 40.13%. The significant impact of guar gum in terms of the percentage of DPPH scavenging activity (antioxidant activity) of dried white bitter gourd has a positive outcome, as samples WB+1%GG (T5) and 2%SB+0.2%KMS+1%SC+1%GG (T16) showed higher antioxidant activity in comparison to the control (T0) and the other pretreatments, both in solo and combination, respectively. Overall, samples pretreated with guar gum performed better than salt or KMS or sodium carbonate and their combinations. Therefore, the application of guar gum as a pretreatment with blanching turns out to be beneficial for higher AA, thereby maintaining product quality as a whole. The present results are in concurrence with the findings of Islam and Jalaluddin (2019) in bitter melon, Ghosh et al. (2017) in Indian olive and Jankar et al. (2019) in raisins, indicating that white bitter gourd has promising antioxidant compounds capable of reducing free radicals, which diminish cellular damage by neutralising reactive oxygen. Many researchers have reported a positive correlation between the total antioxidant capacity and the total phenolic content (Tan, et al., 2014; Sorifa, 2018). According to Kubola and Siriamompun (2008) and Sorifa (2018), the DPPH radical scavenging activity assay for the determination of AA showed the highest correlation with the TPC. However, Sorifa (2018) reported no correlation between AA and TPC due to a different ratio of antioxidant content than the total phenolic content. Thus, bitter gourd fruits are an excellent source of antioxidants and free radical scavenging activity (Sorifa, 2018).

Total phenolic content of dried white bitter gourd
The results indicate the positive influence of using guar gum as a pretreatment and blanching on the retention of the TPC of dried white bitter gourd rings, both individually and in combination with other pretreatments. The mean value of TPC varied between 26.13 and 39.55 mg GAE/100 g of DW, as shown in Table 1. In general, the control sample retained the lowest TPC compared to the pretreated samples. Among the pretreated samples, guar gum treatments viz. WB+1%GG (T5) and 2%SB+0.2%KMS+1%SC+1%GG (T16) reported the maximum TPC, individually and in combination, respectively. Similar findings were reported by...
The present results on the TPC support the findings mentioned earlier on the TPC of the dried white bitter gourd product obtained. According to Tan et al. (2016), bitter gourd is a good source of TPC with high AA (Sorifa, 2018). The quantification of total phenolic compounds in bitter gourd fruits helps to understand the food quality, antioxidant capacity, and better market prospects of the product (Otero et al., 2020). Among the two blanching methods, salt blanching gave comparatively better results with pretreatment solution than water blanching. This might be due to the uniform flow of heat and water due to osmosis in salt blanching rather than the water blanching method. Salt Blanching with pretreatment solution helps to inactivate browning enzymes, remove air from tissues, hydrolyse and solubilise structural polymers, expand intracellular air flowing through intracellular lamella, and stabilise the colour of the product. The drying process results in water loss in vapour form, thereby accelerating the reaction between product constituents. As a result, white bitter gourd loses moisture faster, causing an increase in the concentration of nutrients in the final dried product. Dehydration causes the bridging of reactive polymer groups to close together and increases the crystallisation of polysaccharide gels. The hydroxyl groups lose hydration causes the bridging of reactive polymer groups to close together and increases the crystallisation of polysaccharide gels. The hydroxyl groups lose

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Treatment</th>
<th>Total phenolic content in mg GAE/100 g of DW</th>
<th>Total antioxidant activity in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>WB (Control)</td>
<td>26.13&lt;sup&gt;a&lt;/sup&gt; (0.20)</td>
<td>18.16&lt;sup&gt;i&lt;/sup&gt; (0.14)</td>
</tr>
<tr>
<td><strong>Solo Treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>WB+2% SS</td>
<td>26.13&lt;sup&gt;j&lt;/sup&gt; (0.20)</td>
<td>18.81&lt;sup&gt;s&lt;/sup&gt; (0.14)</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>WB+0.2% KMS</td>
<td>27.52&lt;sup&gt;c&lt;/sup&gt; (0.20)</td>
<td>19.70&lt;sup&gt;y&lt;/sup&gt; (0.15)</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>WB+1% SC</td>
<td>26.83&lt;sup&gt;d&lt;/sup&gt; (0.20)</td>
<td>19.11&lt;sup&gt;b&lt;/sup&gt; (0.14)</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>WB+1% GG</td>
<td>30.77&lt;sup&gt;e&lt;/sup&gt; (0.23)</td>
<td>20.29&lt;sup&gt;g&lt;/sup&gt; (0.15)</td>
</tr>
<tr>
<td><strong>Combination Treatment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T&lt;sub&gt;6&lt;/sub&gt;</td>
<td>WB+2% SS+0.2% KMS</td>
<td>28.80&lt;sup&gt;a&lt;/sup&gt; (0.21)</td>
<td>20.00&lt;sup&gt;a&lt;/sup&gt; (0.15)</td>
</tr>
<tr>
<td>T&lt;sub&gt;7&lt;/sub&gt;</td>
<td>WB+2% SS+1% SC</td>
<td>29.68&lt;sup&gt;e&lt;/sup&gt; (0.22)</td>
<td>20.21&lt;sup&gt;c&lt;/sup&gt; (0.15)</td>
</tr>
<tr>
<td>T&lt;sub&gt;8&lt;/sub&gt;</td>
<td>WB+2% SS+1% GG</td>
<td>32.35&lt;sup&gt;d&lt;/sup&gt; (0.24)</td>
<td>22.27&lt;sup&gt;d&lt;/sup&gt; (0.17)</td>
</tr>
<tr>
<td>T&lt;sub&gt;9&lt;/sub&gt;</td>
<td>WB+0.2% KMS+1% SC</td>
<td>31.66&lt;sup&gt;b&lt;/sup&gt; (0.24)</td>
<td>21.48&lt;sup&gt;e&lt;/sup&gt; (0.16)</td>
</tr>
<tr>
<td>T&lt;sub&gt;10&lt;/sub&gt;</td>
<td>WB+0.2% KMS+1% GG</td>
<td>32.65&lt;sup&gt;d&lt;/sup&gt; (0.24)</td>
<td>24.04&lt;sup&gt;c&lt;/sup&gt; (0.18)</td>
</tr>
<tr>
<td>T&lt;sub&gt;11&lt;/sub&gt;</td>
<td>WB+1% SC+1% GG</td>
<td>32.65&lt;sup&gt;d&lt;/sup&gt; (0.24)</td>
<td>24.63&lt;sup&gt;d&lt;/sup&gt; (0.18)</td>
</tr>
<tr>
<td>T&lt;sub&gt;12&lt;/sub&gt;</td>
<td>2%SB+1% SC</td>
<td>33.73&lt;sup&gt;c&lt;/sup&gt; (0.25)</td>
<td>25.42&lt;sup&gt;e&lt;/sup&gt; (0.19)</td>
</tr>
<tr>
<td>T&lt;sub&gt;13&lt;/sub&gt;</td>
<td>2%SB+1% GG+1% SC</td>
<td>37.88&lt;sup&gt;a&lt;/sup&gt; (0.28)</td>
<td>34.30&lt;sup&gt;c&lt;/sup&gt; (0.25)</td>
</tr>
<tr>
<td>T&lt;sub&gt;14&lt;/sub&gt;</td>
<td>2%SB+0.2% KMS+1% SC</td>
<td>37.28&lt;sup&gt;c&lt;/sup&gt; (0.28)</td>
<td>28.19&lt;sup&gt;e&lt;/sup&gt; (0.21)</td>
</tr>
<tr>
<td>T&lt;sub&gt;15&lt;/sub&gt;</td>
<td>2%SB+0.2% KMS+1% GG</td>
<td>39.06&lt;sup&gt;e&lt;/sup&gt; (0.29)</td>
<td>38.74&lt;sup&gt;g&lt;/sup&gt; (0.29)</td>
</tr>
<tr>
<td>T&lt;sub&gt;16&lt;/sub&gt;</td>
<td>2%SB+0.2% KMS+1% SC+1% GG</td>
<td>39.55&lt;sup&gt;e&lt;/sup&gt; (0.29)</td>
<td>40.13&lt;sup&gt;a&lt;/sup&gt; (0.30)</td>
</tr>
<tr>
<td>C.V.</td>
<td></td>
<td>1.29</td>
<td>1.33</td>
</tr>
</tbody>
</table>

<sup>*Means with different letters in the same column are significantly different (p<0.05); Abbreviations: WB—Water Blanching; SS—Salt Solution; KMS—Potassium meta bisulphite; SC—Sodium carbonate; GG—Guar gum</sup>

Zhu et al. (2012) and Islam et al. (2011) for air-drying bitter gourd powder and oven-dried bitter gourd tissues. According to Islam and Jalaluddin (2019), overdried samples of white bitter gourd exhibited higher phenolic content, which is also directly related to the antioxidant properties of the white bitter gourd. The dehydrated white bitter gourd exhibited higher antioxidant properties due to a substantial increase in simple phenols during the drying process (Sorifa, 2018). The positive impact on antioxidant properties is attributed to the fact that heating increases the naturally present compounds, releasing the bound phenolic compounds by the breakdown of cellular constituents and forming novel compounds such as Maillard reaction products with antioxidant activity (Tan et al., 2013). The present results on the TPC support the findings mentioned earlier on the TPC of the dried white bitter gourd product obtained. According to Tan et al. (2016), bitter gourd is a good source of TPC with high AA (Sorifa, 2018). The quantification of total phenolic compounds in bitter gourd tissues. According to Islam and Jalaluddin (2019), overdried samples of white bitter gourd exhibited higher phenolic content, which is also directly related to the antioxidant properties of the white bitter gourd. The dehydrated white bitter gourd exhibited higher antioxidant properties due to a substantial increase in simple phenols during the drying process (Sorifa, 2018). The positive impact on antioxidant properties is attributed to the fact that heating increases the naturally present compounds, releasing the bound phenolic compounds by the breakdown of cellular constituents and forming novel compounds such as Maillard reaction products with antioxidant activity (Tan et al., 2013). The present results on the TPC support the findings mentioned earlier on the TPC of the dried white bitter gourd product obtained. According to Tan et al. (2016), bitter gourd is a good source of TPC with high AA (Sorifa, 2018). The quantification of total phenolic compounds in bitter gourd fruits helps to understand the food quality, antioxidant capacity, and better market prospects of the product (Otero et al., 2020). Among the two blanching methods, salt blanching gave comparatively better results with pretreatment solution than water blanching. This might be due to the uniform flow of heat and water due to osmosis in salt blanching rather than the water blanching method. Salt Blanching with pretreatment solution helps to inactivate browning enzymes, remove air from tissues, hydrolyse and solubilise structural polymers, expand intracellular air flowing through intracellular lamella, and stabilise the colour of the product. The drying process results in water loss in vapour form, thereby accelerating the reaction between product constituents. As a result, white bitter gourd loses moisture faster, causing an increase in the concentration of nutrients in the final dried product. Dehydration causes the bridging of reactive polymer groups to close together and increases the crystallisation of polysaccharide gels. The hydroxyl groups lose
their noncovalent water due to dehydration, causing shrinkage of plant cells and enabling the adjacent molecules to get closer to fulfilling the hydroxyl group’s valency (Bhattacharjee et al., 2016).

Guar gum is a novel agrochemical that has the characteristic ability to form strong hydrogen bonds with water molecules, thereby reducing shrinkage during dehydration (Mudgil et al., 2014). According to Jia et al. (2017), coating food products with guar gum has shown the potential to affect moisture migration. Pretreatment with guar gum reduces intermolecular forces, thereby improving the biochemical and textural properties of the product. Guar gum forms a cross-linked network with other food additives, such as salt, KMS, and sodium carbonate, thereby increasing the strength of the cell wall and middle lamella of the white bitter gourd, resulting in protection of the surface from shrinkage and loss of nutrients during dehydration (Hua et al., 2015). This cross-linkage also decreases nonenzymatic browning, favouring the high sensory appeal of the dried product (Sumonsiri et al., 2020). Among all 16 treatments (T1 to T16), the best treatment was 2%SB+0.2%KMS+1% SC+1%GG (T16), which demonstrated that guar gum could be used for the first time appreciably as a pre-treatment for the maximum retention of total phenols and antioxidant activity in cabinet-dried white bitter gourd. The outcome of the study will serve as a prerequisite for proposing white bitter gourd as a functional food with bioactive properties.

Conclusion

The present study concluded that as a functional food with bioactive properties, white skin-colored bitter gourd (M. charantia) has great potential in the food industry from a commercial point of view. Based on the outcome of the present study, guar gum treatment in the sole category viz. WB+1%GG (T8) and in the combination category viz. 2%SB+0.2%KMS+1%SC+1%GG (T16) has given very encouraging results on the retention of TPC and AA. Therefore, marketing white bitter gourd with less bitterness will be a promising food option for bitterness-conscious consumers. More studies can be conducted on this type of bitter gourd with a future application as a functional food and nutraceutical to maintain food quality and combat lifestyle diseases.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Founder President Hon’ble Dr. Ashok Kumar Chauhan and Dr. Nutan Kaushik, Director General, Food & Agriculture Foundation, Amity University, Sector-125, Noida-201313 (Uttar Pradesh) for constant support and encouragement.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES


