

## GREEN ENZYMES FROM PLANTS AND MICROBIAL SOURCES USED IN INDUSTRIES: AN OVERVIEW

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### ABSTRACT

Green chemistry, an emerging field of science, focuses on the prevention of waste and the use of hazardous substances. It also emphasizes the elimination or reduction of the use of harmful elements in various stages of the manufacturing process or a product development setting. The unpredictable climatic changes and the environmental hazards produced by the industries made the researcher's attention in this arena. At present, industries make an effort to produce their products greener. As a consequence, several biotechnological and biological applications join hands with green chemistry, making the manufacturing process not only greener but also economical. Enzymes are the result of the combination of green chemistry and biotechnology. In the current scenario, there is a need for new versatile enzymes to develop sustainable and economically competitive production processes. The present article reviews the types of enzymes obtained from plants and microorganisms used in different industries and their vital role in the conservation of an eco-friendly environment.

**Keywords:** Green Chemistry, Enzymes, Eco-friendly, Industries, Microorganisms.

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### INTRODUCTION

Green chemistry is a progressing branch of science that focuses on reducing waste and the use of harmful chemicals or substances at the source rather than at the end. Anastas and Warner in the 1990s formulated a set of twelve guiding principles<sup>1,2</sup> for green chemistry, which leads to being applied to multidisciplinary domains of research and development.<sup>3</sup> The concept of ecologically friendly items<sup>4</sup> is emphasized by the twelve principles. To date, green chemistry has covered almost all the interdisciplinary chemistry aspects such as synthetic chemistry, biochemical studies, materials chemistry, material sciences, and technical chemistry.<sup>5</sup> The emancipation of a large number of wastes by the industries is highly carcinogenic and deteriorates the ecosystem. To alleviate this, the green chemical principle is used. It paves a way for the industries in developing a large number of greener products. This topic is recently gaining attention as the world is facing rapid climate change and several regulations set forth to tackle it. In practice, green chemistry is a novel idea, which may balance climate change and an economical tool for the industries. Although this field is developing, there are many problems like high manufacturing costs and usage of toxic substances still exist.<sup>6</sup> A catalyst is used to accelerate the chemical process in the production of organic chemicals. These catalysts do not interfere with the chemical reaction; however, their use can lead to the generation of various inorganic compounds. This is due to the proper incorporation of stoichiometric inorganic reagents for the synthesis of organic compounds.<sup>7</sup> The generation of wastes has been reduced by the usage of greener catalysts and is still being developed. One of the greener catalysts called enzymes can be obtained from eco-friendly resources like plants, and microorganisms. The enzymes which are derived from the plant named cellulose have their potential uses in the food, pulp, and paper industries.<sup>8</sup> The increasing demand for enzymes is highly noticeable. Microbes such as bacteria, and fungi occur everywhere and can survive even in extreme environmental conditions, are termed extremophiles.<sup>9</sup>

The life cycle and processes of such extremophiles are unique to the environmental conditions they are present. Due to their unique nature, the enzymes obtained from them can be very useful in industries and to the environment, as they are naturally sourced and withstand conditions such as temperature, pressure, etc. This review article gives an overview of the plant and microbial enzymes used in industries that are applicable to the principles of green chemistry.

## Green Enzymes from Plant Source

### Food Industries

Food industries use enzymes for making ingredients and texture modification of food products.<sup>10</sup> The prime goal is to find an enzyme that offers high performance at an economical cost. But the lack of awareness and application areas of enzymes shows a pitfall in the advancement of enzymes in the food industry.<sup>11</sup>

### Bromelain

Bromelain is a protease enzyme present in the *Ananas comosus* plant also known as pineapple. It is highly found in pineapple stem extracts. It can also be extracted from the fruit, crown, core, and leaves of the pineapple.<sup>12,13</sup> Isolation of such enzymes can be done from the stem juices<sup>14</sup> by utilizing precipitation and centrifugation and can be subsequently refined using gel filtration and ion-exchange chromatography.<sup>15</sup> The significant characteristic of meat is a tenderizer.<sup>16</sup> Use of bromelain as an enzyme, has been recorded by its historical use in the tenderizing of meat. Comparing bromelain to ficin, it is available in public markets under the guise of “McCormick and Knorr”.<sup>17</sup> This enzyme has the function to refine the rating scale of the sensory evaluation. It also positively affects the tenderization of meat in comparison to other enzymes from *Ananas comosus*. Sullivan and colleagues reported that bromelain acts on proteins present in sausages especially those of myosin.<sup>18</sup> Bromelain is a better alternative compared with HCl for the production of sauce from the meat of oysters as it receives increased tolerable ratings in the sensory evaluation test.<sup>19</sup> This finding shows that bromelain is a better hydrolyzing medium.

### Papain

Papain is a proteolytic plant enzyme that is isolated from papaya latex (*Carica papaya L.*) The greener the fruit, the more active is the papain.<sup>20</sup> Papain is thermodynamically stable and does not easily inactivate, hence it protects the texture of the product from worsening even after cooking.<sup>21</sup> The commercial value of papain stems due to its significant proteolytic activity and its wide variety of operational conditions.<sup>22</sup> Papain is utilized in the manufacture of soft cheeses such as cream cheese.<sup>23</sup> Since it displays the high activity of proteolysis in milk, it shows an inconsistent formation of clots and is not able to process hard cheese. If the clotting is controlled, the papain might also be used as a milk clotting enzyme for the replacement of the production of cheeses such as pecorino. Papain is analogous to the glutamine and proline-rich patterns, and therefore it is utilized to make hypoallergenic flour suited for hypersensitive patients.<sup>24</sup> The papain promotes the consistency of the dough by keeping it from constricting, and by enhancing its liquidity and elasticity while baking.<sup>25,26</sup> Papain possesses an excellent property of preventing turbidity while being chilled, hence it is utilized in beer manufacturing. Treating beer with papain results in the breakdown of several proteins formed during the manufacturing process of the beverage. Under suitable conditions, papain is noticed as a wine stabilizing agent and a biocatalyst in the wine industry.<sup>27</sup> In contrast to its major rivals, papain is the most widely utilized enzyme in the industry because of its economical properties.<sup>28</sup> It has been recognized from the studies, that papain is an allergen and can cause adverse immunological reactions to human beings. But, the FDA of the United States recognized papain as a safer enzyme.<sup>29</sup>

### Paper and Pulp Industries

Hemi cellulolytic enzymes have a massive increase in interest in recent years, especially in the paper and pulp industry. To produce a pulp that has pollution-free chemical properties, an efficient method, such as the usage of enzymes, is employed to substantially ease the process of kraft production. This is without a doubt, an extraordinary capacity for an environmentally secure method.

### Xylanase

Xylanases are hydrolytic enzymes that degrade xylans. After cellulose, it's the succeeding enzyme that has maximum ample biopolymer and a predominant hemicellulosic polysaccharide observed in molecular walls

of plants. The hydrolytic property of a selected xylan offers a better shape to the fibers with greater permeability. It in turn improves the removal of lignin residues.<sup>30</sup> The elimination of lignin from chemical pulps is called bleaching. In modern times, the whitening of kraft pulp makes use of a huge amount of chlorine resulting in toxic wastes, mutagens, and creating several dangerous conflicts within the ecosystem.<sup>31</sup> By enzymatic treatment, the brightness of pulp was increased, and this improved brightness could make use of the reduction of chlorine consumption.<sup>32</sup> With the usage of xylanases, a nearly quarter percent decline in the intake of chlorine may be attained by facilitating the diminish of toxicity levels, color, and the halides that are absorbed in the waste effluent.<sup>33</sup> These enzymes assist in the rise of the pulping process, aids in retaining water, and decrease the beating time of pulp virgins. They are employed in the process of de-inking and repairing bonding with recycled fibers. Xylanase has a wide range of applications and is employed as a greener tool in different industries.

### Cellulose

Cellulose is an organic compound, obtained from wood pulp and other natural sources like annual plants, trees, and algae.<sup>24</sup> Cellulose is a generally overflowing biodegradable polymer on the planet.<sup>34,35</sup> The main reason why the paper is made out of wood is due to the presence of cellulose. The newly engineered cellulose structure falls into micro and nano-fibrillated cellulose. Micro-fibrillated cellulose (MFC) and nano-fibrillated cellulose (NFC) are recognized for their excessive mechanical properties like hardness and stiffness and are used to enhance durability in products with a paper base.<sup>36</sup> The unique property of nanocellulose is its high strength and stiffness in papermaking and the predominant property is the moist density that is necessary for manufacturing tissue paper and paper towels.<sup>37</sup> Companies are interested in new products by using nanocellulose technology and they recommend using NFC instead of paper as they have high opacity and excellent smoothness.<sup>38</sup> Fiber lean Imerys researchers used a pulverizing technique and produced a low-cost MFC, which is utilized in printing and writing papers.<sup>39</sup> Universities and agencies have invested a considerable amount of money in wood-based nanocellulose technology for the manufacturing and commercialization of NFC and MFC.<sup>40</sup> Therefore, the usage of enzymes namely xylanase and cellulose provide an easy and economical way to reduce the usage of chlorine and its compounds. It also lessens the cost and energy consumption of high-yield wood pulp during refining processes.

### Textile Industry

In the fabric industry, the use of enzymes is an instance of white/commercial technology, which enables an ecological environment for fiber processing and in the improvisation of the output quality. The utilization of energy and the usage and disposal of chemicals in the textile process industry are primary reasons for the enzyme's demand.<sup>41</sup> Textile industry has advanced to the eco-friendly environment and improved the quality of the product, but only 75 enzymes are majorly used in the industry from the existing thousands.<sup>42</sup>

### Cellulase

Cellulases are hydrolytic enzymes that pulverize cellulose into glucose. Cellulase is used to diminish fibrils and fibers and has effectively launched cotton into the fabric industry.<sup>24</sup> Garments are put through to washing treatment to give a barely worn appearance, for instance - stone washing of jeans, where the color blue becomes slightly dull due to the process of pumice at the surface. An alternative technique is to initiate cellulase which can terminate the use of pumice and can prevent it from fabric damage and little pumice dust is exposed to nature. Through this process, the production efficiency can be raised as the process of washing will have minimal usage of stones and more garments.<sup>43</sup> Bio-polishing is the disposal of micro and woolly fibrils from the fabric by the activity of cellulase enzymes. It enhances several properties namely appearance, pigment, luminosity, and water absorption, as well as giving a smooth surface that is less frizzy. These elements offer the functionality to adjust cellulosic fibers in a composed and favored manner. Bio-treatment of cotton and wool combination fabric makes use of cellulase to enhance dyeability with flexibility and softness.<sup>44</sup> This treatment provides the process finishing with extended softness.<sup>45</sup> Bio-washing with cellulase activity is ecological and highly used in detergents that improve appearance, color, and dirt removal from cotton garments. For effective stain elimination and to regain the luminosity of pigmented fabrics endoglucanase-rich cellulase can be used in detergent formulations.<sup>46,47</sup> Cellulase

enzyme causes a non-homogeneous surface that eliminates indigo dye confined in the fibers, which fabricates a dim and worn appearance.

### Laccase

Laccases are multi-copper-containing oxidoreductase enzymes that utilize oxygen for the oxidation of phenols and phenolic amines.<sup>48</sup> In the textile industry, enzymatic bleach using laccase act as an alternative. The biological bleaching operation is found in laccases that only target the color of the substances.<sup>49</sup> In the textile industry, the usage of laccase is to decolorize dye effluents and to reform fabric textures. This is due to the fact that the laccase has the ability to break down the dyes of various organizational layouts, including artificial dyes.<sup>50,51</sup> Laccase pre-treatment improved the color and clarity of the cotton fabrics by flavonoid oxidation<sup>52</sup> and reduced the H<sub>2</sub>O<sub>2</sub> concentration in bleaching using artificial chemicals. For textile wet processing, several commercial solid laccases have been introduced into the market.<sup>53</sup> The flavonoid oxidation with laccase can colorize the cellulosic fibers with the generation of pigments onto cotton fabrics.<sup>54</sup> Enzymes can be employed in the textile industry to produce an ecologically acceptable alternative to chemical procedures, like how cellulase and laccase are used in bio washing and dye decolorization.

Table -1: Plant Enzymes from Various Sources

Enzyme	Plant Source	Properties/Uses	References
Bromelain	Pineapple plant ( <i>Ananas comosus</i> )	Enhance the sensory assessment rate and the meat tenderness	12,18
Papain	Papaya latex ( <i>Carica papaya L.</i> )	Production of semi-soft cheese, hypoallergenic flour, light, and clear beers.  Used as a wine stabilizing agent and biocatalyst in the wine industry	20,23,55  27
Xylanase	Found in cell structure of various plant sources	Brightening of pulp, decrease in chlorine intake, and De-inking	33
Cellulose	Found in wood pulp and other natural sources	NFC - high strength and stiffness in paper-based products. MFC - used in printing and writing papers.	37  39
Cellulase	Found in various plant sources	Bio-polishing, Bio-washing, Bio-treatment, and for effective stain removal and garment washing	44-47
Laccase	Found in various plant and vegetable fibers	Decolorize dye effluents, improve the whiteness of cotton, and denim bleaching	50-53

### Green Enzymes from Microbial Sources

Enzymes from various microorganisms are useful in several industries (Table-2.). Enzymes procured from microbial sources tend to be more stable than plant sources and are also conveniently extractable from microorganisms by fermentation. This makes the process of using such enzymes as economical and can be reused in industries.<sup>56</sup> Different and novel strains of microorganisms can be researched and can be genetically tailored to meet industry requirements. Advancements in enzyme research and immobilization of enzymes have led to the optimum gain of the use of an enzyme in the industry.<sup>57</sup>

Table-2: Microbial Enzymes from Various Sources

Enzyme	Source Microorganism	Properties/Uses	References
Thermostable $\alpha$ -amylase	<i>Geobacillus bacterium</i>	Waste cake reduction, Production of ethanol	62,81
Naringinase	Several fungal spp., <i>Eurotium</i> , <i>Aspergillus niger</i> , <i>Circinella</i> , <i>Penicillium</i> , etc., and several bacterial spp., <i>Pseudomonas</i> <i>paucimobilis</i> , <i>Thermomicrobium</i> <i>roseum</i> , etc.	Debittering in juice process industries, Production of Rhamnose	66
Laccases	Various fungal spp. and some bacteria	Removal of phenolic substances in fruit juices, delignification of wood in paper production	69,70
Xylanases	<i>Thermoascus aurantiacus</i>	Lignocellulosic material degradation	72
Non-heme iron oxygenases; Heme- containing oxygenases; flavin-dependent monooxygenases	<i>Pseudomonas putida</i> G7; <i>Bacillus</i> <i>megaterium</i> ATCC 14581; <i>Methylophaga aminisulfidivorans</i>	Production of the indigo dye	77
Endoglucanase	<i>Pyrococcus horikoshii</i>	Hydrolyzing cellulases in textile industries	81

### Food Industry

The food industry is the major user of microbial enzymes, and usually can be seen in food production, brewing, and other such fields of the industry. Several enzymes such as amylases, glucoamylases, proteases, lactases, lipases, phospholipases, and others have been used for a variety of applications in beer production.<sup>58</sup>

#### Amylases

Waste generated by the food industries occurs in the form of waste cakes, which are organic. Food waste is generally disposed of through landfills, composting, or incinerated resulting in groundwater seepage and harmful gas emissions.<sup>59-61</sup> The waste cakes can act as a sole substrate in ethanol production. Amylase enzymes help in the breakdown of high carbohydrate substances, hydrolyzing  $\alpha$ -1,4 glycosidic linkages of poly carbohydrates. It in turn forms short-strings of low molecular weight carbohydrates<sup>58</sup> and its optimum temperature is 60-100°C, making it suitable for industrial settings. Waste cakes have a rich source of carbohydrates that can be broken down into monomers to produce ethanol. The use of amylases, along with the microorganism *Saccharomyces cerevisiae* showed maximum production of ethanol of 46.6 g/L. The output of the same alcohol was shown to be around the value of 1.13 g/g RS. The  $\alpha$ -amylase could be successfully used to its full potential by releasing the reducing sugar within the span of 80 minutes. Reduction of the waste cakes by hydrolysis may reach 84% to 85.2% which makes it a favorable option when compared to other means of solid waste treatment such as composting and anaerobic fermentation.<sup>62</sup> This prevents unnecessary wastage and saves the environment by providing the food industries with a greener pathway of waste disposal.

#### Naringinase

Naringin, along with limonin, and neohesperidin constitute the major citrus-flavored compounds found in citrus fruits.<sup>63,64</sup> To reduce the bitterness caused by this compound, food industries use naringinases to hydrolyze naringin<sup>65</sup> present in citrus juices of fruits such as oranges, grapefruit, etc. Naringinases can be

obtained from several fungal species such as *Eurotium*, *Aspergillus niger*, *Circinella*, *Penicillium*, *Fusarium*, *Trichoderma*, and *Rhizopus* and also from certain bacterial species such as *Bacteroides distasonis*, *Burkholderia cenocepacia*, *Pseudomonas paucimobilis*, *Thermomicrobium roseum*, and so on, and several others from plants, yeasts and even from pig liver<sup>66</sup>, though fungal isolates are preferred in industries as they provide more yield. Other than just being used as a debittering enzyme, naringinases have other uses, namely enzymatic production of rhamnose and kinnow peel treatment.<sup>66</sup> The processing of juice in industries leads to the generation of citrus peels as a waste product. The rhamnosidase extracted from the citrus peels has great value as it can be used to produce rhamnose and prunin. Vila Real formulated a novel and developed an effective method in 2010<sup>67</sup>, by inactivation of  $\beta$ -glucosidase expressed by the naringinase. From the kinnow peel, naringin can be extracted. From *E. coli* cells, recombinant  $\alpha$ -L-rhamnosidase was purified and used for substrate hydrolysis. Naringinase activity was then calculated and then shown and shown its use and capability of producing rhamnose.<sup>68</sup>

### Laccases

Laccases are oxidoreductases that are essentially environmentally friendly and have been gaining significantly increasing interest. When this enzyme is reacted with air, it produces water, being the sole reaction product.<sup>69</sup> This property alone shows the importance and potential of laccases in the view of conserving the environment. These enzymes are not too hard to find and are omnipresent in nature. Sources of laccases include plants and just about every fungal species to have ever been discovered.<sup>48</sup> Laccases in food industries generally contribute to the processing of fruit juices. When juices are being manufactured, phenolic substances that occur naturally, over time, can cause unwanted changes in the color and smell of the fruit juice. This can cause the juice to look darker due to several enzymatic reactions due to phenols, in the process. Therefore, as laccases are known to break down phenols, this darkening<sup>70</sup> and spoiling of the fruit juices can be prevented. Uses of laccases can also be attributed to the beer manufacturing industries. While beer is being made, a cloudy texture can arise because of the presence of phenol groups in the liquid. In beer manufacturing, the removal of phenol is done by using PVPP treatment. But the cost of PVPP is higher, hence the use of laccases is the safest, cheapest option. These enzymes not only remove the phenolic substances but also enhance the shelf life of the beer to a greater extent.<sup>71</sup>

### Paper and Pulp Industries

We can see paper everywhere and it is an integral part of our lives. This reveals that the paper industry is one of the biggest manufacturing industries and we can accomplish that the waste from the industry has a significant impact on the environment. Laccases, as previously discussed, also have their uses in the paper industry. To produce paper pulp, lignin present in wood fibers (acting as a sort of natural gum) must be broken down, and to aid this process, chemical and mechanical methods can be used. A chemical method that makes use of chlorine to "bleach" the paper is one of the methods used. However, bleaching the paper leads to the generation of harmful toxins with the presence of chlorine. To prevent this, laccases along with other oxidative enzymes can be used in the delignification process. Laccases are seen on white-rot fungi and have natural delignifying properties, however, it involves the use of several other enzymes to delignify wood properly.<sup>69</sup> Xylanases obtained from the *Thermoascus aurantiacus*, a thermophilic fungus show promising results when used for lignocellulosic material degradation.<sup>72</sup> The fungus naturally produces enzymes that are thermostable, as the enzyme remains considerably stable in the presence of higher than usual temperatures. The wastes from the paper and pulp industries cause loss of aesthetics, thermal difference, formation of scum, and slime in the surrounding environment. The waste that the paper and industry produce toxic substances in the form of effluents are released into water bodies present close to the industry. Since the toxins are in the form of wastewater, it is imperative that we treat them to reuse the water. Microorganisms in addition to techniques such as UV treatment can be utilized along with enzymes to treat the wastewater. Forming an amalgamation of these techniques along with microorganisms can pave the way for a sustainable future.<sup>73</sup>

### Textile Industry

Textile industries release a large amount of toxic wastewater due to the various processes in the fabric dyeing. The wastewater consists of high BOD or COD levels and the presence of huge amounts of total

dissolved salts that can cause an imbalance in the ecosystem of the surrounding water bodies.<sup>74</sup> The presence of naphthol, acetic acid, soaping chemicals, and radioactive metals can lead to the formation of an oily froth combined with an unpleasant odor in the water bodies. This can even lead to the clogging of soil pores and can affect marine life.<sup>75</sup> Physical methods of dumping wastes from the textile industry, such as landfills and incineration, can lead to the pollution of the atmosphere. These two methods of waste disposal can lead to emissions of methane, CO<sub>2</sub>, and nitrous oxide production that can lead to global warming. It can also lead to the formation of carcinogens, cause respiratory problems, and can increase ecotoxicity.<sup>76</sup> Due to these reasons, treating textile wastewater with the help of enzymes in a green biology-based approach can reduce the negative environmental impact caused by the industry.

### Indigo Producing Enzymes

Indigo is a dye that is used to impart its color onto jeans and denim clothing. It was originally made by fermenting the plant *Indigofera tinctoria*, which can largely be found in India. In later 1870, Adolf von Baeyer developed a method to chemically synthesize indigo from isatin. The processes developed from his methodologies did not account for the environmental impact of the chemically produced dye, as the wastes from production were not environmentally friendly. Hence, such features led to the research and development of a greener yield of the indigo dye. In recent years microbial enzymes, indigo been produced through a combination of oxygenation of indoles and with the help of peroxygenases, produce indigo. This led to the discovery of enzymes that can be classified into three groups: heme-containing oxygenases, flavin-dependent monooxygenases, and non-heme iron oxygenases.<sup>77</sup> Production of indigo by using these enzymes has yielded favourable results, although much research has not yet been done in an industrial environment. Finally, large amounts of toxic wastes from denim manufacturing can be stopped at the beginning of production of the indigo dye, hence we can avoid pollution at the source.

### Hyperthermostable Endoglucanase

Cellulases are enzymes that are used in the biopolishing of textile fibers. These enzymes are employed to make the product soft and to remove the fuzz. The cellulase enzyme is obtained from the mesophilic fungi and the use of this enzyme in the biopolishing process gives it a change in tensile strength, adds weight to the fabric, and even gives it a clean look to fabric due to the hydrolysis reaction. The optimum temperature of cellulases was found to be around 55°C, which is inferior to the biopolishing process as higher temperatures enable the better removal of starch in the process, and thus a better end product is obtained.<sup>78,79</sup> Since there is no thermostable form of the cellulase enzyme, we must look at the endoglucanase enzyme. This hyper thermostable endoglucanase enzyme is isolated from *Pyrococcus horikoshii* found at a hydrothermal vent in Okinawa, Japan. Due to its tolerance at high temperatures, the enzymes isolated from this bacterium are highly thermostable even around 95°C.<sup>80</sup> Due to its capacity for hydrolyzing celluloses at intense heat, textile processing in the biopolishing step can be carried out using steam instead of a water bath.<sup>78</sup> By using thermostable enzymes, the time and the cost of production can be reduced. Moreover, using steam when compared to a water bath can greatly reduce the amount of water used in biopolishing and textile production. Microorganisms are an excellent source of green enzymes. These enzymes can be used as-is or with the help of other microorganisms, such as yeast, in industrial processes.

## CONCLUSION

The use of green enzymes and the development of proper green pathways can be very beneficial to the industries in terms of profitability and to the environment in terms of sustainability. More industries should employ enzymes extracted from plants and microorganisms, as they are easily available and ubiquitous. The enzymes obtained from nature can be manipulated using gene-editing techniques to provide the enzymes to the industries as per requirement. The enzymes act as replacements for the vast number of harsh and hazardous chemicals that are used in various industries. By considering the use and by an understanding of such enzymes, more research and development can be carried out to incorporate them into the manufacturing and production plants. The use of enzymes as an alternative for chemicals in industrial processes prevents the release of approximately 700 million kg of carbon dioxide into the atmosphere per year.<sup>82</sup>

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## CONFLICT OF INTERESTS

The authors declare that there is no conflict of interests.

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All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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