**Bulletin of Environment, Pharmacology and Life Sciences**
Bull. Env. Pharmacol. Life Sci., Vol 10 [4] March 2021 : 129-134

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

**ORIGINAL ARTICLE** **OPEN ACCESS**

# Antioxidant and Anti-inflammatory xylooligosaccharides produced from lignocellulosic materials.

**Rajani J Thanekar[1], S D Mali[2], M M Bendigeri[3], Y C Attar[4] ***
Full Address: 1,2,3: Research student, Department of Microbiology, Rajaram College Kolhapur.
4: Head, Department of Microbiology, Rajaram College Kolhapur
* corresponding author –Dr Y C Attar. E-mail Id – ycmicro@gmail.com;ycamicro@gmail.com

**ABSTRACT**
*The present study aims at production of xylooligosaccharides from lignocellulosic materials like wheat bran and Pistia stratiotes by bacteria Massilia timonae B2YR followed by the study of in vitro antioxidant activity anti-inflammatory activity of xylooligosaccharides. The total phenol content of XOS produced was 1236.914mg/ml of Gallic acid equivalent determined by folinciocalteau method. The antioxidant activity demonstrated by DPPH assay ranged from 15% to 74 % for the concentration 0.2 -1mg/ml. The IC50 value for antioxidant activity was 0.6 mg/ml of XOS.The anti-inflammatory studies for xylooligosaccharides showed maximum percent inhibition of denaturation of egg albumin 74.79 % at 400 µg/ml with a IC50 values 238.400 µg /ml. The percent inhibition of bovine serum albumin denaturation to was 70.67 % at 400 µg/ml concentration with the IC50 value 213.36 µg/ml.*
***Keywords:*** *Xylooligosaccharides, Anti-inflammatory activity, Egg albumin denaturation, Bovine serum denaturation*

Received 12.12.2020 Revised 23.02.2020 Accepted 05.03.2021

## INTRODUCTION

Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation isa chemical reaction that can produce free radicals, like Reactive oxygen species (ROS) is a free radicals or molecular species capable of generating free radicals. Most of the reactive oxygen species including superoxide anion (O2.-), hydrogen peroxide (H2O2) and hydroxyl radical (OH) are inevitably produced as by-products of normal aerobic metabolisms and are increased during infections, exercise, stress conditions, radiations etc. leading to chain reactions that may damage cells.Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. The term "antioxidant" is mainly used for two different groups of substances: industrial chemicals which are added to products to prevent oxidation, and natural chemicals found in foods and body tissue which are said to have beneficial health effects. Amongst the ROS, H2O2 is an important molecule as although it is not toxic by itself.The generation of H2O2 by activated phagocytes is known to play an important role as bactericidal and antifungal since it also acts as mediators of inflammation by activation of signal transduction pathways[1].

Inflammation is caused due to injury, infection or destruction characterised by heat, redness, pain, swelling and disturbed physiological functions. Inflammation is a protective measure produced by tissue in response to physical trauma, noxious chemical or microbial agents. It helps body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair. Inflammation leads to increase in vascular permeability, membrane alteration and protein denaturation. Protein denaturation is main cause of inflammation[2]. The Steroidal and Non-steroidal anti-inflammatory drugs (NSAID's) are used for treating inflammation, but long term use of NSAID's can cause gastric erosions and stomach ulcers.[3]. The in-vitro anti-denaturation activity of heat treated Bovine serum albumin and egg albumin can be used for detecting anti-inflammatory compounds[4].

Xylooligosaccharides are oligomers of xylose sugars. Xylooligosaccharides are produced through hydrolysis of xylan. Xylan is major component of hemicellulose present in lignocellulosic materials[5].Xylooligosaccharides when incorporated in diets offer antioxidant activity, anti-inflammatory properties, antibacterial properties, anti-allergic activity and positive effects on the immune system. The present study aims at evaluating the antioxidant activity and anti-inflammatory activity of Xylooligosaccharides produced by organism *Massilia timonae* B2YR from lignocellulosic biomass like wheat bran and *Pistia stratiotes*. The wheat bran is composed of Arabinoxylan (19-25%),

Starch(17-29%), protein(14-18%), Lignin(~3%), β-glucans (1-3%), Phytic acid(3-5%), Feluric acid(0.3-5%), ferulic acid being the most common phenolic compound[6]. While Pistia comprises of 92.9% H2O, 1.4% protein, 0.3% fats, 2.6% carbohydrates, 0.9% crude fiber and 1.9% minerals (mostly potassium and phosphorous)and many more bioactive compounds like vitamin A vitamin C stigma steroland phenols etc[7]. The antioxidant activity was demonstarted by DPPH assay, while the anti-inflammatory activity of xylooligosaccharides produced fermentatively was studied by in-vitro method of albumin denaturation inhibition assay and bovine serum albumin denaturation inhibition assay for the first time without sacrificing any animal[8].

## MATERIAL AND METHODS

### Materials and methods

**Production of xylooligosaccharides**: The production of xylooligosaccharides was carried out in modified medium of horokoshi containing 1% w/v wheat bran and 1%w/v *Pistia sp.* The sterilized medium was inoculated with *Massilia timonae* B2YR KY942185 isolated from soil collected from saw mill industry at Badhule district of Kolhapur, Maharashtra,India[9]. The production broth was incubated at room temperature for 72 hours.

**Purification of xylooligosaccharides**: After fermentation of 72 hrs the fermented broth was added with two volumes of ice cold iso propanol to stop the reaction and precipitate unused xylan after. The precipitated xylan was removed by centrifugation broth at 7000 rpm for 15 minutes. The broth was kept for evaporation of isopropanol at room temperature. Then broth was passed through activated charcoal for removal of discoloration. Then it was added with ethanol to precipitate xylooligosaccharides. The precipitate of xylooligosaccharides formed was used for studying anti-inflammatory activity.

**Detection of Xylooligosaccharides formed**

The Xylooligosaccharides precipitated were ground to fine and reconstituted in distilled water at concentration of 1mg/ml and loaded on to Silica gel 60 along with standard sugars xylose and xylooligosaccharides like xylobiose and xylotriose in same concentration. The chromatography was carried out in solvent system of ratio butanol: acetic acid: water (2:1:1) the chromatogram was then developed using orcinol reagent[9].

**Qualitative estimation of phenol:**

1 ml aqueous extract of XOS was prepared in a concentration of 5mg/ml in isopropanal to this 3ml of 10% lead acetate solution[10]**.**

**Estimation of total phenol content**:

The total phenol content of XOS was determine by modified protocol of Bastola et al., 100 µl of XOS extract treated with 0.5 ml of folin ciocalteau reagent to this 2 ml of 2% $Na_2CO_3$ was addedthen incubated for 30 min at 28±2°C in dark and then absorbance measured at 660nm[11] . The phenol content was expressed as Gallic acid equivalent per liter of XOS extract.

**Antioxdant activity by DPPH assay**

DPPH(2,2-diphenyl-1- picrylhydrazyl is reduced by antioxidant substance which leads to discoloration which is measured as DPPH scavenging ability of substance. The produced XOS was checked for its antioxidant ability by investigating its DPPH scavenging ability. The XOS extract in was prepared in a concentration ranging frmm 0.2mg-1mg/ml distilled water. The assay was performed following the protocol given by Veenashri following slight modifiation were the concentration of XOS was varried and the reaction mixture contained 1ml of XOS extract and 1ml of freshly prepared DPPH solution in ethanol ( 0.1mMol/ml). After vigorous shaking mixture was incubated in dark for 30 minutes. The absorbance was measured at 517 nm. Control mixture contained DPPH solution and water while ethanol solution was used as blank.[12] The percent of antioxidation calculted using

$$\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100 \qquad \text{eqn} \quad 1$$

### *In vitro* Anti-inflammatory studies

**a. Inhibition of albumin denaturation**

The produced xylooligosaccharides were dissolved in distilled water to prepare a concentration ranging from 80 µg /ml to 400 µg /ml. Similarly, standard reference drug diclofenac was used in study with concentration 80 to 400 µg /ml. The reaction mixture contained 0.2 m egg albumin 2.8 ml phosphate buffer saline solution of 6.8 pH and 2 ml of Xylooligosaccharides concentration. The reaction mixture was then incubated at 37°C for 10-15 minutes followed by heating at 70°C for 10 minutes [13].After cooling the absorbance was observed spectrophotometrically at 660nm. The percent inhibition was calculated using following equation.

$$\frac{Absorbance\ negative\ control - Absorbance\ test\ sample}{Absorbance\ negative\ control} \times 100 \qquad \text{eqn 2}$$

**b. Bovine serum albumin denaturation inhibition assay**

2% w/v Bovine serum albumin solution was prepared in sodium phosphate buffer of pH 6.8. The stock of Xylooligosaccharides and reference drug Diclofenac was prepared in concentration of 1000 µg/ml in Distilled Water A concentration ranging from80 -400 µg /ml for both xylooligosaccharides and diclofenac was studied for Anti-denaturation of Bovine serum albumin. A modified method of William et al was used. 1.8 ml of BSA solution was reacted with 1.2 ml of sample and drug for 10-15 minutes at 37°C. Then, the reaction mixture was heated at 70°C for 10 minutes. After cooling absorbance was read at 660nm [8].The percent inhibition was calculated using equation mentioned above.

## RESULTS AND DISCUSSION

### Production of Xylooligosaccharides

Production of xylooligosaccharides by *Massilia timonae* B2YR KY942185 from 100 ml broth with 1% w/v concentration of wheat bran and *Pistia stratiotes* yielded 0.125mg of precipitate of xylooligosaccharides.

### Detection of Xylooligosaccharides

The produced xylooligosaccharides showed similar rf value as the standard xylobiose and xylotriose. Thus conforming the production of xylooligosaccharides presented in fig 1. Similar studies reported by [14] two distinct spots separated correspond to xylobiose and xylotriose.


Figure 1: TLC image of Xylooligosaccharides produced by *Massilia timonae* B2YR from wheat bran and *Pistia stratiotes* Lane X-xylose, Lane XOS-standard mixture of xylobiose and xylotriose, Lane B2X- XOS produced by organism.

### Estimation of phenol qualitatively

Formation of white bulky precipitate in the reaction mixture of XOS extract and lead acetate confirms presence of phenols**.**

### Quantification of Phenol content in XOS extract

The phenol content of XOS extract was quantified to 1236.914±48.50mg Gallic acid equivalent/lit of extract this being the highest report of from the reports of 116.86 mg GAE/ litre of extract. [15]. The most of the yield of phenols in the XOS extract contributed by *Pistia stratiotes.*

### Antioxidant activity of XOS:

The antioxidant activity of XOS presented in the fig 2 it can be seen that the antioxidant activity is concentration dependent which increases with the increase in concentration of extract from 0.2 mg/ml showing 15.38% to 1mg showing 74.86% antioxidant ability. Similar reports were the antioxidant activity showed concentration dependency was given by Jiang et al they observed nearly 62.1% at 0.8mg whereas present study shows 68.9% activity[16]. The IC50 value studied from the slope was0.6mg/ml.

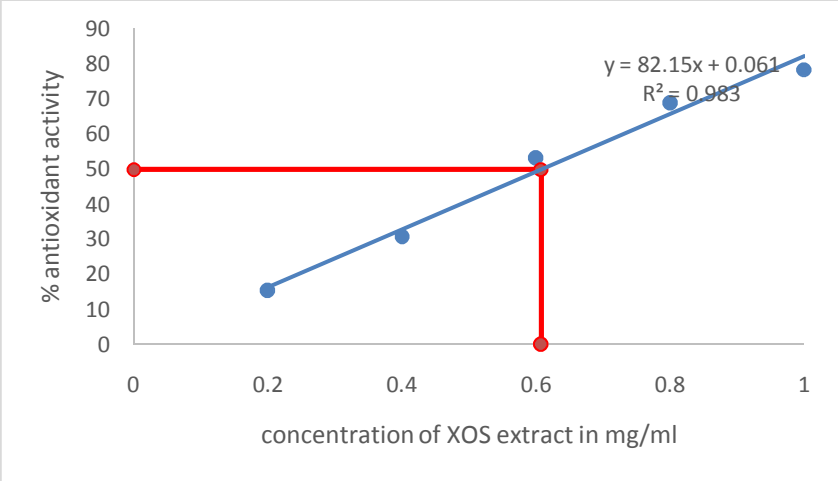


**Figure 2 Antioxidant study by DPPH assay of XOS extract**

**Table 1. The anti-inflammatory effect of Xylooligosaccharides on egg albumin and bovine serum albumin denaturation**

| Sr.no | Concentration in µg/ml | Percent inhibition of Egg albumin | |
|---|---|---|---|
| | | Diclofenac | XOS |
| 1 | 80 | 30.04± | 28.04 |
| 2 | 160 | 47.32 | 37.80 |
| 3 | 240 | 58.02 | 49.18 |
| 4 | 320 | 71.19 | 61.38 |
| 5 | 400 | 80.86 | 74.79 |
| | R squared | 0.996851 | 0.99654 |
| | IC 50 value | 192.25 µg/ml | 238.40 µg/ml |

All experiment performed in triplicates. The IC50 and R squared values obtained by regression analysis

**Table 2. The anti-inflammatory effect of Xylooligosaccharides on egg albumin and bovine serum albumin denaturation**

| Sr.no | Concentration in µg/ml | Percent inhibition of Bovine serum albumin | |
|---|---|---|---|
| | | Diclofenac | XOS |
| 1 | 80 | 40.31 | 33.33 |
| 2 | 160 | 50 | 43.33 |
| 3 | 240 | 61.25 | 52 |
| 4 | 320 | 73.75 | 62 |
| 5 | 400 | 85.62 | 74.66 |
| | R squared | 0.997884 | 0.995347 |
| | IC 50 value | 155.32µg/ml | 213.36 µg/ml |

All experiment performed in triplicates. The IC50 and R squared values obtained by regression analysis

***In-vitro* Anti-inflammatory studies:**

One of the cause of inflammation is protein denaturation and any compound preventing the denaturation of protein can be used for the treatment of inflammatory diseases. The compound which inhibit protein denaturation more than 20% could be studied as potential anti- inflammatory agent [17].Protein denaturation observed in present study was dependent on concentration of xylooligosaccharides, and the Diclofenac used as standard reference.

**a. Inhibition of albumin denaturation:**

The inhibition of egg albumin was maximum at 400 µg /ml of xylooligosaccharides which was 74.79%, While standard reference drug used diclofenac showed 80.86% at same concentration. The regression analysis for inhibition of egg albumin denaturation showed a p value of 0.003622and that of diclofenac was 0.004. The results are summarized in table 1. the IC50 values of xylooligosaccharides and diclofenac were 238.400 µg /ml and 192.256 µg /ml respectively. The *Cynodon dactylon* extract showed inhibition of 56.3% at 400µg/ml [13]

**b. Bovine serum albumin denaturation inhibition assay:**

The xylooligosaccharides showed maximum denaturation inhibition of bovine serum albumin 70.67% at 400 µg/ml, diclofenac showed 84.37% denaturation inhibition at 400 µg/ml. The IC50 value was 155.32 µg/ml and 213.36 µg/ml for diclofenac and xylooligosaccharides respectively as represented in table 2 . Similar studies have been carried out with extracts of *Enicostemmaaxillare* which showed maximum inhibition of 71% at 500 µg/ml [18].

The use of in-vitro studies for studying anti-inflammatory ability of xylooligosaccharides is reported for the first time. An anti-inflammatory effect of Xylooligosaccharides on RAW 264.7 , showed that LPS stimulated cells inhibited macrophage mediated cytokines production like TNF-α, IL-β and IL-6[19].

## CONCLUSION

An increase in health awareness has led to development of many health benefiting products xylooligosaccharides being one of them. The present work focusses on the antioxidant and anti-inflammatory study of Xylooligosaccharides which was produced from lignocellulosic biomass like wheat bran and *Pistia stratiotes.* The study is being reported for the first time for a xylooligosaccharides showing an anti-denaturation of both Egg albumin and Bovine serum albumin which confirms the anti-inflammatory property of xylooligosaccharides. Thus XOS produced from lignocellulosic wastes like wheat bran and *Pistia stratiotes* can be used as an antioxidant and anti-inflammatory agent

## ACKNOWLEGEMENT

We would like to express gratitude to Principal DrKhemner Sir for providing facilities for performing experiments. First author would like to thank the Head of the department and supervisor Dr Mrs. Y C Attar for her support, guidance. Also acknowledge BARTI Pune, for awarding fellowship and providing financial assistance.

## CONFLICT OF INTEREST

Authors declare no conflict of interest.

**Abrv**- XOS- Xylooligosaccharides

## REFERENCES

1. Yu X, Yin J, Li L, Luan C, Zhang J, Zhao C, et al. (2015). Prebiotic potential of xylooligosaccharides derived from corn cobs and their In Vitro antioxidant activity when combined with Lactobacillus. J Microbiol Biotechnol;25:1084–92. https://doi.org/10.4014/jmb.1501.01022.
2. Chandra S, Chatterjee P, Dey P, Bhattacharya S.(2012). Evaluation of in vitro anti-inflammatory activity of coffee against the denaturation of protein. Asian Pac J Trop Biomed:2011–3. https://doi.org/ 10.1016/S2221-1691(12)60154-3.
3. Gadamsetty G, Maru S, Sarada NC. (2013). Antioxidant and anti-inflammatory activities of the methanolic leaf extract of traditionally used medicinal plant mimusops elengi L. J Pharm Sci Res;5:125–30.
4. Tatti PN, Anitha S, Shashidhara S, Deepak M, Bidari S. (2012). Evaluation of in-Vitro Anti-Denaturation Activity of Isolated Compound of Butea Monosperma Bark . Pharma Sci Monit;3:2314–20.
5. Samanta AK, Jayapal N, Jayaram C, Roy S, Kolte AP, Senani S, et al. (2015). Xylooligosaccharides as prebiotics from agricultural by-products: Production and applications. Bioact Carbohydrates Diet Fibre ;5:62–71. https://doi.org/10.1016/j.bcdf.2014.12.003.
6. Babu CR, Ketanapalli H, Beebi SK, Kolluru VC, Bran W, Endosperm C. (2018). Wheat Bran-Composition and Nutritional Quality : A Review. Adv Biotechnol Microbiol;9. https://doi.org/10.19080 /AIBM.2018.09.555754.
7. Tulika T, Mala A. (2014). Pharmaceutical Potential of Aquatic Plant Pistia stratiotes (L.) and Eichhornia crassipes. J Plant Sci ;3:10. https://doi.org/10.11648/j.jps.s.2015030101.12.
8. Williams LAD, O'Connar A, Latore L, Dennis O, Ringer S, Whittaker JA, et al. (2008). The in vitro anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (Immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals. West Indian Med J;57:327–31.
9. Thanekar RJ, Attar YC. (2017). Study of Xylanolytic Microorganisms by using Xylan Extracted from Natural Waste. Int J Res Appl Sci Eng Technol;5:482–8. https://doi.org/10.22214/IJRASET.
10. Sangeetha G, Vidhya R.(2016). In vitro anti-inflammatory activity of different parts of Pedalium murex (L.). Int J Herb Med IJHM;4:31–6.
11. Bastola KP, Guragain YN, Bhadriraju V, Vadlani PV. (2017). Evaluation of Standards and Interfering Compounds in the Determination of Phenolics by Folin-Ciocalteu Assay Method for Effective Bioprocessing of Biomass. Am J Anal Chem;08:416–31. https://doi.org/10.4236/ajac.2017.86032.
12. R VB. (2015). Xylo-oligosaccharides isolated from ragi ( eleusine coracana ) bran: determination of their structure and function. Department of Biocheistry and Nutrition , Central Food Technological Research Institute .Mysore-020.

13. Suchita G. (2017). Assessment of In-vitro Anti-Inflammatory Activity of Cynodon Dactylon and Acyclovir Showing Synergistic Effect by Albumin Denaturation and Membrane Stabilization Assay. Mod Approaches Drug Des ;1:1–5. https://doi.org/10.31031/madd.2017.01.000506.
14. Hauli I, Sarkar B, Mukherjee T, Chattopadhyay A, Mukhopadhyay SK. Alkaline extraction of xylan from agricultural waste, for the cost effective production of xylooligosaccharides, using thermoalkaline xylanase of thermophilic Anoxybacillus sp. Ip-C. Int J Pure Appl Biosci 2013;1:126–31.
15. Rashad MM, Mahmoud AE, Nooman MU, Mahmoud HA, El-Torky AEDMM, Keshta AT. Production of antioxidant xylooligosaccharides from lignocellulosic materials using Bacillus amyloliquifaciens NRRL B-14393 xylanase. J Appl Pharm Sci 2016;6:30–6. https://doi.org/10.7324/JAPS.2016.60606.
16. Bian J, Peng F, Peng XP, Peng P, Xu F, Sun RC. (2013). Structural features and antioxidant activity of xylooligosaccharides enzymatically produced from sugarcane bagasse. Bioresour Technol ;127:236–41. https://doi.org/10.1016/j.biortech.2012.09.112.
17. Drăgan M, Stan CD, Iacob A, Profire L. (2016). Assessment of in vitro antioxidant and anti-inflammatory activities of new azetidin-2-one derivatives of ferulic acid. Farmacia;64:717–21.
18. Leelaprakash G, Mohan Dass S. (2011). Invitro anti-inflammatory activity of methanol extract of enicostemma axillare. Int J Drug Dev Res 3:189–96.
19. Chen HH, Chen YK, Chang HC, Lin SY. (2012). Immunomodulatory effects of xylooligosaccharides. Food Sci Technol Res;18:195–9. https://doi.org/10.3136/fstr.18.195.

**CITATION OF THIS ARTICLE**

R J Thanekar, S D Mali, M M Bendigeri, Y C Attar· Antioxidant and Anti-inflammatory xylooligosaccharides produced from lignocellulosic materials. Bull. Env.Pharmacol. Life Sci., Vol10[4] March 2021 : 129-134