International Journal of Pharmacy and Biological Sciences
ISSN: 2321-3272 (Print), ISSN: 2230-7605 (Online)
IJPBS | Volume 8 | Issue 1 | JAN-MAR| 2018 | 623-631
Research Article | Biological Sciences | Open Access | MCI Approved| ज्ञान-विज्ञान विमुक्तये
|UGC Approved Journal |

ANTI-INFLAMMATORY ACTIVITY OF THE EXOPOLYSACCHARIDES (EPS) PRODUCED FROM POLLUTED SOIL

Ravi Gangalla[1], Baswaraju Macha[2], Sarika Kasarla[2], Rakesh Eerla[3], Raja komuraiah Thampu[*]

[1, 2,3 *] Department of Microbiology, Kakatiya University, Warangal-506009, Telangana, India

[2]Medicinal Chemistry Division, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Telangana, India.

***Corresponding Author Email: rkthampukuc@yahoo.com**

ABSTRACT

Anti-Inflammatory activity of the exopolysaccharides produced from the polluted soil bacteria. Isolate the Exopolysaccharides were purified by recrystalization techniques and estimated by total carbohydrates by the phenol-sulphuric acid method. Exopolysaccharides (GR-1 to GR-21) were evaluated for In-vitro, In-vivo anti-inflammatory activity the isolated Exopolysaccharides GR-2-65.33±0.14, GR-5- 61.24±0.15, GR-1 -58.23±0.38, considered to possess potent anti-inflammatory activity when compared to standard drug indomethacin.

KEY WORDS

Anti-inflammatory activity, exopolysaccharides (EPS), indomethacin.

INTRODUCTION

Cyclooxygenases (COX) or prostaglandin endoperoxide synthases are the key enzymes in the synthesis of prostaglandins, the main mediators of inflammation, pain, and increased body temperature (hyperpyrexia). The body produces two main isoforms of COX proteins, that is, cyclooxygenases- 1 (COX-1) and cyclooxygenases-2 (COX-2). The COX-1 is responsible for formation of important biological mediators such as prostanoids, including prostaglandins, prostacyclin, and thromboxane, and involved in pain causing, blood clotting, and protecting the stomach (S. S. Chhajed, P. B. Hiwanj, V. A. Bastikar et al., 2010), whereas COX-2 is involved in the pain by inflammation and plays a major role in prostaglandin biosynthesis in inflammatory cells and central nervous system (P. McGettigan and D. Henry 2006). When COX-1 is inhibited, inflammation is reduced, but the protection of the lining of the stomach is also lost. This can cause stomach upset as well as ulceration and bleeding from the stomach and even the intestines. Whereas COX-2 is usually specific to inflamed tissue, there is much less gastric irritation associated with COX-2 inhibition together with the decreased risk of peptic ulceration (C. J. Hawkey 1999). Therefore, selective COX-2 inhibitors such as celecoxib and rofecoxib had been developed for ease of inflammation associated with COX (R. P. Mason, M. F. Walter, H. P. McNulty et al., 2006) .The use of coxib drugs such as rofecoxib (VioxxW) and valdecoxib (BextraW) was withdrawn from the market in 2004 and 2005, respectively, because of increased risk of heart attacks and strokes with long-term use (K. Han, Y. Zhou, F. Liu et al., 2014). At present, celecoxib (CelebrexW) is the only COX-2 inhibitor available in the United States. Hence, there is a need for COX-2 inhibitor with no adverse effects Exopolysaccharides are one of the well-known sourced of bio-macromolecules. It widely exists in the plants, microorganism, algae, and other animals (Y. Yu et al., 2018; J. H. Xie et al., 2016; F. Zhang et al., 2016). Polysaccharides derived from various fungi have been developed for edible and medicinal use, especially polysaccharides for the different mushrooms (X. Meng et al., 2016). Bacterial Exopolysaccharides can also be divided into exopolysaccharide, intracellular

exopolysaccharide, and cell wall polysaccharide according to their cellular storage. Animal polysaccharides are widely distributed, which exist in almost all tissues and organs.

Natural exopolysaccharides have a broad spectrum of biological effects, such as anti-tumor, anti-cancer, antibiotic, antioxidant, anti-mutant, anticoagulant, and immune-stimulation activities (Z.J. Wang et al., 2018; Z. Wang et al., 2017; J.-H. Xie et al., 2016; J.H. Xie et al., 2015) can directly influence the metabolism and display medicinal applications (J. H. Xie et al., 2016). Among them, the anti-tumor effect of polysaccharides has attracted increasing attention. Multiple polysaccharides have been recognized as the safe and non-toxic adjuvants in tumor treatment (J. H. Wang et al., 2017; Y. Yu et al., 2017; J. H Wang et al., 2010). Polysaccharides exposing to gastric fluid, rather than monosaccharide, only a few have lower molecular weights. Other carbohydrates, such as starch, are hydrolyzed thoroughly in the small intestine and absorbed into the portal blood (A. Lovegrove et al., 2017). Exopolysaccharides can regulate the whole tumor microenvironment, in which the human body immunity is a target of vital role (L. Liu et al., 2016). The immune organ indices are increased in the host (L. F. Xia et al., 2016) besides, immune cells, including lymphocytes (F. N. Razali et al., 2016), macrophages, and NK cells (M. S. Shin et al., 2017), are also activated in infiltrating tumor tissues with exopolysaccharide intervention. In mice with breast cancer, Solanumnigrum polysaccharide fraction can elevate the level of TNF and control the relationship between pro-inflammatory and anti-inflammatory cytokines (F. N. Razali et al., 2016), it could possibly enhance the host immune response in fighting cancer. Exopolysaccharides-immune crosstalk is also linked with the problems of specificity and sensitivity. Exopolysaccharide has been extensively used for immune modulator in anti-tumor therapy, which can not only improve the therapeutic effect but also reduce the toxic effect also. Supplementation with polysaccharides-based enteral formula from Crassostreahongkongensis would be beneficial for patients undergoing 5-fluorouracil chemotherapy (B. Cai et al., 2014).

Bacterial exopolysaccharides (EPS) produced by polluted soil bacteria have found their most valuable application in the improvement of the rheology, texture, stability and sensory properties of fermented foods (Ko et al., 2016). Polluted soil bacteria (PSB) produce a wide diversity of EPS, with unique chemical compositions and structures, comprising homopolysaccharides and heteropolysaccharides (Sanlibaba and Çakmak 2016). Among the homopolysaccharides, glucans and fructans are most frequently found and are mainly produced by the genus Leuconostoc, Lactobacillus, Streptococcus and Weissella (Sanlibaba and Çakmak 2016; Zannini et al. 2016). Extracellular enzymes synthesize a variety of α-glucans such as dextrans from sucrose (Leemhuis et al., 2013). The biological function of the EPS was studied, focusing on its Anti-Inflammatory activity, for exploring the application of the EPS as a functional ingredient in the food industry.

MATERIAL AND METHODS:

Isolation and purification of Exopolysaccharide producing bacteria (EPS):

Isolation of bacteria producing an exopolysaccharide (BPE) was performed on several samples of soil were taken from different sugarcane polluted soil (Gayathri sugarcane factory, Kamareddy, Telangana, India). Soil material was taken from a depth of 5-20cm. A total of ten gram of soil material aseptically suspended in physiological saline solution (0.85%) and serial dilutions were made to 10^{-l} to 10^{-6} and incubated in medium ATCC (per liter of medium): 0.2 g KH 2 PO4 ; 0.8 g K2 HPO4 ; 0.2 g MgSO4.7H 2 O; 0.1 g CaSO4 .2H2 O; 2.0 mg FeCl3 ; Na2MoO4.2H2O (trace); 0.5 g Yeast Extract; 20 g sucrose; and 15 g agar bacto with pH 7.0 and NAM medium for four days at a temperature of 28ºC (Remel, 2005; Santi et al., 2008). Bacteria that produce EPS characterized by colonies of various bacteria that form thick slime (mucoid) subsequently selected (Tallgren et al., 1999) and purified by restreaking colonies until obtaining single colonies. Selection of bacterial exopolysaccharide-producing potential by setting the dry weight of bacterial exopolysaccharide produced according to the method proposed by (Emtiazi et al., 2004).

Screening bacteria producing exopolysaccharide:

Selection and screening bacterial exopolysaccharide-producing potential by setting the dry weight exopolysaccharide produced by bacteria in broth medium ATCC (per liter of medium): 0.2 g KH 2 PO4; 0.8 g K2 HPO4; 0.2 g MgSO4.7H 2 O; 0.1 g CaSO4 .2H2 O; 2.0 mg FeCl3; Na2MoO4.2H2O (trace); 0.5 g Yeast Extract; 20 g sucrose; with pH 7.2 using sucrose as a main carbon source method proposed by Emtiazi et al., (2004) and Santi et al., (2008). Colonies of bacteria that form thick slime (mucoid) on solid medium ATCC were grown in 250 ml broth medium ATCC and incubated at a temperature of 28ºC for three days at the top of the machine shaker with 100 rpm rotation (MAXQ 6000 USA). After incubation culture were heated with 100ºc in 2minuts for enzyme inactive. At the end of incubation, cells were harvested with 1 mM EDTA by adding 500µg/l then shaken until homogeneous heat generated while adding and then centrifuged at 12000 rpm for 12 min. The supernatant was separated from the bacterial cell deposition was taken, coupled with cold acetone solution with a ratio of 1:2. Then performed again with the speed centrifugation 15000 rpm for 15 minutes. Deposition of biomass in the form of exopolysaccharide then washed with distilled water three times and dried at 60ºC for 24 hours or until dry weights obtained were fixed.

Phenol-Sulphuric Acid Method for EPS detection (Total Carbohydrate):

Exopolysaccharides were estimated by total carbohydrates by the phenol-sulphuric acid method (Dubois et al., 1956). Concentrated sulphuric acid causes hydrolysis of glycosidic linkages, these hydrolyzed neutral sugars are then partially dehydrated with the elimination of three molecules of water to form furfural or furfural derivatives. The color compounds developed by the condensation of furfural or furfural derivatives with phenol are measured at 490 nm.

Reagents:

Phenol - 5 % (w/v)

Sulphuric acid – Concentrated (35.7 N)

Procedure:

1 ml of Eps sample, 1ml 5 % (w/v) phenol was added followed by 5 ml concentrated sulphuric acid. The sample tubes were kept in ice cubes while adding sulphuric acid. The mixture was incubated at room temperature for 25 min and the absorbance read at 490 nm. The Glucose was used as the standard in the range of 0-100 µg/ml concentration from 1 mg/ml stock solution. A standard graph was plotted with absorbance at 490 nm against the concentration of glucose. A blank was also prepared in the same way (0 mg glucose).

Table.1 Isolation of bacteria producing exopolysaccharide (EPS)

No	Isolate strain Code	Colony Color Media ATCC	Gram Test Positive	Gram Test Negative	Description
1.	1	White turbid			*-Slimy*
2.	2	White turbid	+		*VerySlimy*
3.	3	White turbid			*-Slimy*
4.	4	Yellowish white	+		Not Slimy
5.	5	White turbid	+		*Somewhat Slimy*
6.	6	Yellowish white			*- Slimy*
7.	7	White turbid	+		Not Slimy
8.	8	White			*-Somewhat Slimy*
9.	9	White turbid	+		Not Slimy
10.	10	White turbid	+		Not Slimy
11.	11	Yellowish white	+		Not Slimy
12.	12	Yellowish white	+		Not Slimy
13.	13	White turbid	+		Not Slimy
14.	14	Yellowish	+		Not Slimy
15.	15	White turbid	+		Not Slimy
16.	16	White turbid			*-Somewhat Slimy*
17.	17	White turbid			*-Slimy*
18.	18	White	+		Not Slimy
19.	19	Translucent white	+		Not Slimy
20.	20	White turbid	+		Not Slimy
21.	21	Yellowish white	+		Not Slimy

*** Very Slimy = - - - - Slimy = - - - Enough Slimy =- - Somewhat Slimy =-**

Table.2 Dry matter exopolysaccharide in exopolysaccharide production medium for 72 h of incubation

S.No	Isolates Code	Dry matter EPS (mg/ml)
1	GR-1	1.49
2	GR-2	1.63
3	GR-3	0.23
4	GR-4	0.19
5	GR-5	2.13
6	GR-6	0.18
7	GR-7	0.09
8	GR-8	0.03
9	GR-9	1.67
10	GR-10	1.72
11	GR-11	0.45
12	GR-12	0.53
13	GR-13	0.83
14	GR-14	0.45
15	GR-15	0.49
16	GR-16	0.23
17	GR-17	0.75
18	GR-18	0.39
19	GR-19	0.42
20	GR-20	0.47
21	GR-21	0.56

Fig-1: Exopolysaccharide

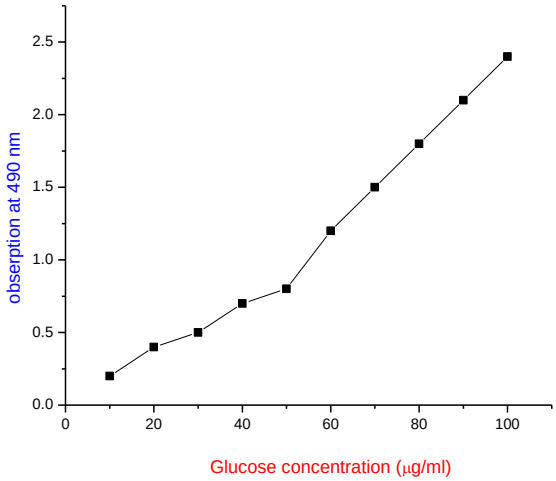

Fig-2: Absorbance V/s Concentration (for glucose standard graph)

BIOLOGICAL ACTIVITY:

The Isolation compounds were evaluated for In Vitro *Anti-Inflammatory Activity* by using TMPD Assay method for this Cayman's Colorimetric COX (Ovine) Inhibitor screening Assay Kit. The results were depicted in **table-3.**

Evaluation of anti-inflammatory activity

***In vitro* Anti-inflammatory activity**

The Isolation compounds were evaluated for their *in vitro* anti-inflammatory activity by TMPD assay method This assay is based on chromogenic assay based on oxidation of N, N, N', N, -tetra methyl-p-phenylenediamine (TMPD) during the reduction of Prostaglandin H2 by COX-2 enzyme. This measures the peroxides component of cyclooxygenases. The peroxide activity is assayed calorimetrically by monitoring the appearance of oxidized N, N, N', N, -tetramathyl-p-phenylenediamine (TMPD) at 590nm. The final volume of the assay was 220µl. All the wells background wells contain 160µl of assay buffer and 10µl of heme and 10µl of enzyme. The inhibitor wells contain150µl of assay buffer and 10µl of heme, 10µl of enzyme and 10µl of inhibitor. The plate was shaken for a few seconds and incubated for five minutes at 25ºC.Then 20µl of colorimetric substance, 20µl of arachidonic acid were added. The plate was again shaken for a few seconds and incubated for five minutes at 25^0C.Then the absorbance was noted at 590nm using plate reader.

Table: 3: *In vitro* anti-inflammatory activity:

S.No	Compound	COX-2 Inhibition
1	**GR-1**	**58.23±0.38**
2	**GR-2**	**65.33±0.14**
3	GR-3	41.19±0.24
4	GR-4	39.23±0.14
5	**GR-5**	**61.24±0.15**
6	GR-6	37.21±0.23
7	GR-7	31.16±0.35
8	GR-8	28.23±0.22
9	**GR-9**	**56.24±0.35**
10	GR-10	34.17±0.33
11	GR-11	35.15±0.22
12	GR-12	41.18±0.11
13	GR-13	28.21±0.23
14	GR-14	33.16±0.35
15	GR-15	28.23±0.22

S.No	Compound	COX-2 Inhibition
16	GR-16	11.24±0.35
17	GR-17	14.17±0.33
18	GR-18	25.15±0.22
19	GR-19	41.18±0.11
20	GR-20	33.16±0.35
21	GR-21	27.23±0.22
22	**Indomethacine**	**73.32±0.32**

***IN VIVO* ANTI-INFLAMMATORY ACTIVITY:**

The compounds which are showing best activity by in-vitro anti-inflammatory activity 4 Compounds were selected and were evaluated for in vivo anti-inflammatory activity at a dose range of 100mg/kg body weight by carragenan induced rat paw edema method, From the data it was reveals that all the tested compounds significantly reduced carrageenan induced edema and the results were presented in **Table.4 and 5**.

Table 4: In-vivo anti-inflammatory activity

S.No	Compound	Mean Paw Edema Volume in ml ± SD			
		1h	2h	3h	4h
1	GR-1	0.38	0.31	0.28	0.24
2	GR-2	0.37	0.35	0.31	0.28
3	GR-5	0.32	0.29	0.25	0.20
4	GR-9	0.35	0.31	0.29	0.24
5	**Control Group**	0.40	0.45	0.50	0.55
6	**Indomethacin**	**0.35± 0.070**	**0.33 ± 0.120**	**0.28 ± 0.080**	**0.21 ± 0.062**

Table 5: *In-vivo* anti-inflammatory activity

S.No	Compound	% Inhibition of Paw Edema			
		1h	2h	3h	4h
1	GR-1	28.31	38.27	42.52	54.75
2	GR-2	16.07	25.75	32.66	44.75
3	GR-5	21.0	33.1	40.7	57.5
4	GR-9	19.63	38.1	42.11	43.75
5	**Indomethacin**	**37.5**	**47.6**	**60.5**	**73.7**

RESULTS AND DISCUSSION

A total of 21 isolates were obtained from the polluted soil material (Table 1). The bacterial growth in the medium ATTC shows the diversity of morphology of bacteria that grow both shape and color of a single colony and mass so that the selected 21 isolates suspected to produce exopolysaccharide to characterize the bacterial colony forming slimy slime. A number of these isolates based on test results produce gram-negative 6 isolates and 15 isolates of gram-positive bacteria. Gram-negative bacteria produced marked with slimy bacteria after reacted with KOH and vice versa gram-positive bacteria are not slimy.

In table1 above shows that the difference in the growth of bacteria isolated in the semi-selective medium is medium ATCC (American Type Culture Collection) by the general media are NAM (Nutrient Agar Medium). Where the media NAM diverse bacterial growth that must be screened to select a slimy bacteria or gram-negative bacteria to specific media tested exopolysaccharide. While in ATCC medium which is a semi-specific medium so that the bacteria are grown is a bacterial exopolysaccharide.

Five of potential bacterial exopolysaccharide producing each of the isolates code GR1, GR2, GR5, GR9, and GR10 can produce dry weight exopolysaccharide from 1.49 to 2.13 mg/ml of medium. Dry weight exopolysaccharide weighing results indicate that the bacterial isolates code GR5 resulted in a higher dry weight than the other isolates

of bacteria with the code. Excreting bacteria growth exopolysaccharide around the related bacteria.

Polluted soil matrix is a plant root development, production of root exudates internal metabolic outcomes plants which generally contain a lot of carbon compounds, organic and the growth of macro and micro polluted soil. Some organic compounds with low molecular weight such as simple sugars and polysaccharides (arabinose, fructose, glucose, maltose, mannose), oligosaccharides, amino acids (arginine, asparagine, aspartate, cysteine, cystine, glutamine), acid Organic (acetic, ascorbic, benzoic acid, and malic) and phenolic compounds. Some of these compounds can enhance the growth and development of soil microorganisms. Five isolates that have a value of potential in producing exopolysaccharide are GR1-GR21 isolates compounds were screened for in *vitro* anti-inflammatory activity. The assay was performed using colorimetric COX (ovine) inhibitor screening assay kit (Cyaman chemical, MI, USA). The colorimetric COX (ovine) inhibitor screening assay utilizes the peroxidase activity of ovine cyclooxygenase to oxidize the colorimetric substrate, N, N, N', N'-tetramethyl-p-phenylenediamine (TMPD). The results were presented in Table 3. Among the tested compounds GR-1, GR-2, GR-5 and GR-9 are considered to possess more potent anti-inflammatory activity when compared to standard drug indomethacin.

From the compounds which have shown best in-vitro anti-inflammatory activity, 4 Compounds were selected and were evaluated for in vivo anti-inflammatory activity at a dose range of 100mg/kg body weight by carrageenan induced rat paw edema method, From the data it was revealed that all the tested compounds significantly reduced carrageenan induced edema and the results were presented in Table4 and 5. Among the tested compounds are GR-1, GR-2, GR-5 and GR-9 considered to possess potent anti-inflammatory activity when compared to standard drug indomethacin.

CONCLUSION:

A total of 21 isolates were obtained from the polluted soil material (Table 1). Purified recrystalization techniques and characterization by biochemical tests and evaluated for In-vitro, In-vivo anti-inflammatory activity. GR-1, GR-2, GR-5 and GR-9 considered to possess potent anti-inflammatory activity when compared to standard drug indomethacin.

REFERENCE

A. Lovegrove, C.H. Edwards, I. De Noni, H. Patel, S.N. El, T. Grassby, C. Zielke, M. Ulmius,

L. Nilsson, P.J. Butterworth, P.R. Ellis, P.R. Shewry, Role of polysaccharides in food, digestion, and health, Crit. Rev. Food. Sci. Nutr. 57 (2017) 237-253.

Bertin, C., Yang, X., Weston, L.A. 2003. The role of root exudates and allelochemicals in the rhizosphere. *Plant Soil,* 256: 67 83.

C. J. Hawkey, "COX-2 inhibitors," *The Lancet*, vol. 353, no. 9149, pp. 307–314, 1999.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers and F. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

Emtiazi, G., Ethemadifar, Z., Habibi, M.H. 2004. Production of extracellular polymer in Azotobacter and biosorption of metal by exopolymer. *Afr. J. Biotechnol.,* 3(6): 330 333.

F. N. Razali, S. K. Sinniah, H. Hussin, N. Z. Abidin, A. S. Shuib, Tumor suppression effect of

*Solanumnigrum*polysaccharide fraction on Breast cancer via immunomodulation, Int. J. Biol.

Macromol. 92 (2016) 185-193.

F.Zhang, L.H. Lin, J.H. Xie, A mini-review of chemical and biological properties of polysaccharides from *Momordicacharantia*, Int. J. Biol. Macromol. 92 (2016) 246-253.

H. Xie, F. Zhang, Z.J. Wang, M.Y. Shen, S.P. Nie, M.Y. Xie, Preparation, characterizationand antioxidant activities of acetylated polysaccharides from *Cyclocaryapaliurus*leaves, Carbohyd. Polym. 133 (2015) 596-604.

J.-H. Xie, Z.-J. Wang, M.-Y Shen, B. Gong, H.-S. Li, Q. Zhao, W.-J. Li, M.-Y. Xie, Sulfatedmodification, characterization and antioxidant activities of polysaccharide from *Cyclocarya paliurus*, Food Hydrocolloid. 53 (2016) 7-15.

J. H. Wang, S. R Zuo, J. P. Luo, Structural Analysis and Immuno-Stimulating Activity of an

Acidic Polysaccharide from the Stems of *Dendrobiumnobile*Lindl. Molecules. 22 (2017), 611.

J. H Wang., J. P. Luo, X. Q. Zha, B. J. Feng, Comparison of antitumor activities of different polysaccharide fractions from the stems of *Dendrobiumnobile*Lindl. Carbohydr. Polym. 79 (2010) 114-118.

J. H. Xie, M.L. Jin, G.A. Morris, X.Q. Zha, H.Q. Chen, Y. Yi, J.E. Li, Z.J. Wang, J. Gao, S.P.

Nie, Advances on bioactive polysaccharides from medicinal plants, Crit. Rev. Food. Sci. Nutr.
56 (2016) S60-S84. B. Cai, H. Chen, H. Sun, P. Wan, H. Sun, J. Pan, Production of immunoregulatory polysaccharides from *Crassostreahongkongensis*and their positive effects as a nutrition factor in modulating the effectiveness and toxicity of 5-FU chemotherapy in mice, Food Func. 7 (2016)
390-397.
K. Han, Y. Zhou, F. Liu et al., "Design, synthesis and in vitro cytotoxicity evaluation of 5-(2-carboxyetheny isatin deriva-tives as anticancer agents," *Bioorganic & Medicinal Chemistry Letters*, vol. 24, no. 2, pp. 591–594, 2014.
Ko, J.A., Ryu, Y.B., Park, J.Y., Kim, C.Y., Kim, J.S., Nam, S.H., Lee, W.S. and Kim, Y.M. (2016) Glucosylrubusosides by dextransucrases improve the quality of taste and sweetness. *J MicrobiolBiotechnol* **26**, 493-497.
L. F. Xia, S. H. Liang, H. Wen, J. Tang, Y. Huang, Anti-tumor effect of polysaccharides fromrhizome of *CurculigoorchioidesGaertn*on cervical cancer, Trop. J. Pharm. Res. 15 (2016)
1731-1737.
Leemhuis H, Pijning T, Dobruchowska JM, van Leeuwen SS, Kralj S, Dijkstra BW, Dijkhuizen
L (2013) Three-dimensional structures, reactions, mechanism, a-glucan analysis and their impli-
cations in biotechnology and food applications. J Biotechnol 163:250–272.
L. Liu, S. Nie, M. Xie, Tumor Microenvironment as a New Target for Tumor Immunotherapy of Polysaccharides, Crit. Rev. Food. Sci. Nutr. 56 (2016) S85-S94.
M. S. Shin, S. H. Hwang, T. J. Yoon, K. S. Shin, Polysaccharides from ginseng leaves inhibit tumor metastasis via macrophage and NK cell activation, Int. J. Biol. Macromol. 103 (2017)1327-1333.
P. McGettigan and D. Henry, "Cardiovascular risk and inhibi-tion of cyclooxygenase: a systematic review of the observational studies of selective and nonselective inhibitors of cyclooxyge-nase 2," *The Journal of the American Medical Association*, vol. 296, no. 13, pp. 1633–1644, 2006.
Remel, 2005. Microbiology products: instructions for use of MacConkeyAgar. (2013).
Santi, L.P., Ai Dariah, danGoenadi, D.H.2008. Increased mineral soil aggregate stability by exopolysaccharide-producing bacteria. *Tower Estates,* 76(2): 93 103.
R. P. Mason, M. F. Walter, H. P. McNulty et al., "Rofecoxib increases susceptibility of human LDL and membrane lipids to oxidative damage: a mechanism of cardiotoxicity," *Journal of Cardiovascular Pharmacology*, vol. 47, no. 1, pp. S7–S14, 2006.
Sanlibaba, P. and Çakmak, G.A. (2016) Exopolysaccharides production by lactic acid bacteria. *Applied Microbiol***2**, 1000115.
S. S. Chhajed, P. B. Hiwanj, V. A. Bastikar et al., "Structure based design and in-silico molecular docking analysis of some novel benzimidazoles," *International Journal of ChemTech Research*, vol. 2, no. 2, pp. 1135–1140, 2010.
Tallgren, A.H., Airaksinen, U., von Weissenberg, R., Ojamo, H., Kuusisto, J., Leisola, M. 1999. Exopolysaccharide-producing bacteria from sugar beets. *Appl. Environ.Microbiol.*65 (2): 862 864.
X. Meng, H. Liang, L. Luo, Antitumor polysaccharides from mushrooms: a review on the structural characteristics, antitumor mechanisms and immunomodulating activities, Carbohydr.
Res. 424 (2016) 30-41.
Y. Yu, M.Y. Shen, Z.J. Wang, Y.X. Wang, M.Y. Xie, J.H. Xie, Sulfated polysaccharide from
*Cyclocaryapaliurus*enhances the immunomodulatory activity of macrophages, Carbohyd.
Polym. 174(2017) 669-676.
Y. Yu, M.Y. Shen, Q.Q. Song, J.H. Xie, Biological activities and pharmaceutical applications of polysaccharide from natural resources: A review. *Carbohydr. Polym.* 183 (2018) 91-101.
Zannini, E., Waters, D.M., Coffey, A. and Arendt, E.K. (2016) Production, properties, and industrial food application of lactic acid bacteria-derived exopolysaccharides. *ApplMicrobiolBiot* 100, 1121-1135.
Z.J. Wang, J.H. Xie, M.Y. Shen, S.P. Nie, M.Y. Xie, Sulfated modification of polysaccharides:
Synthesis, characterization and bioactivities, Trends Food Sci. Tech. 74(2018) 147-157.
Z. Wang, J. Xie, Y. Yang, F. Zhang, S. Wang, T. Wu, M. Shen, M. Xie. Sulfated *Cyclocarya*
Paliurus polysaccharides markedly attenuates inflammation and oxidative damage inlipopolysaccharide-treated macrophage cells and mice, Sci. Rep. 7 (2017) 40402.

***Corresponding Author:**
Raja komuraiah Thampu*
Email: **rkthampukuc@yahoo.com**