ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

ANTIMICROBIAL AND DYEING POTENTIAL OF PRODIGIOSIN OBTAINED FROM MULTI-DRUG RESISTANT *SERRATIA NEMATODIPHILA* EXHIBITING LOW TOXICITY

Chandrima Ghosh, Shraddha Patel, Shubhada Nayak*

Department of Microbiology, Rayat Shikshan Sanstha's Karmaveer Bhaurao Patil College (K.B.P), Vashi, Navi Mumbai, Maharshtra, India

**Corresponding author: shubhadanayak@kbpcollegevashi.edu.in*

ABSTRACT

The industrial production of a commodity varies proportionally with its demand in the commercial market. Recently, a strong inclination of consumers towards green products has led to the adaptation of sustainable production processes for various products including pigments. Prodigiosin is one such pigment that has raised curiosity among researchers for its dyeing potential as well as its antioxidant and anticancer activities. In the current study, the biomedical applications and dyeing potential of prodigiosin, obtained from *Serratia nematodiphila*, was studied. The antimicrobial resistance profile of the prodigiosin producer was also carried out owing to the widespread antibiotic resistance in nature. Interestingly, *S. nematodiphila* isolated from garden soil was found to be a multi-drug resistant strain. The pigment extracted from this isolate also showed broad spectrum antibacterial activity. Antifungal activity was also observed against one test culture used in our study. The pigment showed a considerably high LC_{50} value of 0.5mg/mL, indicating its possible use for various biomedical applications below the LC_{50} concentration. Furthermore, it showed good dyeing potential without the use of a mordant. The dye was retained even after treatment with a mild detergent. Moreover, the antibacterial activity of prodigiosin was confirmed after dyeing and treatment of the cloth with mild detergent, based on SASMIRA test. Thus, our results indicate considerable potential of prodigiosin for industrial application.

Keywords: Optimization, Pigment, Prodigiosin, *Serratia nematodiphila*, Sustainable.

1. INTRODUCTION

The interest in sustainable approach to industrial manufacturing processes is increasing worldwide. Since synthetic dyes and colorants are used extensively in several industries, the use of natural pigments has evidently attracted the interest of researchers. For instance, the cosmetic and textile industries almost exclusively depend on dyes for marketing of their finished goods. The food and pharmaceutical industries also rely on food grade colorants to improve the appearance of products. Although natural pigments can be obtained from two basic sources i.e. plants and micro-organisms, the latter is often a convenient choice for manufacturers. This is due to the stability and easier scale up of production that allows better management and downstream processing of microbial pigments as against other sources [1]. Several microbial pigments are identified and reported in literature. Few examples of natural pigments include carotenoid, melanin, flavin, quinone, monascin, violacein, indigo and prodigiosin [2].

Prodigiosin is a red pigment commonly produced, as a secondary metabolite, by *Serratia marcescens* and other *Serratia* spp. Less commonly, it is also produced by the genus *Pseudomonas*, *Vibrio, Alteromonas*, *Rugamonas*, *Monascus*, *Rhodotorula*, *actinomycetes* and *Pseudoalteromonas* [3, 4]. While pigments like β-carotene, riboflavin and phycocyanin find extensive application in food industries, prodigiosin is specifically in demand by pharmaceutical industries. The natural pigments in food industries are basically selected based on their color diversity, preservative and antioxidant nature. Besides, they are safe and biodegradable. In addition to these properties, the promising applications of prodigiosin in pharmaceutical industries are due to several pharmacological properties exhibited by this compound. Published studies have reported antimicrobial, anti-tumor, anticancer and cytotoxic activities of prodigiosin and prodigiosin like pigments [5]. The low cytotoxicity of prodigiosin in noncancerous cells and high apoptotic activity observed in cancer cell lines are ideal properties of an anti-cancer drug. Several reports on promising

anticancer activity of prodigiosin has prompted the discovery of possible anticancer drugs in near future [5-7]. Moreover, the antimalarial and antifouling activities of prodigiosin are also documented [8, 9]. Prodigiosin also shows potential application in other sectors like immunology. A key role of this compound has been identified in cell signalling and virulence of bacteria [10]. Prodigiosin produced by strains of *Vibrio* has shown better survival on exposure to UV irradiation as compared to non-pigmented strains, suggesting its protective action against the same [11].

The choice between prodigiosin, or other natural pigments, and synthetic dyes becomes more comprehensive considering the stability of the former compound to light, heat and pH [12]. The synthetic pigments on the other hand, are less stable and identified as a probable mutagenic/carcinogenic agent. They also persist in the environment for decades causing environment pollution [13]. Furthermore, the raw material for currently used colorants isnon-renewable resource such as fossil oil [14].

The pigment obtained, *from Serratia nematodiphila*, was characterized as prodigiosin and optimised for maximum production in a previous study [15]. In the current study, various applications of prodigiosin including its antibacterial, antifungal, cytotoxic and dyeing potential were studied.

2. MATERIAL AND METHODS

2.1. Isolation, screening and identification of pigment producing bacteria

The isolation and screening of pigment producing bacteria was carried out in a previous study. The isolate was identified as *S. nematodiphila* and the pigment was characterised asprodigiosin based on UV-visible spectrophotometry [15].

2.2. Extraction and purification of prodigiosin

Prodigiosin was extracted using the solvent (methanol) extraction method [16]. For carrying out the extraction procedure, *S. nematodiphila* was grown in sterile Nutrient Broth (NB), previously optimized for pigment production, for 48h [15]. The culture broth was centrifuged at 10,000rpm for 15min. The pellet thus obtained was washed with distilled water and centrifuged again at 10,000rpm for 15min. The centrifuged and washed pellets were mixed thoroughly with acidic methanol (prepared by mixing 0.1N HCl to methanol until the pH dropped to 3), by vortexing, and the cells were lysed using a sonicator (Ultrasonic homogenizer, BioLinx with 6mm probe). After sonication, the pigment released in the supernatant was collected as crude pigment extract. This extract was purified using the separating funnel extraction method [17]. In this method, the above supernatant and petroleum ether was added to a separating funnel in equal proportion. This mixture was mixed vigorously by intermittent shaking and then allowed to rest under Room Temperature (28°C, RT), to allow separation of solvent layers. The cellular impurities were retained in the upper layer and the extracted pigment settled at the bottom of the separation funnel in the methanol layer. The separated pigment was collected carefully and the solvent was allowed to evaporate at RT. The procedure was repeated again to obtain a considerably pure powdered form of prodigiosin pigment.

2.3. Evaluation of antibiotic sensitivity profile of *S. nematodiphila*

The antibiotic sensitivity profile of *S. nematodiphila* was evaluated with the help of Antibiotic Sensitivity Test (AST) using disc diffusion method. The AST was performed using a Mueller Hilton (MH) agar plate pre-swabbed with *S. nematodiphila* ($0.1O.D_{540nm}$). Four antibiotics discs were placed on these plates at uniform distance from each other, and the plates were incubated at RT for 24h. The observations were interpreted based on a standard NCCLS chart and the test isolate was classified as antibiotic resistant, intermediate or sensitive [18]. The antibiotics used in our study are indicated in table 1.

2.4. Qualitative determination of antibacterial and antifungal activity of prodigiosin

The antibacterial and antifungal activity of prodigiosin was evaluated qualitatively using a ditch plate technique. To carry out this technique, sterile Nutrient Agar (NA) and Sabourauds Agar (SA) plates, for bacterial and fungal cultures respectively, were prepared and a ditch (1.5 x 7cm) was made in the center of the plate. The extracted pigment was diluted (approximately 100mg/mL) with methanol and added to the ditch in NA plate. The antibacterial activity of prodigiosin was determined against 7 laboratory cultures including *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus aureus* 6538p, *Bacillus subtilis*, *Proteus vulgaris* and *Proteus mirabilis*. The cultures were streaked perpendicular to the ditch and the NA plates were incubated at 37°C for 24h. The fungal cultures used in our study were *Aspergillus niger, Rhizopus,*

Penicillium citrinum and *Candida albicans*. The fungal isolates were streaked similarly on SA plates and incubated at RT for 24h.

2.5. Evaluation of antimicrobial activity of prodigiosin by well diffusion method

The antibacterial and antifungal activity of prodigiosin was evaluated using the agar well diffusion method. To carry out this method, the NA and SA plates, pre-seeded with bacterial and fungal cultures respectively, were prepared. Two wells of approximate 6-8mm were punched in these plates using a sterile cork borer. The pigment sample dissolved in DMSO was added to one well and DMSO was added to the other well that served as control. The pigment was allowed to pre-diffuse for 20min on NA and SA plates and then incubated at 37°C and RT, respectively, for 24h to determine the antimicrobial activity of prodigiosin.

2.6. Cytotoxicity assessment of prodigiosin by MTT assay

The cytotoxicity assessment of prodigiosin was carried out on *Rattusnorvegicus* Kidney epithelial cell line- NRK-52E. The (3-(4, 5-Dimethylthiazol-2-Yl)-2, 5-Diphenyltetrazolium Bromide (MTT) assay was carried out at Animal Biotechnology and Biochemistry Division, KET's Scientific Research Centre, Mulund (E), Mumbai-400081, India. A 96 well plate was seeded with NRK-52E cell line and used for carrying out MTT assay. The cells were maintained in Dulbecco`s Modified Eagle Media (DMEM). After incubation, the cells were observed for monolayer under the microscope. Suitable dilutions (prepared in DMSO) of the pigment sample were prepared using a stock solution of 1mg/mL and added to the above wells. Wells with negative control (containing DMEM), positive control (containing DMSO) and a vehicle control, with the final volume of 100 µL per well, were also set up. The plate was incubated in a 5% humidified CO_2 incubator at 37°C for 48h. After incubation, the DMEM media was discarded and 100µL of MTT reagent (5 mg/mL, prepared in DMEM medium) was added to each well including the controls. The plates were further incubated at 37°C for 3h in dark. Later, 100µL of a stop mix solution (DMSO) was added in each well to dissolve the formazan precipitate formed during incubation. The plates were read at 550nm using microplate reader. The results were reported as % viability which was observed from the dose response curve using the MTT Assay.

2.7. Evaluation of dyeing potential of prodigiosin

In order to check whether the pigment extracted can be used as a dye, different pieces of cloth material (cotton, mixed cotton and muslin cloth) were stained with prodigiosin. For this purpose, the pigment extracted in solvent phase was kept in contact with the cloth until the solvent was completely evaporated in dark. The dyed cloth was then dried, washed with 1% detergent (Surf powder) and checked for color stability.

2.8. Evaluation of antibacterial activity of cloth sample after dyeing with prodigiosin

A cotton cloth sample was cut in 3 pieces of size 0.5x8cm, dipped in undiluted solutions of prodigiosin, heat treated prodigiosin, and control (DMSO) respectively. To investigate whether the cloth retains the antibacterial activity after dyeing, it was placed on NA plates pre-streaked with 2 gram positive and 2 gram negative cultures parallel to each other with a distance of 1.5cm on each plate. The plates were incubated for 24h at 37°C and the results were noted. The cloth samples were further washed with detergent and rinsed in warm water (60°C for 5min). The above experiment was repeated with washed strips thrice, and the change in activity of prodigiosin was recorded. A clear zone of inhibition, around and underneath the cloth after incubation, indicated antibacterial activity. In order to further confirm the above activity, the prodigiosin sample was provided to test lab of SASMIRA- 'The synthetic & art silk mills research association' to carry out standard industrial technique AATCC 100 (Antibacterial Activity Assessment of Textile Materials: Parallel Streak Method). This technique uses the same protocol mentioned above, except the use of standard cultures *i.e., S.aureus* ATCC 6538 and *K. pneumoniae* ATCC 4352.

3. RESULTS AND DISCUSSION

The pigment produced by *S. nematodiphila*, was extracted using methanol solvent and further purified by separating funnel extraction method. Various applications of the extracted pigments are reported in this section.

3.1. Evaluation of antibiotic sensitivity profile of *S. nematodiphila*

Table 1 represents the antibiotic sensitivity profile of prodigiosin producer. This study was carried out based on the assumption that any antibiotic producing

microorganism, also possesses mechanisms to overcome the detrimental effect of the same. Since prodigiosin, itself is reported to have antimicrobial properties, the curiosity to determine the inherent resistance of *S. nematodiphila*, if any, towards the commonly used antibiotics led to the evaluation of AST profile of the prodigiosin producer. As observed from the table 1, *S. nematodiphila* was found to be inherently resistant to eleven out of the 22 antibiotics tested in our study. It also showed intermediate resistance to kanamycin. As expected, it showed resistance to antibiotics including vancomycin, methicillin, penicillin, bacitracin and clindamycin that are particularly active against gram positive organisms. However, interestingly, it also showed resistance to broad spectrum antibiotics like doxycycline and ampicillin, which is exclusively used for treatment of infections caused by gram negative bacteria. In addition, *S. nematodiphila* was also found to be resistant to novobiocin, neomycin and tobramycin that are active against gram positive as well as gram negative bacteria.

Table 1: Antibiotic resistance profile of *S. nematodiphila*

Sr. No.	Antibiotic	Zone of inhibition (mm)	Susceptibility based on NCCLS chart
1	Kanamycin (30µg)	15	Intermediate
2	Nitrofurantoin (300µg)	23.5	Susceptible
3	Tetracycline (30µg)	22	Susceptible
4	Linezolid (30µg)	23	Susceptible
5	Chloramphenicol (30µg)	32	Susceptible
6	Polymixin-B (30µg)	No zone	Resistant
7	Vancomycin (30µg)	No zone	Resistant
8	Methicillin (30µg)	No zone	Resistant
9	Doxycycline hydrochloride (30µg)	10	Resistant
10	Penicillin-G (10U)	No zone	Resistant
11	Sulphamethoxazole(75µg)	44	Susceptible
12	Clindamycin (30µg)	No zone	Resistant
13	Sulfisoxazole (0.25mg)	30	Susceptible
14	Ciprofloxacin (5µg)	25	Susceptible
15	Bacitracin (10U)	No zone	Resistant
16	Piperacillin (100µg)	30	Susceptible
17	Ampicillin (10µg)	10	Resistant
18	Streptomycin (10µg)	15	Susceptible
19	Neomycin (10µg)	11	Resistant
20	Novobiocin (30µg)	No zone	Resistant
21	Tobramycin (10µg)	12	Resistant
22	Gentamicin (10µg)	15	Susceptible

With the increased spread of antibiotic resistance in nature, it is imperative that we study the antibiotic resistance profile of environmental isolates. This is necessary, especially for isolates intended to be used for industrial applications. Once the characteristics of these bacteria are studied, proper measures can be followed for its disposal after recovery of the product of interest. It is also important to note here, that with the extreme widespread antibiotic resistance, even in non-clinical isolates, rejecting the industrial application of a microbial product may not be a feasible choice. To the best of our knowledge, none of the published studies have verified the role of pigment production in acquiring drug resistance in Serratia species (except that virulent strains are generally non-pigmented). However, a study reported significant relation between pigment production and multi-drug resistance in *Pseudomonas aeruginosa*. They also indicated a possible relation between pigment production and presence and/or expression of virulence factors in *P. aeruginosa* [19].

3.2. Evaluation of antibacterial and antifungal activity of prodigiosin

The ditch plate technique was used to determine the antibacterial activity of prodigiosin against laboratory

isolates. The pigment showed antibacterial activity against all gram positive isolates *i.e., S. aureus*, *S. aureus* 6538p and *B. subtilis* as well as gram negative isolates *i.e., E. coli*, *K. pneumoniae*,*P. vulgaris*, *P. mirabilis*. However, it showed antifungal activity only against one of the three fungal isolates (i.e., *Penicillium citrinum*) used in our study. Hence, prodigiosin isolated from *S. nematodiphila* may be considered as a broad spectrum antibacterial agent. The results of ditch plate technique were further confirmed by the observed zones of inhibition against test isolates by well diffusion assay (Table 2).

Table 2: Zones of inhibition of test isolates against prodigiosin

Sr. No.	Test isolates	Zone of inhibition mm)
1	*Escherichia coli*	14
2	*Klebsiella pneumoniae*	17
3	*Staphylococcus aureus*	15
4	*Staphylococcus aureus 6538p*	19
5	*Bacillus subtilis*	22
6	*Proteus vulgaris*	15
7	*Proteus mirabilis*	12
8	*Aspergillusniger*	No zone
9	*Rhizopus*	No zone
10	*Penicilliumcitrinum*	11
11	*Candida albicans*	No zone

Our observations are in concordance with a previous study by Darshan and Manonmani [20], that reported broad spectrum antibacterial activity of prodigiosin, isolated from *S. nematodiphila*, against *Bacillus cereus*, *S. aureus*, *P. aeruginosa* and *E. coli*. They further reported that prodigiosin inhibited the motility of test isolates at sub-lethal concentrations, and induced programmed cell death when used at optimum concentrations. Contrary to our findings, Lapenda *et al*. [21] reported the inhibitory activity of prodigiosin isolated from *Serratia marcescens* UFPEDA 398 against gram positive bacteria *i.e. S. aureus*, *Enterococcus faecalis* and *Streptococcus pyogenes* with significant zones of inhibition in the range of 14-35mm. Another study indicated the activity of prodigiosin, extracted from *Vibrio ruber* DSM 14379, against *B.subtilis*. They reported that the prodigiosin induced autolysins in actively growing *B. subtilis* cells causing cell death whereas exhibited bacteriostatic activity during the stationary phase [22].

3.3. Evaluation of dyeing potential of prodigiosin

The prodigiosin extracted in our study successfully dyed the cloth pieces. Fig. 1 represents a sample piece of cotton cloth dyed with prodigiosin. The pigment was retained well by the cotton cloth material followed by mixed cotton and muslin cloth. Generally the microbial pigments are mixed with a mordant to retain the color after dyeing. However, as observed in Fig. 1, the cotton cloth was stained considerably after a single processing. Moreover, it retained the color after washing it with a mild detergent. Hence a mordant was not used in our study.

Fig. 1: A sample of cotton cloth dyed with prodigiosin

Similar to our observations, Gulani *et al*. [23] also reported considerable dyeing potential of prodigiosin extracted from *S. marcescens*, without using a mordant. Another study, on optimization of prodigiosin production and its application, reported the suitability of prodigiosin- type biochrome, extracted from *Serratia* sp. KH-1, for the purpose of textile dyeing. The maximum uptake of dye was observed at 50°C when dyed for 50min duration using 4.3g/L dye concentration. The dyeing intensity was further improved on using sodium chloride as a mordant [24]. A recent study reported the suitability of natural pigments obtained from marine bacteria to be used as food colorant in jellies [25].

3.4. Cytotoxicity assessment of prodigiosin by MTT assay

Fig. 3 represents the growth response curve of NRK-52E cell line to increasing concentration of prodigiosin. It clearly indicates a considerable increase in toxicity of

the pigment above LC_{50} value (*i.e.*, ~ 0.5mg/mL). Thus it can be stated that the pigment extracted from *S. nematodiphila* can be used for suitable biomedical applications below the concentration of 0.5mg/mL. Table 3 represents the percent viability of NRK-52E cells observed at different concentrations of prodigiosin. The MTT assay is a simple colorimetric test for determination of cell proliferation and survival which was developed by Mosmann [26] and later adapted by Cole [27].

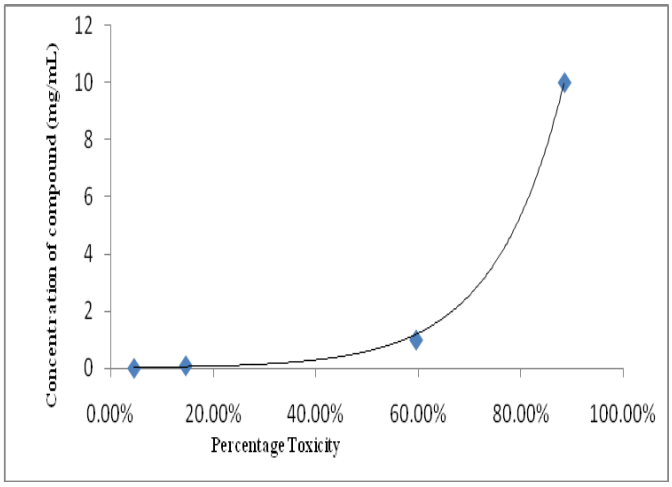

Fig. 2: The growth response curve of NRK-52E cell line to increasing concentration of prodigiosin

Table 3: Percent viability of NRK-52E cells at different concentrations of prodigiosin

Concentration of prodigiosin (mg/mL)	Percent viability (%)
10	21.49
1	40.43
~ 0.5	50
0.1	85.35
0.01	95.43

Initially, it was used to measure the chemosensitivity of human lung cancer cell lines. However, due to its simplicity, the technique was further modified to study the cytotoxicity of various bioactive compounds and toxic materials.A recent study reported the selective cytotoxic and genotoxic activity of prodigiosin loaded halloysite nanotubes on human epithelial colorectal adenocarcinoma and human colon carcinoma [28]. The selective cytotoxicity of prodigiosin has also been reported in human neuroblastoma cell lines [29]. Another study reported the production of a novel prodigiosin called MAMPDM (2, 2' - [3-methoxy-1'amyl -5' -methyl -4 - (1"-pyrryl)]dipyrrylmethene)], which showed selective cytotoxicity on cancer cell lines and reduced toxicity to non -malignant cells. The LC_{50} value of MAMPDM was reported to be 1.59 µM and 0.176 µM for U937 and LS -A cells, respectively [30].

3.5. Evaluation of antibacterial activity of cloth sample after dyeing with prodigiosin

Table 4 represents the antibacterial activity of cotton cloth after dyeing with undiluted solutions of prodigiosin, heat treated prodigiosin, and control (DMSO). As observed, prodigiosin dyed cloth sample showed considerable antibacterial activity against both gram positive as well as gram negative isolates. However, this activity was lost after heat treatment of prodigiosin or washing of the cloth sample with a mild detergent. The investigation carried out in our laboratory, however, confirmed the antibacterial coating effect of prodigiosin on cotton cloth. This can improve the keeping quality of the dyed product, which may become prone to certain infectious agents during storage and handling. Moreover, it may also be useful as coatings for wound dressing. The test report of SASMIRA further confirmed the above results and indicated broad spectrum antibacterial activity of prodigiosin that was retained after dyeing.

Table 4: Inhibition observed along the length of streak of test isolates against prodigiosin

Sr. No.	Test isolates	Length of inhibition (mm)				
		Undiluted prodigiosin	Heat treated prodigiosin	Washed cloth sample	Control (DMSO)	SASMIRA report
1	*Escherichia coli*	10	No zone	No zone	No zone	-
2	*Klebsiella pneumoniae*	8	No zone	No zone	No zone	-
3	*Bacillus subtilis*	9	No zone	No zone	No zone	-
4	*Staphylococcus aureus 6538p*	8	No zone	No zone	No zone	10
5	*Klebsiella pneumoniae* ATCC 4352	-	-	-	-	13

Another study reported the bactericidal activity of a red pigment isolated from marine bacteria i.e. *Vibrio* sp. SKMASRSP9. It had the ability to kill around 50% cells of *S. aureus* and *E. coli* within 16h of contact on silk cloth. The dyed cloth sample of other fabric material like polyester, cotton and satin also showed suitable antibacterial activity [31]. Prodigiosin obtained in another study showed pH sensitive solubility in water that was used to dye cellulose fabric. The dyed fabric also showed antibacterial activity [32].

4. CONCLUSION

The potential of prodigiosin has been recognized in textile industries as well as in biomedical fields. The current study reported the broad spectrum antibacterial, and antifungal activity of prodigiosin obtained from *S. nematodiphila*. Together with high productivity under optimized conditions (reported in earlier study), considerably low cytotoxicity and antimicrobial activity, the prodigiosin pigment appears to be a suitable candidate for commercial applications. The antibacterial activity that is retained after dyeing of fabric can be exploited through bandages and wound dressings. In addition, the current study also proposes a thoughtful insight to the relation between the antibiotic resistance observed between the prodigiosin producer, and the antimicrobial activity of the pigment. To the best of our knowledge, such relation has not been reported in literature. Hence further studies are required to study the inherent or acquired nature of antibiotic resistance observed in *S. nematodiphila*, and whether the antibiotic resistance is affected by loss of pigmentation.

Conflict of interest

Authors declare no conflict of interest

5. REFERENCE

1. Parekh S, Vinci VA, Strobel RJ. *Appl. Microbiol. Biotechnol.*, 2000; **54**:287-301.
2. Dufosse L. Encyclopedia of Microbiology, 3rd Edition. Pigments, Microbial. Elsevier/Academic Press, New-York 2009; p. 457-471.
3. Khanafari A, Assadi MM, Fakhr FA. *Online J. Biol. Sci.*, 2006; **6(1)**:1-13.
4. Harris AKP, Williamson NR, Slater H, Cox A, Abbasi S, Foulds I, et al. *Microbiol.* 2004; **150**:3547-3560.
5. Maheswarappa G, Kavitha D, Vijayarani K, Kumanan K.*Indian J. BasicApplied Med. Res.*, 2013; **3(1)**:257-266.
6. Darshan N, Manonmani HK.*J. Food Sci. Technol.*, 2015; **52(9)**:5393-5407.
7. Prabhu VV, Hong B, Allen JE, Zhang S, Lulla AR, Dicker DT, et al.*Cancer Res.*, 2016; **76(7)**:1989-1999.
8. Castro AJ. *Nature*, 1967; **213(5079)**:903-904.
9. Kumar TS, Aparna H. *Int. J.Curr. Microbiol. Appl. Sci.*, 2014; **3(5)**:712-725.
10. Liu GY, Nizet V.*Trends Microbiol.*, 2009; **17(9)**:406-413.
11. Boric M, Danevcic T, Stopar D.*Microbial Ecol.*, 2011; **62(3)**:528-536.
12. Joshi VK, Attri D, Baja A, Bhushan S. *Indian J.Biotechnol.*, 2003; **2**:362-369.
13. Duran N, Teixeira MFS, Conti R, Esposito E.*Critical Rev. Food Sci. Nutr.*, 2002;**42(1)**:53-66.
14. Alihosseini F, Ju KS, Lango J, Hammock BD, SunG.*Biotechnol. Progress* 2008; **24(3)**:742-747.
15. Ghosh C, Patel S.*Environ. Exp. Biol*. 2020; **18**:153-160.
16. Li D, Liu J, Wang X, Kong D, Du W, Li H, et al.*Int. J. Mol. Sci.*, 2018; **19(11)**:3465.
17. Guryanov ID, Karamova NS, Yusupova DV, Gnezdilov OI, Koshkarova LA. *Russian J.Bioorg. Chem.*, 2013; **39**:106-111.
18. CLSI. Performance standards for antimicrobial discsusceptibility tests; approved standard-11th ed.,4 document M02-A112012.
19. Finlayson EA, Brown PD.*West IndianMedical J.*, 2011; **60(1)**:24-32.
20. Darshan N, Manonmani HK. *AMB Express*, 2016; **6**: Article no. 50.
21. Lapenda JC, Silva PA, Vicalvi MC, Sena KXFR, Nascimento SC.*World J.Microbiol.Biotechnol.*, 2015; **31(2)**:399-406.
22. Tjasa D, Maja BV, Maja T, Masa Z, David S. *Front.Microbiol.*, 2016; **7**.
23. Gulani C, Bhattacharya S, Das A. *Malaysian J.Microbiol.*, 2012; **8(2)**:116-122.
24. Chauhan K, Dalsaniya P, Pathak H. *Fibers Polymers*, 2015; **16**:802-808.
25. Ramesh C, Vinithkumar NV, Kirubagaran R, Venil CK, Dufossé L. *Microorganisms*, 2020; **8(4)**:556.
26. Mossman T. *J. Immunol. Methods*, 1983; **65(1-2)**:55.
27. Cole SP. *Cancer Chemother. Pharmacol.*, 1986; **17(3)**:259-263.

28. Guryanov I, Naumenko E, Akhatova F, Lazzara G, Cavallaro G, Nigamatzyanova L, et al. *Front.Bioeng. Biotechnol.*, 2020; **8**:424.
29. Francisco R, Perez Tomas R, GimenezBonafe P, Soto Cerrato V, Gimenez Xavier P, Ambrosio S. *European J. Pharmacol.*, 2007;**572**: 111-119.
30. Deorukhkar AA, Chander R, Ghosh SB, Sainis KB. *Res. Microbiol.*, 2007; **158(5)**:399-404.
31. Pabba SK, Krishna G, Prakasham RS, Singara Charya MA. *J. MarineBiosci.*, 2015; **1(1)**:1-11.
32. Gong J, Ren Y, Fu R, Li Z, Zhang J.*Polymers*, 2017; **9(10)**:468.