Antioxidant and antimicrobial activity of bioactive prodigiosin produces from *Serratia marcescens* using agricultural waste as a substrate

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Revised: 17 April 2018 / Accepted: 23 April 2018 / Published online: 7 June 2018
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Abstract The objective of this investigation was to explore the antioxidant and antimicrobial property of bioactive prodigiosin produced from *Serratia marcescens* using rice bran. The antioxidant potential of prodigiosin was examined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid (ABTS) radical scavenging method via UV-visible, electron spin resonance spectrum (ESR), cyclic voltammetry and excitation emission spectrum. The antimicrobial activity of prodigiosin was examined against foodborne pathogens. The shelf life extending capacity of prodigiosin was evaluated with meat extract powder (MEP) as a model food material. The DPPH and ABTS radicals were completely scavenged by prodigiosin at the concentration of 10 mg/L. The food spoilage was inhibited by the addition of prodigiosin with MEP and it was compared with conventional preservative. The prodigiosin has prohibited the growth of foodborne pathogens effectively and the shelf life of the food was also extended significantly. The antimicrobial edible preservative developed in this study

inhibited the growth of the microbial populations that produced through storage of the MEP and free radical scavenging activity. The results reveal that the bioactive prodigiosin effectively scavenged the free radical and inhibited the bacterial growth in food stuff.

Keywords Prodigiosin · Antimicrobial · Antioxidant · Food preservative · Foodborne pathogens

Introduction

The prodigiosin is a red coloured secondary metabolite, produced by many bacteria. The prodigiosin has many pharmaceutical values such as antimicrobial, algicidal, anti-inflammatory, anticancer, antimalarial, anti-diabetic and immunomodulatory effects (Arivizhivendhan et al. 2016; Bennett and Bentley 1999; Kim et al. 2008; Nikodinovic-Runic et al. 2014; Ryazantseva and Andreyeva 2014). Many kinds of artificial additives such as preservative and colorants are being used in food processing industries for aesthetic appearance and preservation. There are wide spread doubt regarding the hazardous nature of these additives are free from hazardous chemicals or not. There are many reports on health impairment due to consumption of artificial food additives in modern days (Brannen et al. 2001). One of the main consequence of these additives is cell damage by oxidation which leads to the suppression of immunity in a human being. The free radicals, reactive oxygen species and reactive nitrogen in synthetic additives cause inflammation, metabolic disorders, irregular cell aging, reperfusion damage, atherosclerosis and carcinogenesis (Aruoma 2003; Lee et al. 2001; Lobo et al. 2010; Ramesh et al. 2012; Venturini et al. 2015; Young and Woodside 2001). The intake of natural

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additives through dietary foods may help to maintain an adequate disease defense mechanism by balancing oxidants and antioxidants level in the human body (Khor et al. 2014; Sahiner and Cansin Sackesen 2012; Thangthaeng et al. 2015). Thus, the natural preservative is always found to be advantages for not only aesthetic appearance but also for health benefits of the food products. Thus, there has been a constant research for the development of eco-friendly/natural food additives. Therefore, natural preservatives are gaining significant attention as a food additive and there is much demand for natural additives as a supplementary in food processing industries. Generally, the foodborne pathogens reduce the shelf life of the food stuff. So the antimicrobials potential preservative is required to prevent natural spoilage and to avoid contamination by microorganisms (Liang et al. 2013; Tajkarimi et al. 2010). The addition of natural bioactive compounds can be considered as an alternate additives for the safe functioning of human organs, because they have excellent functions on food than synthetic chemical additives (Joana Gil-Chávez et al. 2013). The bioactivity of prodigiosin was evaluated in the present investigation by free radical scavenging activity and bactericidal activity on foodborne pathogens. The antioxidant potential of prodigiosin was analyzed by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and 2, 2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid (ABTS) scavenging methods. The meat extract powder was selected as a model food material for the determination of food preservative potential of prodigiosin.

Materials and methods

Chemicals

The reagents such as Erythrosine, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2'-azino-bis 3-ethylbenzthiazoline-6-sulfonic acid (ABTS) were purchased from Merck chemicals, India. Meat extract powder (MP) and other microbiological ingredients were procured from HiMedia, India.

Biosynthesis of prodigiosin

Prodigiosin producing bacteria was isolated from animal fleshing and it was characterized by 16S RNA sequencing. Wheat bran and animal protein were used as a substrate for the biosynthesis of prodigiosin. The isolation, production and extraction of prodigiosin were carried out as per the procedure described in our previous study by Arivizhivendhan et al. (2015a, b). In detail, the isolated strain was inoculated in nutrient broth and incubated at 30 °C for 24 h. The fermentation medium was prepared by mixing of

tannery fleshing and wheat bran (ratio of 70:30). 30 g in 15 mL of mineral solution ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 33.90 g/L; KH_2PO_4 , 15 g/L; NaCl , 2.5 g/L; NH_4Cl , 5 g/L) and they were transferred into 250 mL capacity of Erlenmeyer conical flask. The flask was cotton plugged and sterilized at temperature, 121 °C; pressure 15 lbs/inch² for 20 min in an autoclave. The flask with fermentation medium was allowed to cool to reach room temperature and then the medium was inoculated with 5 mL of inoculum from a mother culture. The flasks were incubated for 96 h at 30 °C. Prodigiosin production was optimized with different environmental factors such as time, temperature pH and concentration of substrate which was quantified by UV visible method via prodigiosin standard slope. Acidified methanol was employed for the extraction of prodigiosin from the fermented medium. The extracted prodigiosin was purified by thin layer chromatography (TLC) using methanol: acetonitrile at a mixing ratio of 3:1 and separated by column chromatography. The purified prodigiosin again evaluated through TLC. Hence, the TLC shown one peak correspondence to prodigiosin and no impurity peaks was observed. Then, the solvent was evaporated using rotary vacuum evaporator to get the prodigiosin. The recovered prodigiosin was stored and used for the further experimental studies. The prodigiosin (50 mg) dissolved in deuterated acetonitrile was taken for the characterization of Nuclear Magnetic Resonance (NMR) spectrum. ^1H and ^{13}C NMR experiments were performed at a frequency of 500 MHz on a JEOL ECA 500 spectrometer, at 28 °C.

Antioxidant potential of prodigiosin

Determination of free radical scavenging potential of prodigiosin by UV-visible spectroscopy

DPPH free radical scavenging assay

The free radical scavenging activity of prodigiosin was evaluated using DPPH radicals scavenging assay. The purified prodigiosin was dissolved in 1% (v/v) of ethanol and used for the evaluation of antioxidant activity. DPPH solution of volume 1 mL (10 mg/L of distilled water) was introduced into a glass test tube containing prodigiosin (5 or 10 mg/L) in distilled water and the same volume of methanol alone was considered as control. The solution was vortex mixed at room temperature for 30 min and then the reacted solution was recorded for its absorbance value at λ 515 nm using the UV-Visible spectrophotometer (Nanjo et al. 1996).

ABTS radical scavenging assay

ABTS scavenging activity of prodigiosin was performed as per the methodology given in Miliauskas et al. (2004). The required ABTS stock solution was prepared by dissolving 10 mg of ABTS and 1 mg potassium persulfate in 10 mL of distilled water. The solution was allowed to stand for 12 h in the absence of light. 1 mL of ABTS stock solution was mixed with 9 mL of distilled water and named as working solution. 10 mL of working solution was introduced into a clean glass test tube containing 1 mL of prodigiosin (5 or 10 µg) solution. Then the solution was thoroughly mixed and allowed to react for 30 min. The reacted solution was recorded for its absorbance at 734 nm in the UV–visible spectrophotometer (Koleva et al. 2001). The scavenging efficiency of prodigiosin on free radicals (DPPH and ABTS) was calculated according to the following equation:

ABTS and DPPH radical scavenging percentage

$$= \frac{A_c - A_s}{A_c} \times 100 \quad (1)$$

where A_s absorbance of the sample after reaction time necessary to reach the plateau (30 min); A_c the absorbance of control.

Free radical scavenging activity by electron spin resonance (ESR) spectrum

Radical scavenging activity by EPR was measured as per the method presented by Mensor et al. (2001). In free radical scavenging method 100 µL of prodigiosin (5 or 10 mg) was mixed with 100 µL of DPPH and ABTS (100 µmol/L) in water. The potassium persulphate buffer solution was used only for ABTS radical scavenging method. Then, the solutions were mixed vigorously for 10 s and transferred into a 100 µL Teflon capillary tube and kept in the cavity of the EPR spectrometer. The spin adduct was measured exactly after 2 min in EPR spectrometer. The instrumental measuring conditions were set at central field, 3475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 5 mW; gain, 6.3×10^5 ; temperature, 298 K.

In-vitro antimicrobial susceptibility study of prodigiosin

The antibacterial activity of prodigiosin was studied in disc-diffusion method and compared with chemical food preservative, sodium nitrite (NaNO_2) as a positive control. Six foodborne pathogens such as *Escherichia coli* (MTCC-2939), *Bacillus cereus* (MTCC-8372) *Clostridium botulinum* (ATCC27022), *Vibrio vulnificus* (ATCC27562) and

Salmonella enterica (ATCC29630) were selected and allowed to grow overnight on Muller Hinton broth. The matured colonies after 24 h, (1×10^7 CFU/mL) were used for the analysis of antimicrobial activity by disc diffusion method. The swab technique was used to inoculate pathogens on dried surface of Muller Hinton agar plate by streaking over the agar surface for four times, the plates were rotated to ensure an even distribution of inoculum. Then the medium was allowed to dry for 3 min before the preparation of wells. The prodigiosin solution was prepared by dissolving 5 mg of prodigiosin in 10 mL of dimethoxy sulfonate (1%). Prodigiosin solution (0.3 mL) was impregnated into the disc and allowed to dry at 50 °C for 2 h, DMSO alone referred as control and NaNO_2 (5 mg in 10 mL of DMSO) solution served as positive control. Then, the disc was placed onto the plates and it was incubated at 37 °C for 24 h and the zone of clearance was calculated for the validation of antimicrobial activity.

Food preservative efficiency on introduction of prodigiosin

The increment of food preservative efficiency on introduction of prodigiosin was evaluated on meat extract powder (MEP) as model food material. The model food material was prepared by dissolving commercially available MEP in sterilized water at 1:1 w/v ratio. A known weight of prodigiosin and NaNO_2 as a positive control (0.1% w/w), were added into MEP and sterilized at temperature, 121 °C and pressure, 15 lbs/inch² for 20 min in an autoclave. The MEP alone was served as control. The samples were incubated at 37 °C for 48 h. After the incubation period, 1 g of sample was dissolved in 9 mL of sterile distilled water. The resulting solution was placed on nutrient agar and incubated at 37 °C for 24 h. The bacterial contamination was determined for every 6 h and all the experiments were carried out in triplicate. The bacterial food spoilage was observed by Scanning electron microscopy.

Results and discussion

Optimization condition for prodigiosin production

The isolated red bacterial strain was sequenced and analyzed for 16S ribosomal RNA. The Phylogenetic tree was constructed based on Neighbor-joining method using ClustalW software and it was observed to be 99% similar to *Serratia marcescens* which revealed that the isolated red bacteria was most closely related to *S. marcescens*. The prodigiosin extracted from *S. marcescens* and characterized by nuclear magnetic resonance spectroscopy. The influence

of various process parameters such as incubation time, temperature, pH, tannery fleshing (TF) and glycerol and substrate concentration were studied for optimum production of prodigiosin. After 24 h incubation, the medium colour was changed from light yellow to red, which was indicated the biosynthesis of prodigiosin. Furthermore, the increased in incubation time up to 96 h the intensity of red colour was increased as shown in (Fig. 1a). The solution pH played a significant role on production of prodigiosin. The Fig. 1b shows that the maximum production of prodigiosin was observed at pH 7.0. The prodigiosin concentration was found to be significantly increased with increasing concentration of TF and maximum prodigiosin production observed at 30% (w/v) of TF (Fig. 1c). Similarly, Sumathi et al. (2014) also reported that the prodigiosin production increased with increasing in concentration of TF because TF provided carbon and nitrogen sources to the bacterial biosynthesis of prodigiosin. The temperature also played a major role in the prodigiosin production and the maximum yield of prodigiosin was observed at 30 °C (Fig. 1d). The prodigiosin production was not observed at temperature below 10 °C and above 40 °C. Its known that at low concentration the diffusion of substrate and activity of enzyme are very low, and at high temperature the proteinaceous substrate (TF) might undergo deactivation.

Characterization of prodigiosin by NMR

^1H and ^{13}C NMR spectroscopy

The structure of prodigiosin recovered from SSF process was elucidated through ^1H -NMR (400 MHz, DMSO- d_6) analysis. The chemical shifts observed at δppm : 0.83 (3H, terminal methyl), 0.85 (3H, terminal methyl), 0.9–2.4 (complex unresolved multiple due to number of methylenes and methines), 3.51 (3H, $-\text{OCH}_3$), 3.6–4.8 (protons attached to electronegative groups such as oxygen, nitrogen, etc. and therefore shifted downfield), 5.1 (d, 2H, olefinic protons), 5.4 (broad hump, 2H, $2\times-\text{NH}$ or $-\text{OH}$ or $1-\text{OH}^+1 > \text{NH}$), 5.792 (d, 1H, $=\text{CH}$), 8.09 (broad hump, 2H, $2\times-\text{NH}$ or $-\text{OH}$ or $1-\text{OH} + 1 > \text{NH}$) (Fig. 1e). ^{13}C NMR (100 MHz, DMSO- d_6) δppm : 15.44 (terminal methyl), 22.90–32.80 (number of methylenes and methines), 38.921 ($-\text{OCH}_3$), 40.0–71.90 (oxygen/nitrogen bonded carbons); 130.103 ($> \text{C}=\text{O}$ or $\text{ArC}-\text{O}$), 173.79 ($> \text{C}=\text{O}$ or $\text{ArC}-\text{O}$) conformed the structure of prodigiosin molecule. The ^{13}C -NMR confirmed the presence of 14 carbons in the prodigiosin structure (Fig. 1f).

Antioxidant potential of prodigiosin

The antioxidant potential of prodigiosin was studied by estimating free radicals scavenging assay. *ABTS radical*

Fig. 1 Factors affecting the biosynthesis of prodigiosin, various parameters such as **a** time, **b** pH, **c** mass of TF and **d** temperature. Characterization of prodigiosin by NMR spectroscopy **e** ^1H NMR and **f** ^{13}C NMR

scavenging assay: The measurement of total antioxidant activity of many pure substances are being tested by the generation of radical cation in ABTS scavenging assay. There was a significant decrease in ABTS at all the studied prodigiosin concentration. The scavenging activity of prodigiosin was observed to be 25, 51, 74, 92 and 99% at

the concentration 2, 4, 6, 8, and 10 $\mu\text{g/mL}$ of prodigiosin respectively (Fig. 2a). The results reveal that the ABTS^+ radical scavenging activity was directly proportional to concentration of prodigiosin. The reduction of ESR signals confirmed the radical scavenging activity of prodigiosin in ABTS^+ method by a complex hyperfine structure as shown

Fig. 2 ABTS free radical scavenging activity of prodigiosin by **a** percentage of radical scavenging activity, **c** UV-visible spectrum and **e** electron spin resonance spectrum. DPPH free radical

scavenging activity of prodigiosin, **b** percentage of radical scavenging activity, **d** UV-visible spectrum and **f** electron spin resonance spectrum

in Fig. 2e. The blank experiments confirmed a relatively small decrease in ABTS[•] concentration upon irradiation of prodigiosin free oxygen-saturated aqueous solutions (Fig. 2e). The ABTS[•] radical concentration significantly reduced with the addition of prodigiosin into the ABTS[•] solution. This confirmed that the biosynthesized prodigiosin molecule having effective free radical scavenging potential.

DPPH radical scavenging assay

Generally, DPPH is more stable form of hydroxyl and superoxide radicals, which was used for the determination of antioxidant capacity (Siriwardhana and Shahidi 2002). Figure 2d shows that DPPH is one of a stable free radical with a characteristic absorption peak observed at λ 515 nm (Matthaus 2002). At different concentrations of prodigiosin were tested for the radical scavenging effect against DPPH. The DPPH free radicals scavenging activity of prodigiosin were found to be 29, 58, 86, 99 and 99% and 65% at 2, 4, 6, 8, and 10 $\mu\text{g/mL}$ of prodigiosin respectively (Fig. 2b). The free radical scavenging activity was increased in the solution with increasing the concentration of prodigiosin. ABTS and DPPH radical scavenging methods are the common spectrophotometric procedures for the determination of antioxidant capacities of many components. The ABTS radical cation formation takes place instantaneously by the addition of potassium persulphate in ABTS solution (Miller and Rice-Evans 1997). The ABTS scavenging activity was carried out by decolourisation technique for stable radical generation prior to reaction with putative antioxidants (Gülçin 2006; Said et al. 2003). This could be explained by the fact that, the antioxidants donate protons to the free radicals to neutralize charge in the solution and thus decreases the absorbance intensity at its characteristic wavelength at 515 nm (Cheng et al. 2006). The decrease in absorbance denotes the extent of radical-scavenging activity in DPPH (Nagai et al. 2002). The hydrogen ion donation from an antioxidant involves the decoloration of DPPH radical which was observed upon the addition of higher concentration of antioxidant (Alasalvar et al. 2002; Milaeva et al. 2013).

Electron spin resonance (ESR) spectroscopic study

The DPPH radical scavenging assay of prodigiosin was recorded using ESR spectroscopy (Fig. 2e). The radical scavenging activity of prodigiosin was measured by the radical intensity from the ESR spectrum. The results revealed that, about 99% of radical signal was inhibited on addition of 10 $\mu\text{g/mL}$ of prodigiosin and 60% of radical was inhibited by the addition of prodigiosin at the concentration of 5 $\mu\text{g/mL}$ (Fig. 2f). The signal reduction in

EPR may be explained by the formation of DPPH radicals neutralized by H^+ from antioxidant. The protonated H^+ of the C-ring in prodigiosin react with $\dot{\text{N}}$ radical of the DPPH molecule and thus deviation was captured in EPR spectrum, this confirmed the radical scavenging ability of prodigiosin molecule (Kong et al. 2016). The kinetic order of radicals scavenging activity by prodigiosin molecules was best obeyed by pseudo-second order kinetic model based on the regression coefficient. This may be explained by the radical scavenging processes depend on concentration of prodigiosin and free electron present in oxidative compounds (ABTS and DPPH).

Confirmation of free radical scavenging activity through excitation emission spectrum

The free radical scavenging activity of prodigiosin was analyzed by excitation emission matrix spectrum (Fig. 3). The samples prodigiosin, DPPH and ABTS alone did not exhibited significant fluorescence peaks in the studied spectrum (Fig. 3a, b). However, the addition of prodigiosin into DPPH solution showed emission peak at λ 435 nm (Fig. 3c). The results confirmed that the radical neutralized product is fluorescence in nature. This may be explained by DPPH cleave the N–H bond from C-ring of the prodigiosin molecule. The $\dot{\text{H}}$ radical was entrapped by $\dot{\text{N}}$ of the DPPH molecules and the free radical of the DPPH molecules was neutralized. The delocalization of pi bonded electron might be shifted and the N–H bond from the B-ring was protonated. One more $\dot{\text{H}}$ radical was generated by prodigiosin molecules then it was entrapped by $\dot{\text{N}}$ of another DPPH molecules. The N^+ from the C-ring overlapping with N^- of C-ring and one more pyrrole ring was formed. Due to hyper conjugation and pyrrole ring formation enhanced the emission intensity of the prodigiosin molecule. The peak at 290 nm in UV–visible spectrum of prodigiosin molecules also confirmed the formation of pyrrole ring.

The kinetic order ABTS and DPPH scavenging reactions were assessed based on the regression coefficients and χ^2 values. The experimental data were plotted for pseudo first order and second order kinetic models using the following mathematical expression.

$$\log(q_e - q_t) = \log q_e - \frac{k_1}{2.303} t \quad (2)$$

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (3)$$

The constants k_1 and k_2 were the pseudo first and second order rate constants and q_e is the maximum radical scavenging capacity. The values of k_1 , k_2 and q_e of each process were determined from the slopes and intercepts of the plots and presented in Table 1 along with their relevant

Fig. 3 Fluorescence spectra for the free radical scavenging potential of prodigiosin **a** DPPH, **b** prodigiosin and **c** DPPH + PG

Table 1 Kinetic order analysis of ABTS and DPPH free radical scavenging capacity of prodigiosin

Dosage of prodigiosin	ABTS scavenging				DPPH scavenging			
	First order		Second order		First order		Second order	
	R ²	k ₁	R ²	k ₂	R ²	k ₁	R ²	k ₂
5 mg	0.91 ± 0.001	1.10 ± 0.016	0.5741 ± 0.003	4.81 ± 1.635	0.97 ± 0.013	0.41 ± 0.001	0.78 ± 0.056	1.9 ± 0.076
10 mg	0.99 ± 0.002	0.27 ± 0.001	0.97 ± 0.0002	0.78 ± 0.008	0.98 ± 0.001	0.23 ± 0.0073	0.94 ± 0.012	0.39 ± 0.023

regression coefficients (R^2). The validity of kinetic order for ABTS and DPPH scavenging reaction was assessed based on the regression coefficients and χ^2 values. The experimental data were plotted for first order and second order models. The best obeying order of reaction was identified by the observation of R^2 values. The results confirmed that the radicals scavenging activity of prodigiosin followed the pseudo-second order kinetic model. Thus, the radical scavenging process depends on two parameters such as concentration of prodigiosin and free electron of the oxidative compounds.

In-vitro study on antimicrobial susceptibility of prodigiosin

The antimicrobial activity of prodigiosin was assessed for the six selected foodborne pathogens. The antimicrobial activity was experimented in disc diffusion method by zone of inhibition. Figure 4a shows the antibacterial activity of prodigiosin against six selected food borne pathogenic strains of *E. coli* (0.6 cm), *B. cereus* (0.6 cm), *S. aureus* (0.6 cm), *C. botulinum* (0.7 cm), *V. vulnificus* (0.2 cm) and *S. enteritidis* (0.5 cm) experiment at 10 μ g concentration of prodigiosin. Amongst, the high zone of inhibition was

Fig. 4 **a** Antimicrobial activity of prodigiosin against foodborne pathogens such as *S. aureus*, *E. coli*, *B. cereus*, *C. botulinum*, *V. vulnificus*, *S. enteritis*. **b** Bacterial growth profile in MEP with and without addition of prodigiosin and NaNO_2 by plate count method. Microbial growth inhibition potential of prodigiosin and NaNO_2 incubation time 46 h by scanning electron microscopy, **c** MEP@PG and **d** MEP@ NaNO_2

observed for all the pathogens with prodigiosin molecule than with NaNO_2 and control.

Anti-food spoilage activity of prodigiosin

The meat extract powder (MEP) was preserved with prodigiosin and NaNO_2 and named as (MEP@PG) and (MEP@ NaNO_2) respectively. The MEP without any preservation (MEP@C) was considered as a negative control. The bacterial population was calculated using colony counting method (Fig. 4b). The MEP@C, MEP@PG and MEP@ NaNO_2 were exposed to ambient condition for 48 h. The MEP@C showed 20×10^8 CFU/g (Log 8) of bacterial population contamination and MEP@ NaNO_2 showed 32×10^6 CFU/g (Log 6) of bacterial contamination. The MEP@PG food completely inhibited the bacterial growth and it was about 6×10^1 CFU/g (Log 1). Further, the bacterial contamination was analyzed by scanning electron microscopy (SEM) as shown in Fig. 4c–e. The SEM images of MEP@ NaNO_2 food showed bacterial attachment due to the contamination of foodborne pathogens (Fig. 4e). Similarly, the MEP@C showed the bacterial growth in SEM image exposition time at room temperature (Fig. 4e). There was no bacterial growth observed in MEP which was exposed to PG, which confirmed the bacterial inhibition capacity of prodigiosin molecule (Fig. 4d). The natural bioactive prodigiosin is a potential molecule for the disinfection of pathogens in food stuff. Bacterial growth inhibition capacity of prodigiosin molecules was much effective than chemical preservative of NaNO_2 (Fernández-Sánchez et al. 2002). The effective antimicrobial property of the prodigiosin molecule may be

due to the protonated methoxy group present in the prodigiosin molecule (Arivizhivendhan et al. 2015a, b). Once prodigiosin impregnated on the disc, the leaching ability prodigiosin from disc was observed to be less. This property may suppress the complete bioactivity of prodigiosin however, prodigiosin mixed with MEP showed good effective antimicrobial activity than the commercially available chemical preservative. This observation suggests that the prodigiosin could be considered as an alternative food additive for food processing industries. The shelf life of the food stuff can be extended by preserving with prodigiosin up to twofold than chemical preservative. Hence, prodigiosin can be used as a preservative for its effective antioxidant properties and antimicrobial activity as well as it adds aesthetic colour to the food stuff than chemical/synthetic preservatives. This is the first report on the application of prodigiosin for the preservation of food stuff.

Conclusion

The prodigiosin was successfully produced and applied for the evaluation of food preserving and antioxidant properties. The addition of prodigiosin onto food material not only enhanced the antioxidant properties, food preservation and also gives food coloration for pleasant appearance. The free radical scavenging capacity of prodigiosin molecules elucidated its rancidity and microbial inhibition potential, thereby extending shelf life of food material. Hence, prodigiosin can be considered as alternative food

preservation in food processing industries for its effective antioxidant and bactericidal properties.

Acknowledgements K. V. Arivizhivendhan thanks to Science and Engineering Research Board (SERB), DST, India for the National Postdoctoral Fellow Award (PDF/2016/003726).

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