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Research article

Antimicrobial assessment of polyphenolic extracts from onion (*Allium cepa* L.) skin of fifteen cultivars by sonication-assisted extraction method

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ABSTRACT

Onion skin possesses various health benefits due to its phenolic and antimicrobial components. In this study, sonication-assisted extracts of onion skin of differentially coloured cultivars (dark-red, red, pink and white) were investigated for their antimicrobial activity against six pathogenic bacteria. Antimicrobial efficacy of fifteen different coloured extracts was analysed by agar well-diffusion assay with principal component analysis (PCA) for comprehensive investigation. Result showed skin extracts of pink cultivars (cv.) significantly ($P \leq 0.05$) effective against pathogenic bacteria followed by red and dark red skin. White skin showed least effect on the growth of bacteria. Skin of cv. 'Phursungi Local' (pink) and cv. 'Hissar-3' (pink) showed best range of inhibition against *Staphylococcus aureus, Klebsiella pnuemoniae, Bacillus cereus* and *Solmonella typhimurium* compared to other. Only white skin extracts of cv. 'Bhima Shubhra' and 'Udaipur Local' inhibited the growth of *Pseudomonas aeruginosa* up to 4.0 ± 0.0 mm. Minimum inhibitory concentration (MIC) of the effective extracts was also elucidated in the range between 0.09 – 9.0 mg/mL. Skin extracts of cv. 'Hissar-2' (red) and 'Bhima Shubhra' (white) showed better inhibition at the concentration of 0.45 and 0.72 mg/mL against *Streptococcus agalactiae* and *Pseudomonas aeruginosa*, respectively. As per correlation analysis, positive correlation was obtained between total flavonoids and inhibition rate of all the bacteria while a weak correlation ($R^2 = 0.3967$) was observed against *Pseudomonas aeruginosa*. The waste skin of the analysed cultivars can be utilised in food and health sector as natural preservative and antimicrobial agent.

1. Introduction

Onion (*Allium cepa* L.) is one of the oldest and important crops from ancient time. It possesses many health benefits such as anti-inflammatory, anti-carcinogenic, cardio-protective and anti-oxidative (Santas et al., 2010; Sagar et al., 2018). Onion is a rich source of flavonoids like quercetin and kaempferol than any other crop (Hollman and Arts, 2000; Mojzer et al., 2016). Moreover, onion skin contains higher amount of flavonoids than the edible part of the onion (Skerget et al., 2009) because quercetin flavonol is oxidized into 3,4-dihydroxybenzoic acid and 2,4,6- trihydroxyphenylglycosilic acid and concentrated in dry onion skin to protect bulb from soil microbes (Takahama and Hirota, 2000). Several studies confirmed that the onion skin contained higher amount of quercetin, kaempferol, luteolin, and other quercetin derivatives which have been examined as antifungal and antibacterial agents (Lachman et al., 2003; Wiczkowski et al., 2008; Benítez et al., 2011; Duan et al., 2015). Hence, these natural compounds have attracted food industry as antimicrobial agents for improving food stability (Sofia et al., 2007). Traditionally, food products have been protected from microbial spoilage using chemical compounds, however, in recent years, consumers showed interest towards chemical free food products (Viuda-Martos et al., 2008). The resistant nature of pathogens against traditional preservatives and antibiotics has also been attracted scientists for alternative sources of antimicrobial agents (Xu and Lee, 2001). Onion skin can be used as a natural preservative to replace synthetic components in food products.

Enteric bacteria such as *Salmonella* sp., *Pseudomonas* sp., *Klebsiella* sp. and *Staphylococcus aureus* are the main micro-organisms causing sporadic diarrhoea in children and adults (Induja and Geetha, 2018). Previous investigations revealed that onion bulb extract and onion oil extract had significant effect on various microbes (Yang et al., 2004; Cushnie and Lamb, 2005). A few studies are available on onion skin extract as an antimicrobial agent. Skerget et al. (2009) investigated antibacterial potential of red onion skin extract and reported good inhibition against

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Escherichia coli, Bacillus cereus and *Pseudomonas fluorescens* than onion bulb. Likewise, skin extract of red onion was found effective for the inhibition of two pathogenic bacteria such as *Staphylococcus aureus* and *E. coli* up to 12 mm (Masfria et al., 2019). Quercetin flavonol has been extensively studied as a key flavonoid for the inhibition of bacteria (Wu et al., 2008) and certainly, the flavonoids content may be correlated with the antimicrobial activity of the onion. Besides this, extraction is an important step to obtain bioactive compound from the plant materials. It is proven that sonication-assisted method is significantly better and efficient than the conventional methods such as Soxhlet extraction and maceration for better recovery of the flavonoids from onion (Ren et al., 2020).

Therefore, the aims of the present study were to analyse: 1) the antimicrobial potential of skin extracts of different coloured onions followed by principal component analysis (PCA) for a comprehensive view of inhibition, 2) the minimum inhibitory concentration (MIC) of the extracts, and 3) to find out the correlation between the total flavonoids of the extracts and the inhibition rates of the bacteria.

2. Materials and methods

2.1. Plant material

Fifteen onion cultivars were procured from Indian Agricultural Research Institute (IARI), New Delhi, India. According to the skin colour, these cultivars were grouped in to four categories: dark red ('Agri Found Dark Red', 'NHRDF Red'), red ('Arka Kirtiman', 'Hissar-2', 'Pusa Red', 'Pusa Riddhi', 'Sukh Sagar'), pink ('Agri Found Light Red', 'Bhima Kiran', 'Bhima Shakti', 'Hissar-3', 'Phursangi Local', 'Pusa Madhavi'), and white ('Bhima Shubhra', 'Udaipur Local').

2.2. Chemical and reagents

HPLC (high-performance liquid chromatography) grade methanol and trifluoroacetic acid were procured from Thermo Fisher Scientific (Waltham, MA, US). Flavonoid standards such as kaempferol, luteolin, quercetin, and quercetin 3-β-D-glucoside were purchased from Sigma-Aldrich Ltd. (New Delhi, India) with ≥97.0% purity. Nutrient agar, amoxicillin, potassium acetate, aluminium chloride and other chemicals were obtained from Himedia Laboratories (Mumbai, India).

2.3. Sample preparation

Onion skin of each cultivar was collected (100 g each) and washed with chlorinated water (0.5%) followed by distilled water for complete removal of impurities, then kept in deep freeze (Vestfrost Solutions, Denmark) at –40 °C for 24 h. Samples were freeze-dried at -50 °C temperature and 0.039 mbar pressure up to 48 h using lyophilizer (Mini Lyodel, Delvac Pumps, Chennai, India). Freeze-dried skin was ground using mixer-grinder (3053 Colt, Usha International Ltd, India) followed by the sieving (400 microns) for the formation of onion skin powder (OSP) with homogenous particle size and stored at -30 °C in air tight plastic containers for further use.

2.4. Sonication-assisted extraction

Extraction was carried out according to Jang et al. (2013) with minor changes. OSP (1 g) of each cultivar was mixed separately with 25 mL of methanol (extraction ratio 1:25, w/v) into beakers. All beakers were left overnight at 5 °C temperature. Samples were sonicated at 50% amplitude and 20 kHz frequency for 10 min using sonicator (QSonica, Newtown, US) and vortexed for 5 min repeatedly two times. Centrifugation was carried out for 10 min at 5,000 *g* at 5 °C using refrigerated centrifuge (3-18KS, Sigma, Hossein Shakeri, Germany). Then, supernatants were collected in capped tubes and stored at -25 °C for further use.

2.5. Quantification of flavonoids

Flavonoid quantification was carried by a HPLC (Prominence UFLC, Shimadzu, Kyoto, Japan) equipped with photodiode array (PDA) detector and an auto-sampler. A reverse phase Zorbax Eclipse plus C18 column (100 mm × 4.6 mm, 5 μm, Agilent, CA, US) was used for the separation of flavonoids. Methanol (absolute) and trifluoroacetic acid (0.1% in water) were used as mobile phase, with the flow rate of 1 mL/min. Volume of 20 μL of OSP extract was used for flavonoids quantification (Sagar et al., 2020). The results are presented in mg/kg dry weight, DW (Table 1).

2.6. Antimicrobial activity

2.6.1. Microorganisms

Six pathogenic bacterial strains were procured from National Collection of Dairy Cultures, National Dairy Research Institute, Karnal, India: *Bacillus cereus* (NCDC 240), *Klebsiella pnuemoniae* (NCDC 138), *Pseudomonas aeruginosa* (ATCC 27853), *Salmonella typhimurium* (ATCC 23564), *Staphylococcus aureus* (NCDC 109) and *Streptococcus agalactiae* (NCDC 118). All cultures were maintained in 30% glycerol stock at -80 °C. Working cultures were prepared from sub-cultures 18 h before analysis and incubated at optimum conditions. Bacterial cultures were adjusted equivalent to McFarland standard of 0.5, using spectrophotometer (UV-2600, Shimazdu, Kyoto, Japan) to obtain a final concentration of 1.5×10^8 CFU/mL.

2.6.2. Agar well-diffusion assay

This assay was carried out as per the method of Fazeli et al. (2007) with minor changes. Nutrient agar plates were prepared and bacterial strains were transferred after adjusting the culture to McFarland 0.5 turbidity standard for producing 1.5×10^8 CFU/mL on to the plates by sterile swabs. A sterile cork borer was used to make well (8 mm diameter) in agar plates. On the basis of a preliminary study with 10 μL, 15 μL, 20 μL, 25 μL and 30 μL extracts of all the cultivars. The extract concentration volume of 25 μL and 30 μL showed best and approximately same inhibition against the bacteria (data not shown) therefore, 25 μL was taken as test concentration for the studies. Each extract (25 μL) was poured into different well using micropipette, left for 30 min for proper diffusion followed by incubation for 24 h at 37 °C. The zone of inhibition (ZOI) was measured in mm. Absolute Methanol and amoxicillin (20 μL concentration) was used as negative and positive controls, respectively.

2.6.3. Micro-well dilution assay for minimum inhibitory concentration (MIC)

96 Well plate method was selected for MIC determination by dilution method as per Barry and Brown (1996) with slight modifications. Extracts were serially diluted in broth after sterilizing with Millipore filter (0.20 μm) to get 0.09–9.0 mg/mL DW concentration. Inocula (50 μL) of the bacteria was poured into each well and incubated for 24 h at 37 °C. Another culture medium was prepared without adding microorganisms, used as negative control. MIC value was determined as the concentration of the sample where no visible growth of tested organism was found.

2.6.4. Correlation analysis

Correlation analysis was performed between total flavonoids content (TFC) and inhibition rate of six pathogens (agar-well diffusion assay). TFC was measure by the method of Benítez et al. (2011) with minor modifications. OSP extract was taken in volume of 0.5 mL in test tube from different samples. 1.5 mL methanol (80%) was added into each test tube. Further, 0.1 mL aluminium chloride (10%) and 0.1 mL of potassium acetate (1 M) was poured. Distilled water (2.8 mL) was poured and incubated for 30 min at room temperature (30 °C). The absorbance was measured at 410 nm via UV-Vis Spectrophotometer (UV-2600, Shimazdu, Kyoto, Japan). Quercetin was taken as standard and the results were expressed as mg quercetin equivalent (QE)/g DW. Total flavonoid content of the cultivars is provided in Table 2 (Sagar et al., 2020).

Table 1. Quantified flavonoids in the extracts of onion skin of fifteen cultivars.

Cultivar	Peak	RT* (min.)	Peak assignment	Final concentration (mg/kg DW)	Cumulative concentration (mg/kg DW)	±S.D
Dark Red						
Agri Found Dark Red	1	23.11	Q-3D-G	471.37	7009.12	0.01
	2	24.92	QUE	5655.70		0.01
	3	25.38	LUT	317.20		0.02
	4	25.92	KAEM	564.85		0.01
NHRDF Red	1	22.88	Q-3D-G	694.72	14089.8	0.01
	2	24.73	QUE	11885.02		0.01
	3	25.22	LUT	1082.57		0.02
	4	25.73	KAEM	427.47		0.01
Red						
Arka Kirtiman	1	23.08	Q-3D-G	436.27	4841.75	0.02
	2	24.90	QUE	3738.25		0.01
	3	25.36	LUT	369.15		0.02
	4	25.89	KAEM	298.07		0.02
Hissar-2	1	22.91	Q-3D-G	1432.87	14318.05	0.01
	2	24.74	QUE	11429.30		0.01
	3	25.23	LUT	1032.72		0.01
	4	25.75	KAEM	423.15		0.02
Pusa Red	1	22.86	Q-3D-G	378.07	4570.82	0.01
	2	24.73	QUE	2830.92		0.01
	3	25.21	LUT	956.05		0.01
	4	25.72	KAEM	405.77		0.01
Pusa Riddhi	1	22.87	Q-3D-G	631.70	11394.97	0.01
	2	24.73	QUE	8571.30		0.02
	3	25.22	LUT	1669.92		0.02
	4	25.73	KAEM	522.05		0.02
Sukh Sagar	1	22.93	Q-3D-G	505.52	7860.72	0.02
	2	24.76	QUE	6389.12		0.02
	3	25.24	LUT	495.87		0.01
	4	25.76	KAEM	470.20		0.01
Pink						
Agri Found Light Red	1	23.11	Q-3D-G	305.62	5446.17	0.02
	2	24.91	QUE	3496.50		0.01
	3	25.36	LUT	949.57		0.00
	4	25.89	KAEM	694.47		0.01
Bhima Kiran	1	23.06	Q-3D-G	439.10	4802.22	0.01
	2	24.88	QUE	3188.32		0.01
	3	25.34	LUT	466.57		0.01
	4	25.87	KAEM	708.22		0.00
Bhima Shakti	1	23.02	Q-3D-G	489.55	6462.70	0.02
	2	24.58	QUE	4558.50		0.02
	3	25.32	LUT	704.67		0.02
	4	25.84	KAEM	709.97		0.01
Hissar-3	1	22.91	Q-3D-G	419.17	6022.65	0.02
	2	24.75	QUE	4303		0.00
	3	25.24	LUT	780.37		0.00
	4	25.75	KAEM	520.10		0.01
Phursungi Local	1	22.92	Q-3D-G	599.70	6325.32	0.02
	2	24.76	QUE	4642.12		0.01
	3	25.24	LUT	518.40		0.01
	4	25.76	KAEM	565.10		0.01
Pusa Madhavi	1	23.10	Q-3D-G	68.97	4277.85	0.02
	2	24.73	QUE	2856.42		0.00
	3	25.22	LUT	895.77		0.01
	4	25.73	KAEM	456.67		0.02

(*continued on next page*)

Table 1 (*continued*)

Cultivar	Peak	RT* (min.)	Peak assignment	Final concentration (mg/kg DW)	Cumulative concentration (mg/kg DW)	±S.D
White						
Bhima Shubhra	1	22.96	Q-3D-G	56.17	315.87	0.02
	2	24.79	QUE	89.27		0.02
	3	25.27	LUT	46.20		0.01
	4	28.78	KAEM	124.22		0.02
Udaipur Local	1	22.95	Q-3D-G	60.65	339.37	0.01
	2	24.78	QUE	88.40		0.02
	3	25.25	LUT	45.47		0.00
	4	25.97	KAEM	144.85		0.02

(n = 3, Mean ± S.D); * = Retention time; Peak assignment represents, Q-3D-G: Quercetin 3-β-D-glucoside, QUE: Quercetin, LUT: Luteolin, KAEM: Kaempferol.

2.7. Statistical analysis

All the analyses were carried out in triplicate and significance difference ($P \leq 0.05$) was determined by One Way Analysis of Variance (ANOVA) and Duncan's test (post hoc) using IBM® SPSS statistics (version 20). PCA was applied on agar well diffusion data with unit variance scaling using Factoextra package of R-programming language (Open source).

3. Results and discussion

3.1. Agar well-diffusion assay and principal component analysis (PCA)

All skin extracts were found to be effective against pathogenic strains. In the case of Gram positive bacteria, best results were recorded with the extract of cv. 'Hissar-3' against *Bacillus cereus* and 'Bhima Kiran' against *Streptococcus agalactiae,* while cv. 'Pusa Riddhi', 'Phursungi local', and 'Hissar-3' was found highly effective against *Staphylococcus aureus.* Similarly, in the case of Gram negative bacteria, cv. 'Phursungi Local' and 'Hissar-3' showed the highest inhibition against *Klebsiella pnuemoniae* and *Salmonella typhimurium*, respectively, whereas 'Bhima Shubhra' and 'Udaipur Local' were the only effective cultivars against *Pseudomonas aeruginosa* (Table 3). Extracts of 'Bhima Shubhra' and 'Udaipur Local' were found least effective against the bacterial strains. Negative control (methanol) did not show any antibacterial activity (Figure 1).

Table 2. Total flavonoids content of onion skin extracts of fifteen cultivars.

Cultivars	Total flavonoid content (mg QE/g DW)
Dark Red	
Agri Found Dark Red	92.31 ± 1.93^{h}
NHRDF Red	168.77 ± 0.87^{m}
Red	
Arka Kirtiman	96.46 ± 1.38^{i}
Hissar-2	98.09 ± 0.94^{j}
Pusa Red	89.62 ± 0.70^{g}
Pusa Riddhi	150.94 ± 0.17^{k}
Sukh Sagar	88.11 ± 0.19^{f}
Pink	
Agri Found Light Red	83.67 ± 0.37^{e}
Bhima Kiran	81.48 ± 0.58^{d}
Bhima Shakti	75.36 ± 1.02^{c}
Hissar-3	160.18 ± 0.84^{l}
Phursungi Local	66.58 ± 0.55^{b}
Pusa Madhavi	66.88 ± 0.24^{b}
White	
Bhima Shubhra	1.31 ± 0.32^{a}
Udaipur Local	2.01 ± 0.02^{a}

Values with different superscripts $^{a\text{-}k}$ in same column are significantly different ($P \leq 0.05$).

As per the color of onion skin, pink cultivars were the most effective against gram positive bacteria. For instance, highest ZOI (11.0 ± 0.0 mm) was observed with the skin extract of cv. 'Hissar-3' (pink) against *Bacillus cereus* and lowest ZOI (2.0 ± 0.0) was recorded with cv. 'NHRDF Red' (dark red) and 'Hissar-2' (red). A significant difference ($P \leq 0.05$) was found among cv. 'Sukh Sagar' (red), 'Phursungi Local' (white) and 'Hissar-3' (pink). In addition to this, white cultivars 'Bhima Shubhra' and 'Udaipur Local' also inhibited the growth of *Bacillus cereus* up to 6.0 ± 0.0 mm and 9.0 ± 0.0 mm, respectively. Maximum ZOI (10.0 ± 0.0 mm) was reported with 'Bhima Kiran' (pink) against *Streptococcus agalactiae,* while least inhibition (2.0 ± 0.0 mm) was found with the skin extract of cv. 'Pusa Riddhi' (red). Moreover, cv. 'Phursungi Local' (pink) showed highest inhibition rate (7.0 ± 0.0 mm) against *Staphylococcus aureus,* whereas minimum inhibition up to 2.0 ± 0.0 mm was reported with cv. 'Arka Kirtiman' (red) and 'Pusa Red' (red). Cultivars 'NHRDF Red' (dark red), 'Arka Kirtiman' (red), 'Hissar-2' (red) and 'Bhima Kiran' (pink) were found significantly different from each other at the level of $P \leq 0.05$. Overall, skin extracts of pink coloured onions were found best effective against Gram positive bacteria compared to other cultivars. The variation in the inhibition rate of OSP occurred due to cultivar types and variation in the polyphenols content, like quercetin, kaempferol, and luteolin (Table 1). Skerget et al. (2009) compared skin and edible onion bulb for antibacterial activity and found that skin extract (5 g/100 mL) of red onion inhibited 83.3 ± 3.1% growth of *Bacillus cereus,* while lower inhibition (63.3 ± 3.8%) was observed with the extract of edible part.

Regarding Gram negative bacteria, the extract of cv. 'Phursungi Local' (pink) was found best against *Klebsiella pnuemoniae* with 12.0 ± 0.0 mm ZOI and 'Agri Found Dark Red' (dark red), 'Arka Kirtiman' (red), and 'Agri Found Light Red' (pink) were reported as least effective with 3.0 ± 0.0 mm ZOI. A significant difference was obtained among cv. 'Hissar-2' (red), 'Hissar-3' (pink), 'Sukh Sagar' (red) and 'Phursungi Local' (pink). Only white onions, i.e., 'Bhima Shubhra' and 'Udaipur Local', were found effective against *Pseudomonas aeruginosa* among all with 4.0 ± 0.0 mm and 4.0 ± 0.0 mm (ZOI), respectively. Additionally, 'Hissar-3' (pink) was recorded best cultivar against *Salmonella typhimurium* with 15.0 ± 0.0 mm ZOI and cv. 'Pusa Riddhi' (red) least affected (2.0 ± 0.0 mm) the growth of *Salmonella typhimurium*. A significant difference ($P \leq 0.05$) was found between cv. 'Shukh Sagar' (red), 'Bhima Shakti' (pink), 'Hissar-3' (dark red), and 'Phursungi Local' (dark red). Onion phenols and flavonoids are responsible for antimicrobial activity and it is well documented that onion skin contained higher bioactive components than edible bulb (Benítez et al., 2011). The extract of white and yellow skinned onions was investigated against Gram positive and Gram negative bacteria and it was found that both cultivars inhibited only Gram positive bacteria, i.e. *Bacillus cereus* and *Staphylococcus aureus* with 9.5 ± 0.7 mm and 10.5 ± 0.5 mm ZOI, respectively (Santas et al., 2010), while the skin extracts of Indian white cultivars ('Bhima Shubra' and

Table 3. Antimicrobial activity of skin extracts of different coloured onions against some pathogenic bacteria.

Cultivars	Inhibition Zone (mm)					
	Gram positive bacteria			Gram negative bacteria		
	Bacillus cereus	*Streptococcus agalactiae*	*Staphylococcus aureus*	*Klebsiella pnuemoniae*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*
Dark red						
Agri Found Dark Red	10.0 ± 0.0^{f}	3.0 ± 0.0^{bc}	3.0 ± 0.0^{bc}	3.0 ± 0.0^{b}	N.I	3.0 ± 0.0^{ab}
NHRDF Red	2.0 ± 0.0^{a}	6.0 ± 0.0^{f}	4.0 ± 0.0^{de}	6.0 ± 0.0^{f}	N.I	3.0 ± 0.0^{ab}
Red						
Arka Kirtiman	2.0 ± 0.0^{a}	5.0 ± 0.0^{e}	2.0 ± 0.0^{b}	3.0 ± 0.0^{b}	N.I	3.0 ± 0.0^{ab}
Hissar-2	2.0 ± 0.0^{a}	8.0 ± 0.0^{g}	3.0 ± 0.0^{bc}	5.0 ± 0.0^{e}	N.I	3.0 ± 0.0^{ab}
Pusa Red	4.0 ± 0.0^{b}	3.0 ± 0.0^{bc}	2.0 ± 0.0^{b}	3.0 ± 0.0^{b}	N.I	3.0 ± 0.0^{ab}
Pusa Riddhi	6.0 ± 0.0^{c}	2.0 ± 0.0^{b}	7.0 ± 0.0^{g}	7.0 ± 0.0^{g}	N.I	2.0 ± 0.0^{a}
Sukh Sagar	8.0 ± 0.0^{e}	3.0 ± 0.0^{bc}	3.0 ± 0.0^{bc}	8.0 ± 0.0^{h}	N.I	10.0 ± 0.0^{d}
Pink						
Agri Found Light Red	4.0 ± 0.0^{b}	3.0 ± 0.0^{bc}	3.0 ± 0.0^{bc}	3.0 ± 0.0^{b}	N.I	4.0 ± 0.0^{b}
Bhima Kiran	9.0 ± 0.0^{f}	10.0 ± 0.0^{h}	5.0 ± 0.0^{f}	9.0 ± 0.0^{h}	N.I	2.0 ± 0.0^{a}
Bhima Shakti	7.0 ± 0.0^{d}	3.0 ± 0.0^{bc}	4.0 ± 0.0^{de}	4.0 ± 0.0^{cd}	N.I	8.0 ± 0.0^{c}
Hissar-3	11.0 ± 0.0^{g}	3.0 ± 0.0^{bc}	7.0 ± 0.0^{g}	10.0 ± 0.0^{i}	N.I	15.0 ± 0.0^{f}
Phursungi Local	10.0 ± 0.0^{f}	4.0 ± 0.0^{d}	7.0 ± 0.0^{g}	12.0 ± 0.0^{j}	N.I	13.0 ± 0.0^{e}
Pusa Madhavi	3.0 ± 0.0^{ab}	3.0 ± 0.0^{bc}	4.0 ± 0.0^{de}	4.0 ± 0.0^{cd}	N.I	4.0 ± 0.0^{b}
White						
Bhima Shubhra	6.0 ± 0.0^{c}	N.I	N.I	N.I	4.0 ± 0.0^{b}	4.0 ± 0.0^{b}
Udaipur Local	9.0 ± 0.0^{f}	N.I	N.I	N.I	4.0 ± 0.0^{b}	3.0 ± 0.0^{ab}
P.C*	19.0 ± 0.0^{h}	16.0 ± 0.0^{i}	6.0 ± 0.0^{g}	21.0 ± 0.0^{k}	18.0 ± 0.0^{a}	12.0 ± 0.0^{g}
N.C**	N.I	N.I	N.I	N.I	N.I	N.I

Values are mean ± standard deviation (n = 3), * = Positive control (Amoxicillin 20 μL); ** = Negative control (Methanol); N.I = No inhibition.
Values with different letters^{a-k} in same column are significantly different ($P \leq 0.05$) by Duncan's multiple range test.

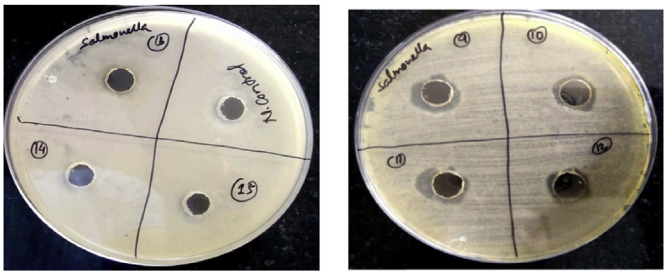

Figure 1. Agar plates of well-diffusion assay against *Salmonella typhimurium*: 9. Hissar-3, 10. Phursungi Local, 11. Sukh Sagar, 12. Positive control, 13. Pusa Riddhi, 14. Bhima Kiran, 15. Udaipur Local and Negative control.

'Udaipur Local') inhibited both Gram positive (*Bacillus cereus*) and Gram negative (*Pseudomonas aeruginosa* and *Salmonella typhimurium*) bacterial strains (Table 3). It revealed that onion skin of Indian cultivars (white) might have higher antimicrobial compounds due to geographical area, cultivar variation and application of sonication-assisted extraction. Additionally, when antimicrobial potential of onion extract (extracted by maceration) was analysed by well-diffusion method, it showed maximum inhibition zone (23 mm) against *Staphylococcus aureus* and 16 mm against *Pseudomonas aeruginosa* at 50 μL of liquid extract (Induja and Geetha, 2018), whereas in the present study, only 25 μL volume of skin extract ('Phursungi Local') inhibited *Staphylococcus aureus* up to 7 mm which confirmed that sonication-assisted extraction method is more effective against pathogenic bacteria due to higher content of flavonoids in OSP (Table 2). When onion bulb extract was investigated against various pathogens, 13 mm and 14 mm inhibition zone was reported for *Salmonella typhimurium* and *Staphylococcus aureus* but maximum inhibition (23 mm) was obtained for *Klebsiella pnuemoniae* (Sharma, 2015). Colours of onion are the key behind the effective inhibition rate. Likewise, Loredana et al. (2019) investigated the extracts of three onion varieties (two copper-coloured skinned and one pink-coloured skin) grown in Mediterranean area (Italy) and found that pink-coloured varieties had best inhibition rate, respectively, 14.3 ± 0.57 mm, 6.33 ± 0.57 mm and 16.66 ± 0.57 mm against *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* which support the present study. Skin of pink onions specifically 'Hissar-3' and 'Phursungi Local' showed a wide range of inhibition against Gram negative bacteria among all the cultivars.

Principal component analysis model was also applied to give comprehensive view of inhibitory effect of onion skin extracts on six pathogenic bacteria. The triplicate values of agar well diffusion assay were considered for PCA to investigate the compact and graphical overview of inhibitory effect. In scatter plot, the focus should be on the direction of variable not on their absolute values because individuals and variables coordinates are not created on the same space (Mandrone et al., 2019). PCA scatter plot (Figure 2) showed antibacterial activity of differently coloured skin extracts. The extracts on the positive side of the plot [Dim1] showed higher inhibition rate against *Bacillus cereus, Klebsiella pnuemoniae* and *Salmonella typhimurium*. Basically, the dots with number are representing pink coloured cultivars followed by dark red and red which maximally inhibited the growth of wide range of bacteria, i.e. *Klebsiella pnuemoniae, Staphylococcus aureus, Bacillus cereus* and *Salmonella typhimurium*, respectively. This showed that dark red, red and pink skin extracts contained higher polyphenolics content which is directly associated with the inhibition rate (Scavo et al., 2019). While on the other side of plot [Dim2], least inhibition rate was found in the case of bacterium *Pseudomonas aeruginosa* by white skin extracts while coloured extract did not exhibit any inhibition.

PCA R-plot was also exhibited to get a clearer picture (Figure 3). R-plot showed individual extract effect on the growth of bacteria in mm. In the graph, bacterial strains were denoted by different colours while minus (-) and zero (0) points denoted no inhibition and increasing

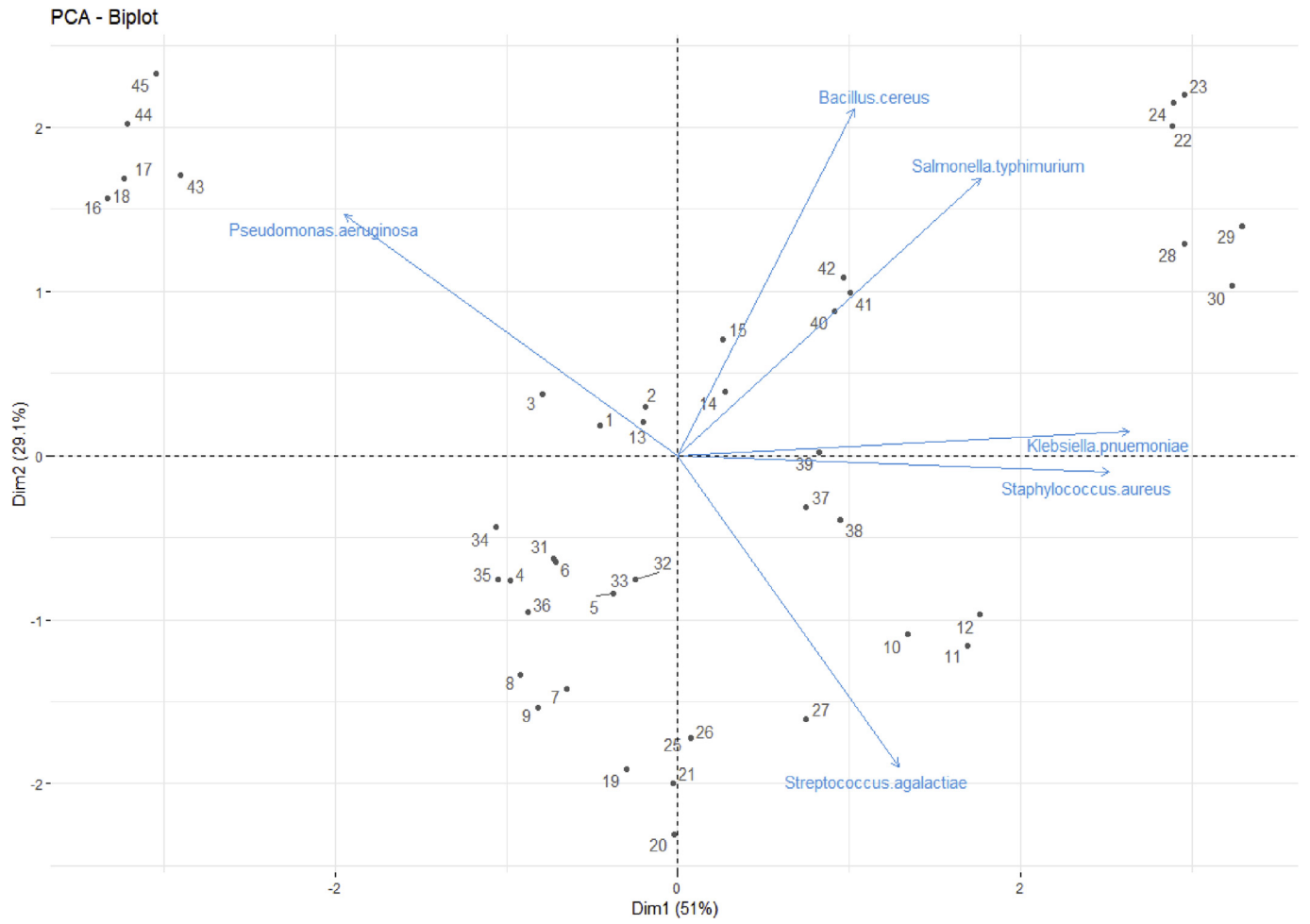

Figure 2. PCA Scatter Plot: Effect of onion skin extracts on bacterial growth. Numbers representing triplicate readings of fifteen cultivars and arrows representing bacterial strains. Extracts reading along the positive component [Dim1] showed higher inhibition rate while reading along the negative component [Dim2] showed less inhibition rate.

numbers showed the increased antibacterial activity. All coloured extracts showed good antimicrobial activity (2 mm–15 mm) against all the bacterial strains except white skin extracts. Graphs showed that pink skin extracts ('Phursungi Local', 'Hissar-3', 'Bhima Shakti') had better inhibition potential. Besides this, extracts of dark red skin ('NHRDF Red') and red skin ('Pusa Riddhi') were also reported as good antibacterial agents. It can be seen that bacterium *Pseudomonas aeruginosa* was found on minus or zero point in most of the graph. Only white cultivars 'Bhima Shubra' and 'Udaipur Local' inhibited *Pseudomonas aeruginosa* growth up to 4 mm.

3.2. Minimum inhibitory concentration (MIC)

MIC values of effective skin extracts are summarized in Table 4. Cultivar 'Phursungi Local' was reported as best material during MIC assay against both types of strains. In case of Gram positive bacteria, cultivars 'Sukh Sagar', 'Hissar-3' and 'Udaipur Local' inhibited *Bacillus cereus* at 0.45 mg/mL during MIC assay. MIC against *Streptococcus agalactiae* was best reported for 'Hissar-2' at 0.45 mg/mL. 'Phursungi Local' was reported to be best against *Staphylococcus aureus* at 0.54 mg/mL in MIC assay.

In case of Gram negative bacteria, lowest MIC (0.36 mg/mL) of cultivar 'Phursungi Local' was recorded against *Klebsiella pnuemoniae*and *Salmonella typhimurium,* while only cultivars 'Bhima Shubhra' and 'Udaipur Local' were found effective against *Pseudomonas aeruginosa* at the concentration of 0.72 mg/mL and 0.81 mg/mL, respectively. In the conclusion, the skin extract of pink coloured onions ('Phursungi Local' and 'Hissar-3') were found highly effective on both Gram positive and Gram negative bacteria, while white skinned cultivars, i.e. 'Bhima Shubhra' and 'Udaipur Local' showed the best MIC against *Pseudomonas aeruginosa.* The differences in inhibitory compounds (polyphenols), cultivar type and bacterial susceptibility are the key reasons behind the MIC. Santas et al. (2010) investigated the antibacterial potential of onion crude extract and found 5 mg/mL and 8 mg/mL as lowest MIC against *Bacillus cereus* and *Staphylococcus aureus*, respectively. Similarly, Ortiz (2015) analysed raw onion extract against *Staphylococcus aureus* and observed 6 mg/mL as MIC. Present study showed lower and better MICs than the results of Santas et al. (2010) and Ortiz (2015) because of the difference in extraction method (sonication-assisted) and higher flavonoids level in utilized onion skin extracts. Moreover, polysaccharides extracted from onions were reported as good antimicrobial agents and inhibited *Staphylococcus aureus* and *Salmonella typhimurium* at 0.10 mg/mL and 0.14 mg/mL of MIC (Ma et al., 2018). Italian onions such as '*Vatolla*' (pink-coloured) and '*Montoro*' (copper-coloured) were investigated for antibacterial activity by MIC assay and it was reported that pink-coloured cultivar had lower MIC value against *Bacillus cereus* (>0.4), *Pseudomonas aeruginosa* (>0.8) and *Staphylococcus aureus* (>1) in mg/mL than dark-coloured (copper) cultivar (Loredana et al., 2019). It revealed that onion extracts specifically pink onion worked as a better antibacterial agent against *Staphylococcus aureus* and other strains because of the higher amount of polyphenols. *Staphylococcus aureus* is responsible for 300 food borne problems annually because it has heat-stable enterotoxins (García-Lomillo et al., 2017). Therefore, onion

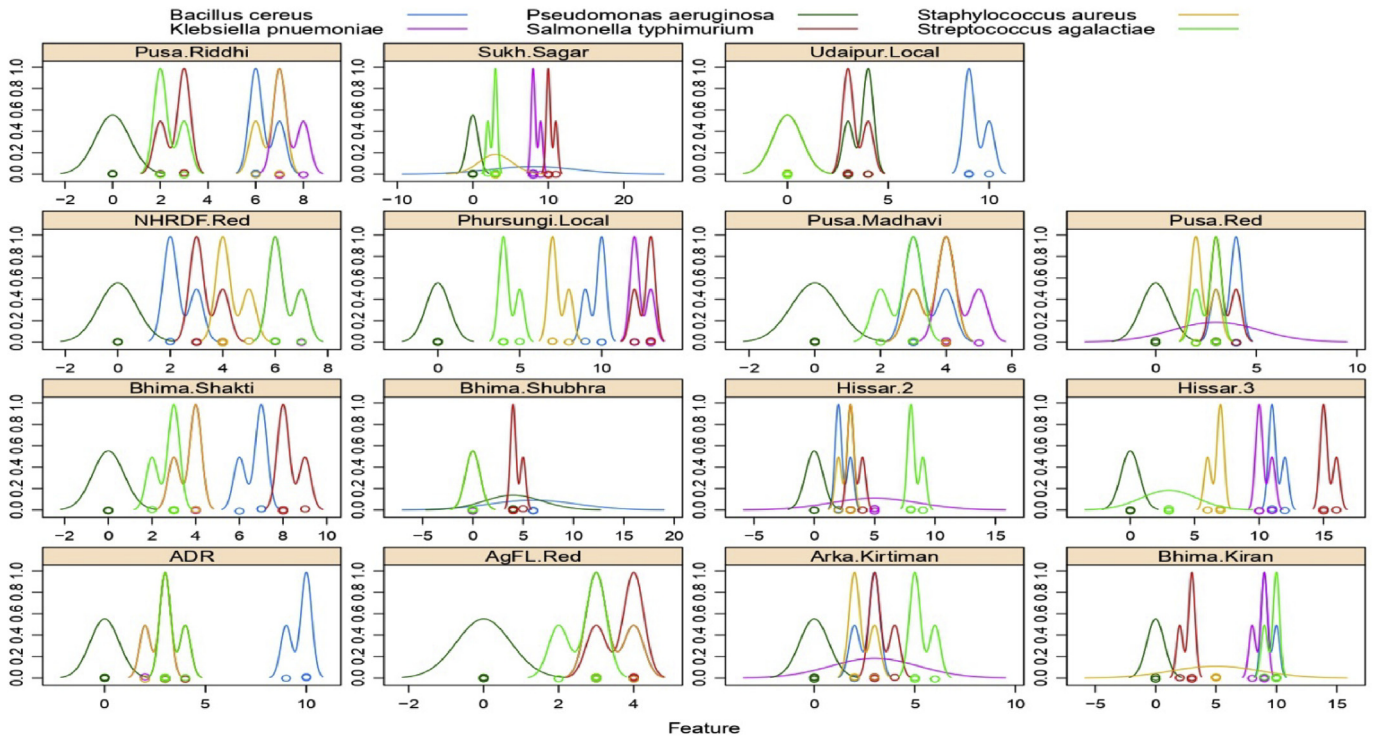

Figure 3. PCA R-plot: Individual effect of fifteen onion skin extracts on different bacteria (0 represents no inhibition while higher values represent higher rate of inhibition).

Table 4. Minimum inhibitory concentration (MICs) of effective skin extracts of different coloured onion.

Cultivars	MICs (mg/mL)					
	Gram positive bacteria			Gram negative bacteria		
	Bacillus cereus	*Streptococcus agalactiae*	*Staphylococcus aureus*	*Klebsiell pnuemoniae*	*Pseudomonas aeruginosa*	*Salmonella typhimurium*
Dark red						
NHRDF Red	>9.0[a]	0.72	0.81	0.72	>9.0	>9.0
Red						
Hissar-2	>9.0	0.45	>9.0	2.7	>9.0	>9.0
Pusa Riddhi	0.63	>9.0	0.72	0.54	>9.0	>9.0
Sukh Sagar	0.45	>9.0	>9.0	0.45	>9.0	0.36
Pink						
Bhima Kiran	0.63	0.63	0.72	0.63	>9.0	>9.0
Bhima Shakti	0.72	>9.0	>9.0	>9.0	>9.0	>9.0
Hissar-3	0.45	>9.0	0.72	0.54	>9.0	0.27
Phursungi Local	0.72	9.0	0.54	0.36	>9.0	0.36
White						
Bhima Shubhra	0.72	>9.0	>9.0	>9.0	0.72	0.90
Udaipur Local	0.45	>9.0	>9.0	>9.0	0.81	0.81

Values are expressed as mean of three experiments (n = 3).

and its skin extracts could be utilised as natural antimicrobials and natural additives which not only increases the shelf-life of perishable items but also enhances the food safety (Vázquez-Armenta et al., 2017).

3.3. Correlation analysis

The results of total flavonoids content and antimicrobial activity (agar well-diffusion assay) were correlated (Figure 4) and most of the cultivar extracts showed positive correlation. A significant positive correlation was observed between TFC and inhibition of Gram positive bacterium, i.e. *Streptococcus agalactiae* (R^2 = 0.7023), *Staphylococcus aureus* (R^2 = 0.7687) and *Bacillus cereus* (R^2 = 0.4983). Similarly, a good correlation was also obtained for Gram negative bacteria viz. *Klebsiella pnuemoniae* (R^2 = 0.6741) and *Salmonella typhimurium* with R^2 = 0.8476 value which showed that the flavonoids content of OSP extracts are responsible for the inhibition of bacteria (Sagar et al., 2020). Inversely, in case of *Pseudomonas aeruginosa* (a Gram negative bacterium) the correlation showed a negative trend (R^2 = 0.3967) because all coloured OSP extracts exhibited no inhibition. It also showed the resistant nature of *Pseudomonas aeruginosa* against these flavonoids. However, only white cultivar extracts showed good inhibition against *Pseudomonas aeruginosa* which indicates that white onion cultivars

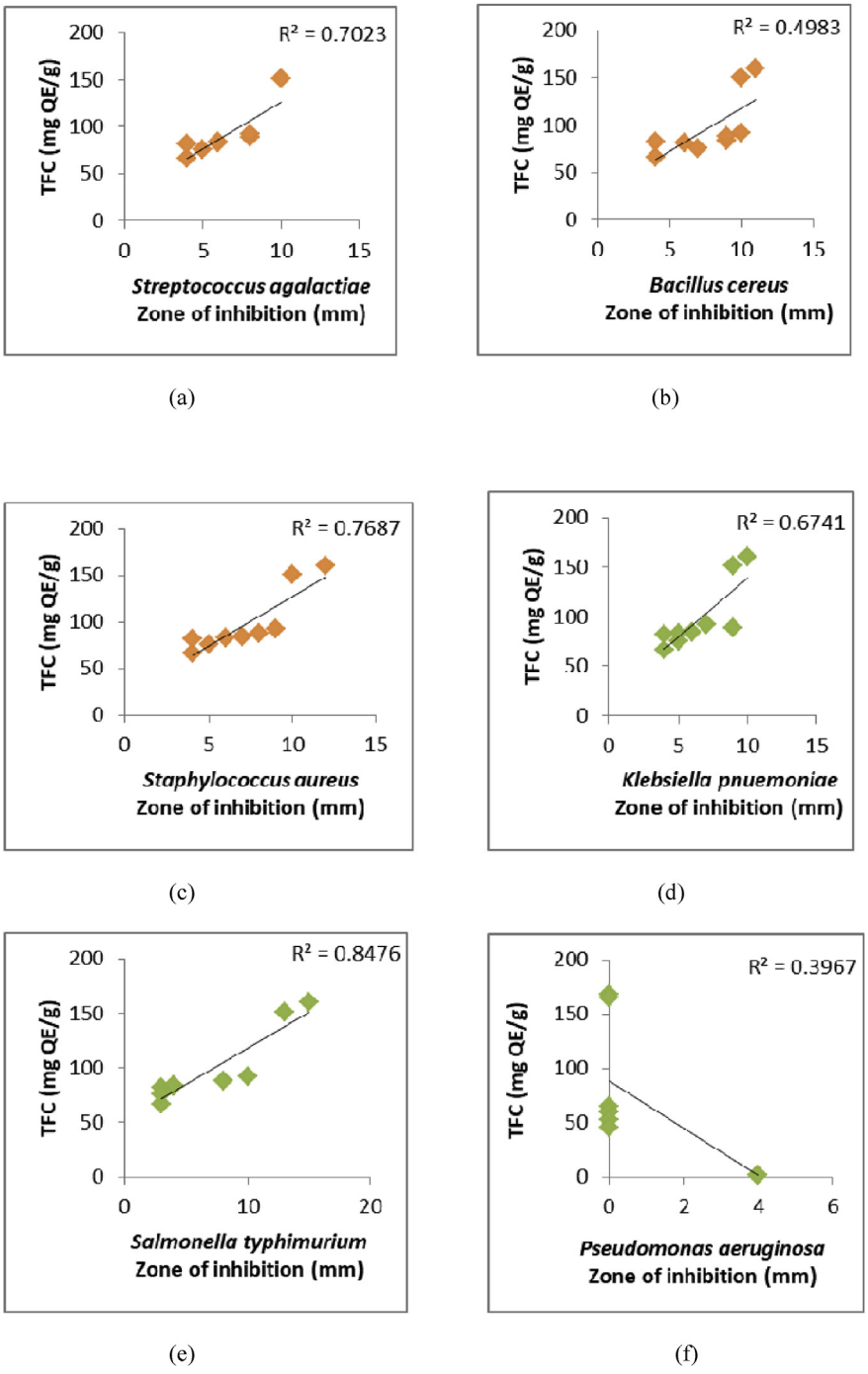

Figure 4. Correlation between total flavonoids content (TFC) and antimicrobial activity of OSP extracts against Gram positive: (a) *Bacillus cereus,* (b) *Streptococcus agalactiae,* (c) *Staphylococcus aureus* and Gram negative: (d) *Klebsiella pnuemoniae,* (e) *Salmonella typhimurium,* (f) *Pseudomonas aeruginosa* bacteria.

contain other effective phenolic compounds like gallic acid which inhibits bacterial growth (Liguori et al., 2017).

4. Conclusions

It can be concluded that skin extract of pink cultivars specifically 'Hissar-3' and 'Phursungi Local' had the higher inhibition potential against both Gram positive and Gram negative bacteria, except *Pseudomonas aeruginosa*. PCA investigation provided an insight view of extracts position on scattered plot with their inhibition role against particular bacterium. Only white skin extracts showed some inhibition in the case of *Pseudomonas aeruginosa*. Skin extracts of cv. 'Phursungi Local' > 'Hissar-3' > 'Bhima Kiran' > 'Sukh Sagar' > 'NHRDF Red' revealed best results against wide range of bacteria in decreasing order when subjected to MIC assay. 'Phursungi Local' exhibited lowest MICs against *Klebsiella pnuemoniae* (0.36 mg/mL) and *Staphylococcus aureus* (0.54 mg/mL), while cv. 'Hissar3' showed lowest MIC against *Salmonella typhimurium* (0.27 mg/mL). Additionally, total flavonoids showed positive correlation

with antimicrobial activity of the extracts. Pink and red coloured onions can be utilised not only as natural preservatives but also as antimicrobial agents in food sector.

Declarations

Author contribution statement

Narashans Alok Sagar: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Sunil Pareek: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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