Assessment of antimicrobial and antioxidant potential of cytoplasmic male sterile lines of pepper

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Abstract

In the present study eight Cytoplasmic Male Sterile (CMS) lines of pepper and one open pollinated commercial variety 'California wonder' were assessed for their antimicrobial and antioxidant potential. For antimicrobial activity, fresh fruits crushed in 10%, 20%, 30% and 40% methanol were analyzed. Thirty per cent and forty percent methanolic extracts were found to be best activator of metabolites exhibiting antibacterial activity. Highest antibacterial activity was shown by 40% methanolic extracts of KCS2A, KCS3A and AVPP9907-S of *Capsicum* CMS lines against different bacteria. The extracts of all lines found effective against *Yersinia pestis*. Phenolic content of all CMS lines was determined by extracting fruit in absolute ethanol, it was found in the range of 594.99–1710.13 µg gallic acid/gfw. Phenolic content was highest in AVPP0710-S (1690.48 ± 19.65 µg gallic acid/gfw) and lowest in KCS 2A. FRAP, CUPRAC and DPPH assay were done to find out antioxidant potential of CMS lines under study. FRAP and CUPRAC were analyzed by crushing fruits in 80% ethanol. FRAP was found in the range of 0.9–3.12 µmol trolox/g, with AVPP0710-S exhibiting highest FRAP value (2.77 ± 0.3522 µmol trolox/g). CUPRAC was found in the range of 0.7–11.22 µmol trolox/g and AVPP9908-S showed highest CUPRAC value (8.55 ± 0.922 µmol trolox/g). Free radical scavenging DPPH assay was done by extraction in absolute methanol and was highest in AVPP9908-S (32.94 ± 3.61%) with 9.91–36.55% overall range. This study will pave the way for the development of genotypes with higher phenol, antioxidant and antimicrobial activity in pepper.

Keywords Cytoplasmic male sterile · Pepper · Antimicrobial activity · Antioxidant · DPPH

1 Introduction

Peppers are an important group of vegetables which are grown on a large scale all over the world. It includes both, mild or less pungent fruit types (sweet pepper) and pungent fruit types (hot pepper) [1]. A wide terminology is used to designate peppers like chili, chile, chilli, aji, paprika and capsicum [2].

Peppers are a rich source of various antioxidants like capsaicinoids, ascorbic acid, carotenoids, Vitamin E, provitamin A etc. [3]. Antioxidants are substances that can prevent or slow damage to cells caused by free radicals,

unstable molecules that the body produces as a reaction to environmental and other pressures. Antioxidants can protect against the cell damage that free radicals cause, known as oxidative stress. They contain high concentration of polyphenols which includes flavonols, flavones, anthocyanins and hydroxycinnamates [4, 5]. These bio-compounds are known to enhance the antioxidant activity of peppers [4, 6, 7]. The antioxidant potential works by preventing the oxidation of lipids by scavenging the free radicals. Vitamin E, C and beta carotene are of special concern in increasing the antioxidant potential of peppers [8]. Hot peppers are reported to show high antioxidant

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potential due to high levels of capsaicinoids in them which also impart pharmacological properties to them [9]. Phenols reduce the risk of lipid autoxidation by scavenging free radicals and thereby inhibit the initiation of oxidation chain [10]. Flavonoids impart organoleptic properties such as taste to the fruits [11]. Anthocyanins are natural pigments which impart diverse colors to the plant life and flavonols are induced by sunlight which shields the plant from harmful effects of ultraviolet rays [12].

In today's scenario, microbial infections and resistance to antibiotic drugs have been the main causes that threaten the health of peoples. Over the decades, it has been observed that the microbial infections are responsible for millions of deaths throughout the world. The antimicrobial activity of plants is mainly attributed to two mechanisms, which include interfering chemically with the synthesis or function of vital components of bacteria, and/or circumventing the conventional mechanisms of antibacterial resistance. These agents can act alone or in combination with antibiotics to enhance the antibacterial activity against a wide range of bacteria. In this regards, phytochemicals have revealed significant activities while many researchers have used natural products to act against bacterial resistance [8–11]. Although synthetic antimicrobial agents have been already approved in many countries, yet the usage of natural compounds that are derived from microbial, animals, or plants attracts the attention of many researchers [13, 14]. These compounds have exhibited promising results in overcoming the emergence of antibiotic resistance in bacterial pathogens [15, 16]. Among all of the available options, plant-derived compounds have displayed more potential applications in combating bacterial infections. Nowadays, people are getting more aware of the type of food they are having and explore the nutritional value of foods before consuming [17–19].

Numerous medicinal plants and many vegetable crops have been reported to possess antimicrobial activity. The antimicrobial potential is generally attributed to the presence of polyphenols in them [20]. Peppers are bestowed to inhibit the growth of many gram positive and gram negative bacteria such as *E. coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus cereus* etc. and fungal strains—*Candida albicans*, *Aspergillus flavus* [21, 22]. Many phytotherapy annuals have emphasized on use of botanicals for treating various diseases of human body such as urinary and skin infection, respiratory and gastrointestinal disorders [23].

In recent times, CMS character has been exploited to develop many hybrids in chilli. The analysis of the antioxidant and antimicrobial potential of CMS lines would help to select healthy lines for hybridization process. Keeping these viewpoints in mind, the present investigation was

formulated to assess the antimicrobial and antioxidant potential of eight cytoplasmic male sterile lines of *Capsicum annuum*. The study would enforce the production of good quality pepper hybrids, the consumption of which can act as robust nutraceutical.

2 Materials and methods

2.1 Collection of plant material

Eight CMS lines of pepper were used for conducting the present investigation. Out of eight CMS lines, five were procured from AVRDC, Taiwan (AVPP9908-S, AVPP9907-S, AVPP0710-S, AVPP9820-S, AVPP0516-S) and three (KCS1A, KCS2A, KCS3A) and a non-CMS variety California wonder (CW) which was used as a reference variety, were obtained from ICAR-IARI, Regional Station, Katrain, Himachal Pradesh, India.

2.2 Bacterial strains

The bacterial strains used for antimicrobial screening were obtained from Department of Microbiology and Biotechnology, Himachal Pradesh University, Shimla, India. The following strains were used for the assay:

Gram negative: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella dysenteriae*, *Yersinia pestis*

Gram positive: *Staphylococcus aureus*, *Bacillus subtilis*.

Microbial strains were inoculated in nutrient broth at 37 °C for 8 h, strains were subcultured on nutrient agar medium at 37 °C and stored at 4 °C.

2.3 Sample collection and extract preparation

Fruits of pepper were collected, washed with distilled water and wiped with 70% ethanol. Fruit of each line was weighed 2 g and crushed in 30 ml of 10%, 20%, 30% and 40% methanol with the help of pestle and mortar. The crude extract was transferred to the centrifuge tube and centrifuged at 7500 rpm for 10 min. The supernatant was collected in a separate tube and was further concentrated by reducing the volume to 15 ml in a water bath at 40 °C for 1–2 h. The resultant extract was stored at –20 °C for further use. Extraction was also done in distilled water (DW) which was used as negative control. Standard antibiotics, streptomycin and bleomycin were used as positive control.

2.4 Determination of antibacterial activity

The antimicrobial activity was determined by agar well diffusion method. 100 µl of 8 h old inoculum was poured

onto nutrient agar plates and evenly spread on the plate using a sterile spreader. Four wells of 6 mm diameter were made with the help of a sterile cork borer in each petri plate. 100 µl of 10%, 20%, 30% and 40% crude extract was carefully added in each well and marked accordingly. Extract was allowed to diffuse through the well into the gel by keeping the plates at the room temperature for 2 h. Plates were then incubated at 37 °C for 18–24 h. Anti-microbial activity was measured as zone of inhibition of bacterial growth. Standard antibiotics streptomycin and bleomycin were used as positive controls and distilled water as negative control. All the tests were performed in triplicates.

2.5 Determination of total phenolic content

The total phenolic content was determined spectrophotometrically following the procedure given by Muresan et al. [24] with slight modifications. In this 2 g of sample was homogenized in 10 ml of 80% ethanol and centrifuged at 5000 rpm for 10 min. Supernatant was stored at – 20 °C for further use. 100 µl of sample was added to 2.9 ml distilled water and 0.5 ml Folin–Ciocalteu reagent. Reaction was neutralized after 3 min by adding 2 ml of 20% sodium carbonate and OD was recorded at 750 nm. Total phenols were calculated as µg gallic acid/gfw.

2.6 Reducing ability using FRAP assay

FRAP (Ferric ion reducing ability of plasma) assay was conducted to determine the reducing power of peppers as described by Benzie and Strain [25] with few modifications. Briefly, the FRAP reagent was prepared by mixing 300 mM acetate buffer, 10 mM TPTZ (prepared in 40 mM HCL) and 20 mM ferric chloride in the ratio of 10:1:1. Ethanolic extract was prepared by homogenizing 2 g of pepper fruits in 10 ml of absolute ethanol. 100 µl of this extract was mixed with 3 ml FRAP reagent and kept in dark for 30 min. Absorbance was recorded at 593 nm in UV–Vis spectrophotometer and reducing capacity was determined as µmol trolox/g.

2.7 CUPRAC assay

CUPRAC (Cupric ion reducing antioxidant capacity) was determined using protocol of Cekic et al. [26]. 100 µl of sample extract prepared previously for FRAP was mixed with 1 ml neocuproine, 1 ml ammonium acetate, 1 ml copper chloride and 1 ml distilled water and absorbance was taken immediately at 450 nm and CUPRAC was determined as µmol trolox/g.

2.8 Estimation of free radical scavenging by DPPH

The antioxidant activity of fruit extracts was determined by free radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) as described by Bruits and Bucar [27]. 2 g of sample fruits were crushed in 30 ml of absolute methanol and final volume made to 15 ml by concentrating in the water bath. 20 µl of methanolic extract of fruits was added to 2 ml of 0.004% freshly prepared DPPH solution in methanol and allowed to stand for 30 min at room temperature. The absorbance was recorded at 517 nm and compared to the control (DPPH solution without sample) and scavenging percentage was determined as follows

$$\text{Free radical scavenging \%} = \{(A_{\text{control}} - A_{\text{sample}})/A_{\text{control}}\} \times 100$$

2.9 Statistical analysis

All experiments were performed in triplicates. The data were analyzed by basic statistics and represented as mean ± standard deviation. The data were analyzed using one way analysis of variance (ANOVA) using SPSS version 11.

3 Results and discussion

3.1 Determination of antibacterial activity

A significant zone of inhibition of methanolic extracts of all eight CMS lines and a non CMS variety CW as observed (Table 1). Among four methanolic extracts (10% to 40%), 30% and 40% were observed to be most effective in activating the metabolites against different pathogens. Extracts of three CMS lines i.e. KCS 2A, KCS 3A and AVPP 9907-S were found most effective in combating the bacterial growth of *Staphylococcus aureus* followed by *Yersinia pestis* and *Bacillus subtilis*. On the other hand, 30% methanolic extract of AVPP0710-S were found more effective than 40% counterparts against *Bacillus subtilis*. 10% and 20% methanolic extracts were also observed to show some inhibition against few bacterial strains such as *Staphylococcus aureus*, *Bacillus subtilis* and *Yersinia* but in majority there was no inhibition at all. California wonder was also seen to inhibit *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus subtilis* and *Yersinia pestis* at different methanolic concentrations. In the present study, *Yersinia pestis* was inhibited by almost all the fruit extracts whereas *Shigella* was found most resistant against many CMS lines of the pepper. Similar results have also been observed by many other researchers using extracts of different types

Table 1 Table showing zone of inhibition of fruit extracts against *Escherichia coli* (EC), *Salmonella typhi* (ST), *Staphylococcus aureus* (SA), *Pseudomonas aeruginosa* (PA), *Shigella dysenteriae* (SD), *Bacillus subtilis* (BC), *Klebsiella pneumoniae* (KP), *Yersinia pestis* (YP) and distilled water (DW)

Line	Extract	Zone of Inhibition (mm)								
			EC	ST	SA	PA	SD	B S	KP	YP
AVPP9908-S	Methanol%	10	0	0	0	0	0	0.4±0	0	0.65±0.21
		20	0.65±0.7	1.45±0.7	1±0.42	0.35±0.49	0	0.5±0.14	0.75±.21	1.1±0.28
		30	0.4±.14	0.9±0.14	0.9±0.42	0.25±0.35	0	0.95±0.07	0.5±.14	0.8±0.28
		40	0	0	0.25±0.07	0	0	0.45±0.07	0	0.3±0.28
	DW	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0.55±0.35	0.5±0.14	0	0.55±0.07
		20	0	0	0	0	0	0.8±0.14	0	0.7±0.14
		30	0.45±0.07	0.75±0.35	0.8±0.28	0.4±0	0	1.2±0.28	0.4±0.14	1.15±0.07
AVPP 0710-S	Methanol%	40	0.85±0.07	1.2±0.42	1.35±0.21	0.65±0.07	0	1.15±0.63	0.65±.07	1.2±0
		DW	0	0	0	0	0	0	0	0
	Methanol%	10	0	0	0.05±0.07	0	0	0.2±0.28	0	0.45±0.07
		20	0	0	0.1±0.14	0	0	0.2±0.28	0	0.6±0.14
		30	0.45±0.07	1.15±0.07	1.1±0.14	0.2±0.28	0	0.65±0.21	0.4±0	1±.14
		40	0.85±0.07	1.45±0.07	1.3±0.14	0.75±0.21	0	1±0.14	0.9±0.14	1.25±0.07
	DW	0	0	0	0	0	0	0	0	0
		10	0	0	0.85±0.35	0	0	0.2±0.28	0.05±0.07	0.3±0.42
AVPP 9907-S	Methanol%	20	0	0	1.35±0.07	0.25±0.21	0	1±0.14	0.1±0	1±0
		30	0.4±0	0.6±0	1.45±0.07	0.45±0.21	0	1.15±0.21	0.35±0.07	1.15±0.07
		40	0.7±0	1.05±0.07	1.45±0.07	0.7±0.05	0	1.35±0.21	0.75±0.07	1.4±0
		DW	0	0	0	0	0	0	0	0
AVPP 9820-S	Methanol%	10	0	0	0.1±0.14	0	0	0.55±0.07	0	0
		20	0	0	0.5±0.70	0	0	0.8±0.28	0	0.6±0.28
		30	0.45±0.07	1.25±0.07	0.8±0.42	0.35±0.07	0	1.1±0.28	0.45±0.07	0.9±0.14
		40	0.6±0	1.5±0	1.05±0.35	0.65±0.07	0	1.15±0.35	0.65±0.07	1.1±0.14
	DW	0	0	0	0	0	0	0	0	0
		10	0	0	0.2±0.28	0	0	0.45±0.21	0	0.6±0.14
		20	0	1±0.07	0.5±0.70	0.05±0.07	0	0.95±0.07	0	0.8±0.14
		30	0.3±0.14	1±0.70	0.5±0.70	0.3±0.28	0	0.95±0.07	0.3±0.14	1.15±0.07
KCS-1A	Methanol%	40	0.75±0.07	1.3±0.70	0.85±0.49	0.55±0.21	0	1.2±0.28	0.6±0.14	1.25±0.07
		DW	0	0	0	0	0	0	0	0
	Methanol%	10	0	0	0	0	0.25±0.35	0.25±0.35	0	0.35±0.49
		20	0	0	0	0	0	0.3±0.28	0	0
		30	0.3±0.14	1.15±0.21	0.9±0.56	0.35±0.07	0.8±0.28	0.75±0.63	0.35±0.21	1.1±0.14
		40	0.6±.14	1.5±0.14	0.95±0.35	0.65±0.07	1.1±0.14	1.4±0.42	0.65±0.07	1.35±0.07
	DW	0	0	0	0	0	0	0	0	0
		10	0	0	0	0	0.35±0.07	0.5±0.05	0	0.5±0.11
KCS-2A	Methanol%	20	0	0.45±0.07	1±0.12	0	0.75±0.07	0.95±0.07	0.1±0.05	0.85±0.21
		30	0.2±0.07	0.85±0.07	1.1±0.14	0.2±0.28	0.85±0.07	1.1±0.14	0.35±0.07	1.05±0.21
		40	0.65±0.07	1.15±0.07	1.2±0.28	0.35±0.21	1.25±0.07	1.3±0.28	0.65±0.07	1.25±0.21
		DW	0	0	0	0	0	0	0	0
KCS-3A	Methanol%	10	0	0	0	0	0	0.3±0.42	0	0.3±0.42
		20	0	0	0	0	0	0	0	0.4±0.56
		30	0	0	0	0	0	0	0	0.46±0.12
		40	0	0.65±0.21	1.1±0	0	0	0	0	0.55±0.21
	DW	0	0	0	0	0	0	0	0	0
		1.1	1.2	1.2	1.2	1.3	1.4	1.1	1.2	1.2
		1.2	1.1	1.3	1.3	1.2	1.1	1.1	1.3	1.3

of pepper and other medicinal plants [28–31]. Plant secondary metabolites such as phenols act as antimicrobial agents and inhibit the growth of gram positive and gram negative bacteria. The efficiency of inhibition depends on the chemical composition, concentration and structure of antimicrobial agents [7]. Methanol extract has been found to be effective in solubilising or activating the antibacterial agents from fruits or various plant parts [28, 32]. Some researchers have reported antimicrobial activity in sterile water extracts but the activity is quite lower as compared to the methanol extracts [21, 22]. The role of solvents in extraction of various metabolites have been well documented by other researchers also [19, 33].

3.2 Determination of total phenolic content

A significant amount of total phenolic content and antioxidant capacities was observed among different CMS lines and a non CMS variety (California wonder) of *Capsicum annum* (Table 2). The total phenolic content ranged from 594.99 to 1710.13 µg gallic acid/gfw in all studied lines. Highest phenolic content was found in AVPP0710-S (1690.48 ± 19.65 13 µg gallic acid/gfw) and lowest in KCS 2A (621.63 ± 26.64 µg gallic acid/gfw). They are the most bountiful antioxidants available in the human food, [34, 35]. Saha et al. [36] have also reported phenols in *Capsicum annum* in the range of 1012–4135 µg gallic acid/g FW. Phenols present in the fruit extract donate hydrogen ion to ferric ions converting them to stable, less reactive ferrous form [37]. This action helps the human body in fighting against oxidative stress which leads to various degenerative processes [17].

Phenolic compounds include a wide range of bioactive natural compounds that are extensively utilized for medical purposes. These compounds, as bioactive molecules, play an important role in enhancing antibiotic activity against resistant pathogens through various mechanisms

[38–40]. In previous studies, the antibacterial activity of *Scutellaria baicalensis* extract has been reported [41]. The antimicrobial activities of different extracts of *S. litwinowii* have been screened against the standard strains of *S. aureus*, *Bacillus cereus*, *P. aeruginosa*, *E. coli* and *C. albicans*. The results of this investigation indicated that extracts obtained from the aerial parts of *S. litwinowii* possessed antioxidant and antimicrobial properties [42]. Many other researchers have also reported good amount of phenolic acids in capsicum cultivars [17–19].

3.3 Reducing ability using FRAP assay

Ferric reducing ability of plasma (FRAP) ranged from 0.9–3.12 µmol trolox/g among CMS cultivars. It was found highest in AVPP0710-S with 2.77 ± 0.35 µmol trolox/g as genotype mean and lowest in AVPP9908-S with genotype mean of 1.06 ± 0.16 µmol trolox/g. Bogusz et al. [18] have also reported high FRAP activity in *C. frutescens*, *C. chinense* and *C. Baccatum*. This assay is based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}) by donation of electrons from antioxidants. The antioxidant potential of plant extract depends upon their reducing power [43] which in turn depends on presence of various metabolites [44]. The phenolic acids have been advocated to offer resistance against various oxidative stresses by scavenging free radicals, inhibiting lipid peroxidation etc. [45–47].

3.4 CUPRAC assay

Copper reducing antioxidant capacity (CUPRAC) ranged from 0.7–11.22 µmol trolox/g with highest in AVPP9820-S (11.06 ± 0.16) and lowest in KCS 1A (0.81 ± 0.11). This activity is based on the reduction of cupric ions (Cu^{2+}) to cuprous ions (Cu^{1+}) by the antioxidant agents present in the extracts. A chelating agent, neocuproine, aids in the above reaction [48]. CUPRAC assay has been employed

Table 2 Mean values and range of phenolic content, CUPRAC, FRAP and DPPH activity of various CMS lines of pepper

Pepper line	Phenolic content (µg gallic acid/gfw)	FRAP (µmol trolox/g)	CUPRAC (µmol trolox/g)	DPPH (%)
AVPP9908-S	725.02 ± 14.19	1.06 ± 0.16	8.55 ± 0.9	32.94 ± 3.61
AVPP 0516-S	952.99 ± 15.77	2.14 ± 0.28	8.41 ± 0.17	14.24 ± 2.71
AVPP 0710-S	1690.48 ± 19.65	2.77 ± 0.35	1.15 ± 0.09	21.28 ± 2.10
AVPP 9907-S	1534.50 ± 20.18	1.53 ± 0.21	1.78 ± 0.19	16.15 ± 2.44
AVPP 9820-S	1499.65 ± 50.76	1.35 ± 0.19	11.06 ± 0.16	11.64 ± 1.73
KCS 1A	1062.61 ± 31.09	1.49 ± 0.24	0.81 ± 0.11	21.80 ± 2.27
KCS 2A	621.63 ± 26.64	1.42 ± 0.28	1.09 ± 0.16	30.27 ± 3.27
KCS 3A	1478.23 ± 46.11	1.31 ± 0.27	4.08 ± 0.15	27.66 ± 3.44
California wonder (variety)	1159.18 ± 38.72	1.47 ± 0.19	1.25 ± 0.09	11.11 ± 1.52
Range	594.99–1710.13	0.9–3.12	0.7–11.22	9.91–36.55

for antioxidant detection in human serum, food and plant extracts [49–52].

3.5 Estimation of free radical scavenging by DPPH

Free radical scavenging DPPH activity ranged from 9.91–36.55% with highest in AVPP 9980-S and lowest in AVPP 9820-S with $32.94 \pm 3.61\%$ and $11.64 \pm 1.73\%$ as their genotype mean respectively. A good amount of scavenging activity has been shown in three capsicum cultivars by Bogusz et al. [18]. CUPRAC, FRAP and DPPH assays analyze the antioxidant potential of fruits and vegetables. DPPH free radical scavenging antioxidant activity depends on the level of phenols in fruits [53, 54] and also on the extracting solvents [55]. More the phenolic content, higher is the antioxidant activity.

4 Conclusion

Over the years, the use of natural compounds derived from microbial, animals, or plants have been seeking the attention of many researchers. In the present investigation, the fruit extract of cytoplasmic male sterile lines of pepper have been found to inhibit the growth of potent bacterial pathogens on growth media. They also have revealed significant antioxidant activities which can help give a boost for consumption of good volume of peppers. The present finding advocate the use of pepper fruits as functional foods which would definitely aid in maintenance of good human health owing to their high antibacterial and antioxidant activities. Moreover, the cytoplasmic male sterile lines could be used in the production of superior pepper varieties in future, consequently would help strengthen the economic status and increase the livelihood security of the farmers.

Today, the huge population in the world to a great extent depend upon agricultural products to meet out the continuous demand which would have serious impact on achieving the sustainable development of agriculture in the coming years. In this direction, this paper may be an important step to achieve sustainable agricultural development in terms of developing value added plant product.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest in this study.

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