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Chemical composition, total phenolic and flavonoid contents, and *in vitro* antimicrobial and antioxidant activities of crude extracts from red chilli seeds (*Capsicum frutescens* L.)

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Abstract

The objectives of present study were to assess the antimicrobial and antioxidant potential of *Capsicum frutescens* L. seeds and to characterize the chemical constituents of the crude extracts. The *n*-hexane and chloroform extracts were analyzed using gas chromatography–mass spectroscopy (GC–MS), which showed the presence of many biologically important volatile constituents, including heterocyclic compounds, *β*-diketones, hydrocarbons, long chain aliphatic carboxylic acids, and their derivatives, such as esters, hydroxy ester, and aromatic compounds. The amounts of the total phenolic content and the total flavonoid content in same the extracts were in the ranges of 7.95–26.15 gallic acid equivalents (GAE mg/g) and 4.64–12.84 rutin equivalents (RU mg/g) of dry weight of extract, respectively. In the determination of the *in vitro* antimicrobial activity, seed extracts prevented the growth of most of the tested pathogens by forming significant inhibition zones. The inhibitory activity was especially remarkable (inhibition zone $\geq$ 13 mm) against *Pesudomaonas aeruginosa*, *Klebsilla pneumonae*, *Staphylococcus aureus* and *Candida albicans*. During the evaluation of the *in vitro* antioxidant activity *via* DPPH assay, *n*-hexane and chloroform extracts showed 26.9% and 30.9% free radical scavenging abilities, respectively, at the concentration of 1 mg/mL. Considering these results, *C. frutescens* seeds can be used as a source of novel antimicrobial and antioxidant compounds.

Keywords: *Capsicum frutescens* L.; GC–MS; Total phenolic content; Total flavonoid content; Antimicrobial activity; Antioxidant activity

1. Introduction

Capsicum peppers are among the oldest cultivated plants in the world [1]. This genus is indigenous to Central and South America from pre-Colombian times and is in the nightshade family Solanaceae. Presently, this genus is believed to consist of 27 species, five of which are domesticated and used as fresh vegetables and spices, along with approximately 3000 varieties [2]. Widespread

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geographic distribution of *Capsicum annuum* and *Capsicum frutescens* from the New World to other continents occurred in the sixteenth century *via* Spanish and Portuguese traders; soon afterwards, they became an integral part of food habits of several countries, including India.

The dried ripened red pod of *C. frutescens* is known to offer the pepper, which is used as a spice to flavour dishes worldwide. In addition to acting as a flavouring and colouring agent, this fruit also has ethnomedicinal prestige and is used to treat a variety of human ailments. Red chilli has been used as an alternative medicine for the treatment of inflammation, diabetes, low back pain and acute tonsillitis [3–5]. Moreover, capsicum plaster containing powdered capsicum and capsicum tincture has been used in Korean hand acupuncture to reduce postoperative nausea, vomiting and sore throat [6,7]. Chilli was an important plant in traditional Mayan medicine to treat various ailments, such as sore throat, earache and skin care [8].

The substances responsible for the pungency of *C. frutescens* pods are the capsaicinoids alkaloids, which are known for their pharmacological, neurological and dietetic effectiveness. These substances have significant antibiotic activity and the ability to reduce the cholesterol level in blood when used at low levels in the regular diet [9].

Although numerous studies have been reported in the literature to justify the medicinal importance of chilli peppers in food, it is notable that the chemical composition and extent of different bioactivities vary considerably with the species and cultivar investigated, along with the extraction conditions used in the experiments. Central India, especially the Malwa region of Madhya Pradesh, is well known for its hot and spicy cuisine; however, to the best of our knowledge, no study has been performed on the locally grown cultivar of *C. frutescence* to understand the role of red chillies in maintaining public health. Thus, in continuation of our work on the identification of pharmacologically active constituents from locally grown plants [10–12], the present study was planned. The goals of the present study on the seeds of dried pods of *C. frutescens* were the following: (i) to investigate the chemical composition of comparatively less explored low polar *n*-hexane and chloroform extracts, (ii) to evaluate its *in vitro* antimicrobial activity against common food borne pathogens, (iii) to determine the total phenolic and flavonoid content, and (iv) to estimate the antioxidant activity by DPPH radical scavenging assay.

2. Materials and methods

2.1. Chemicals

The organic solvents used in the experiments were of analytical grade and purchased from Qualigen Chemicals, India. The Muller Hinton agar culture medium, nutrient agar, Sabouraud dextrose agar medium and antibiotics discs used in study were procured from Himedia Laboratories Pvt. Ltd., Mumbai, India. The other chemicals used were of analytical grade and obtained from Merck, India.

2.2. Plant sample

Air-dried pods of *C. frutescens* were collected from the local spice market of District Ratlam, India. Plant samples were duly authenticated by Dr. V. Gupta, Taxonomist, Department of Botany, Govt. Arts & Science College, Vikram University, Ratlam, India, where a voucher specimen was also deposited for future reference.

2.3. Extraction procedure

The plant samples were washed several times with tap water and finally with distilled water to remove dust. The samples were dried under shade at room temperature. The seeds were separated from dried pods by crumbling and then screening. The shade dried seeds were further ground by means of a mechanical blender (Bajaj GX10, India) to fine powder. One hundred grams of the seed powder was sequentially extracted for 3 days with each solvent *n*-hexane (500 mL $\times$3) and chloroform (500 mL $\times$3) using a Soxhlet apparatus over a water bath. The extracts obtained were filtered through Whatman No. 1 filter paper and then evaporated to dryness by using a rotary evaporator (Buchi, Switzerland). The final crude extracts were collected in an airtight container and then refrigerated at $4 \pm 2\,^{\circ}C$ until further use.

2.4. Gas chromatography–mass spectrometry (GC–MS) analysis

GC–MS analysis of extracts was performed using a Hewlett-Packard 5890 gas chromatograph coupled to a Hewlett-Packard 5970A Mass spectrometer detector (MSD). Compounds were separated on a fused silica capillary column with a column length of 25.0 m, an internal diameter of 0.32 mm and a film thickness of 0.25 μm. The temperature of the injector was 250 °C, and 2 μL of

sample was injected in the split mode with a split ratio of 30:1. Helium (He) was used as a carrier gas, and the flow rate of the gas was 1.1 mL/min. The temperature programme was as follows: initial temperature of 60 °C held for 2 min, followed by the ramping up of the temperature at a rate of 4 °C/min up to 220 °C, which was held for 20 min. The temperature of the MSD transfer line was 300 °C. For mass spectra determination, the MSD was operated in electron ionization (EI) mode, with an ionization energy of 70 eV, while the mass range scanned was 10–400 *m/z*. The temperature of the ion source was 230 °C, and that of the MS quadrupole was 150 °C. The name, molecular weight and structure of the components of the test materials were ascertained by comparing the mass spectra with the known compounds using an automated library search on the NIST MS Search programme 2.0g (National Institute of Standards and Technology, Gaithersburg, MD, USA) [13].

2.5. Determination of the total phenolic contents (TPC)

The concentration of the phenolics in the plant extracts was determined using the Folin Ciocalteau assay [14]. In brief, 1 mL of extracts (1 mg/mL in methanol) or a standard solution of gallic acid (20, 40, 60, 80 and 100 mg/L) was added to a 25-mL volumetric flask containing 9 mL of distilled water. One millilitre of Folin Ciocalteau reagent was added to the mixture and then shaken. After 5 min, 10 mL of a 7% Na_2CO_3 solution was added to the mixture. The final volume was brought up to 25 mL by adding double distilled water and then mixed. After 90 min of incubation at room temperature (23 ± 2 °C), the absorbance was determined against a blank at 750 nm (UV-2550, Shimadzu spectrophotometer). The total phenolic content was calculated using a calibration curve for gallic acid ($R^2 = 0.981$). The results were expressed as the gallic acid equivalent per gram of dry weight of extract (mg of GAE/g of extract). All samples were analyzed in triplicate.

2.6. Determination of the total flavonoid contents (TFC)

The total flavonoid content of the crude extracts was determined using the aluminium chloride colorimetric method [15]. In brief, 5 mL of the extract (1 mg/mL in methanol) or a standard solution of rutin (20, 40, 60, 80 and 100 mg/L) were mixed with 5 mL of 2% $AlCl_3$ in methanol. After a 60-min incubation at room temperature (23 ± 2 °C), the absorbance against blank was determined at 510 nm (UV-2550, Shimadzu spectrophotometer). The total flavonoid content was calculated using a calibration curve for rutin ($R^2 = 0.985$). The results were expressed as the rutin equivalent per gram of dry weight of extract (mg of RU/g of extract). All samples were analyzed in triplicate.

2.7. Evaluation of the antioxidant activity via DPPH radical scavenging assay

The ability of the plant extracts to scavenge DPPH free radicals was assessed using the standard method [16]. Aliquots (2 mL) of various concentrations (62.5–1000 μg/mL) of the plant samples were added to 2 mL of a 0.004% methanolic solution of DPPH. After an incubation period of 30 min in darkness at room temperature (23 ± 2 °C), the absorbance was recorded against a blank at 517 nm (UV-2550, Shimadzu spectrophotometer). Absorption of a blank sample containing the same amount of methanol and DPPH solution acted as the control.

$$\%\ \text{DPPH}\quad \text{inhibition}\quad \text{activity} = [A_0 - A_1/A_0] \times 100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample. Ascorbic acid was used as a positive control. Samples were analyzed in triplicate.

2.8. Antimicrobial activity

2.8.1. Test microorganisms

In the present study, the antibacterial activity of the plant extracts were tested against Gram positive *Bacillus cereus, Staphylococcus aureus, and S. aureus* MRSA and Gram negative *Escherichia coli, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Proteus vulgaris* bacteria, while the antifungal activity was evaluated against *Candida albicans, Candida krusei, Alternaria alternata, Aspergillus flavus, and Aspergillus niger.* All of the microorganisms used in the present study were clinical isolates, obtained from Department of Microbiology, RD Gardi Medical College, Ujjain, India. The isolates were identified in the laboratory using the standard biochemical tests. The bacterial strains were subcultured on nutrient agar at 37 °C, while the fungal strains were subcultured on Sabouraud dextrose agar at 28 °C. All of the microorganisms were preserved at 4 ± 2 °C prior to use.

2.8.2. Antibacterial assay

The antibacterial activity of plant extracts was tested using the well diffusion method [17]. All samples (dry residue) were dissolved in 10% sterile dimethyl sulfoxide to a final concentration of 20 mg/mL. Petri

dishes were prepared with 20 mL of Muller Hinton agar medium and sterilized by autoclaving at 121 °C for 15 min. After inoculation, the Petri dishes were dried for 15 min. Wells of 10-mm diameter, suitably distant, were punched off and subsequently filled with 100 μL of extract. The plates were incubated at 30 °C for 1 h to permit good diffusion before incubation at 37 ± 2 °C for 24 h. Antibiotic ciprofloxacin (5 μg/mL) was used as a positive control, while 10% DMSO was used as negative control. The zone of inhibition (excluding well diameter) that appeared after 24 h was measured (in mm) as a property of the antibacterial activity of the extracts. All of the tests were performed in triplicate.

2.8.3. Antifungal assay

The antifungal activity of plant extracts was determined by the disc diffusion method using Sabouraud dextrose agar medium [18]. To test the antifungal activity, Whatman no. 1 filter paper discs (4-mm diameter), each of which were impregnated with 20 μL of extract (100 mg/mL in 10% DMSO), were placed aseptically on the inoculated agar plates. Plates were incubated at 30 °C for 1 h to permit good diffusion before incubation at 37 ± 2 °C for 48 h. Standard antifungal agent nystatin (100 units/disc) was used as the positive control, while 10% DMSO was used as the negative control. The zone of inhibition (excluding disc diameter), which appeared after 48 h, was measured (in mm) as a property of the antifungal activity of extracts. All tests were performed in triplicate.

2.9. Statistical analysis

The samples were analyzed individually in triplicate, and the data were reported as the mean ± standard deviation. One-way analyses of variance (ANOVA) with the *post hoc* Tukey HSD test was used to compare any significant differences between the extracts and the control at the 5% significance level ($p < 0.05$).

3. Results and discussion

3.1. Chemical composition of the solvent extracts

The GC–MS analysis of *n*-hexane and chloroform extracts of the seeds of *C. frutescens* revealed the presence of a total of 29 compounds (Table 1, Figs. 1 and 2) representing different classes. The major constituents in the *n*-hexane extract were found to be Octadecadienal (*Z*), 3-Carene, Hexadecanoic acid, Tetracosane, Heptadec-8-ene-2,4-dione, 2(3H)-Furanone,dihydro-5-(2-octenyl)-, (*Z*) and Hexadec-8-ene-2,4-dione, while in the chloroform extract, Hexadecanoic acid, 9,12-Octadeca dienoic acid, 1-Hexadecene and 5-Eicosene, (*E*) were found to be present in major quantities. Five compounds, Octadecane, Eicosane, Docosane, 9,12-Octadecadienoic acid, methyl ester and Hexadecanoic acid, were found to be commonly present in both of the extracts. The compounds in GC–MS analysis were identified on the basis of comparison of the retention time and mass spectra with the references present in the NIST mass spectral library.

In a previous study, the most abundant constituents present in the volatile fractions of three varieties of chilli peppers were esters and alcohols in the malagueta chilli pepper (*C. frutescens*), monoterpenes and aldehydes in the dedo-de-moça chilli pepper (*C. baccatum* var. pendulum), and esters and sesquiterpenes in the murupi chilli pepper (*C. chinense*) [19]. Analysis of the fruit volatiles of three Colombian cultivars of *C. frutescens* (cesari amarillo, cesari morado and pajarito) identified isohexyl isohexanoate, isohexyl isovalerate and isohexyl 2-methylbutyrate as the major compounds in cesari amarillo and cesari morado cultivars, while isohexyl isovalerate and hexyl isovalerate were the major compounds in the pajarito cultivar [20]. On isolating the volatile constituents of 16 capsicum accessions, esters and terpenoids were found to be the major compounds, along with a minor presence of nitrogen and sulphur compounds, phenol derivatives, norcarotenoids, lipoxygenase derivatives, carbonyls, alcohols, and hydrocarbons [21]. The volatile composition of *C. frutescens* fruit significantly varies with the variety studied [22] as well as the ripening stage [23].

In the present study, most of the identified volatile compounds were hydrocarbons, fatty acids, fatty esters, and some novel constituents, such as β-diketones. Many of these identified compounds have already been reported to be pharmacologically active. For example, 3-carene has shown anabolic activity in bone metabolism [24], hexadecanoic acid is known to have potential antibacterial and antifungal activities [25,26]; long-chain unsaturated fatty acids, such as linoleic acid, also show antibacterial activity and are the key ingredients of antimicrobial food additives and some antibacterial herbs [27]; unsaturated fatty acids are also suggested to be responsible for the anti-inflammatory activity [28]; and hexadecanoic acid, methyl ester and 9,12-octadecadienoic acid (*Z*,*Z*)-, methyl ester have shown antioxidant and anticancer properties, respectively [29,30]. The presence of such bioactive phytoconstituents and pungent capsaicinoids in the seeds of *C. frutescens* are thought to play a major

Table 1
Chemical composition of crude extracts of *C. frutescens* seeds analyzed by GC–MS.

SI no.	Name of compounds	MW	Formula	Retention time	Peak area (%)
n-Hexane crude extract					
1	3-Carene	136	$C_{10}H_{16}$	5.32	14.88
2	Hexadecane	226	$C_{16}H_{34}$	12.25	2.41
3	Octadecane	254	$C_{18}H_{38}$	14.34	1.55
4	Eicosane	282	$C_{20}H_{42}$	16.16	1.25
5	10-Heneicosene	294	$C_{21}H_{42}$	16.98	0.42
6	Docosane	310	$C_{22}H_{46}$	17.60	0.93
7	9,12-Octadecadienoic acid, methyl ester	294	$C_{19}H_{34}O_2$	18.10	0.84
8	Hexadecanoic acid	256	$C_{16}H_{32}O_2$	18.52	13.76
9	1-Docosene	308	$C_{22}H_{44}$	19.92	0.69
10	9,12-Octadecadienal	264	$C_{18}H_{32}O$	20.97	33.54
11	Heptadec-8-ene-2,4-dione	266	$C_{17}H_{30}O_2$	21.59	7.60
12	Tetracosane	338	$C_{24}H_{50}$	22.19	8.83
13	2(3H)-Furanone,dihydro-5(2-octenyl)	196	$C_{12}H_{20}O_2$	23.60	6.09
14	Hexadec-8-ene-2,4-dione	252	$C_{16}H_{28}O_2$	25.25	6.05
15	Pentadec-8-ene-2,4-dione	238	$C_{15}H_{26}O_2$	27.51	1.17
Chloroform crude extract					
1	1-Phenyloctane	190	$C_{14}H_{22}$	12.41	2.18
2	Phenol,2,4-bis(1,1-dimethyethyl)	206	$C_{14}H_{22}O$	13.65	5.48
3	Octadecane	254	$C_{18}H_{38}$	14.57	3.93
4	1-Hexadecene	224	$C_{16}H_{32}$	14.71	8.76
5	Eicosane	282	$C_{20}H_{42}$	16.41	2.96
6	5-Eicosene	280	$C_{20}H_{40}$	16.54	6.54
7	Hexadecanoic acid, ethyl ester	284	$C_{18}H_{36}O_2$	17.27	3.49
8	Docosane	310	$C_{22}H_{46}$	17.84	2.37
9	1-Dodecene	168	$C_{12}H_{24}$	17.91	4.29
10	9-Hexadecenoic acid, methyl ester	268	$C_{17}H_{32}O_2$	18.10	2.13
11	9,12-Octadecadienoic acid, methyl ester	294	$C_{19}H_{34}O_2$	18.14	4.16
12	Palmitic acid	256	$C_{16}H_{32}O_2$	18.39	25.31
13	Linoleic acid	280	$C_{18}H_{32}O_2$	20.40	23.28
14	9,12-Octadecadienoic acid-2-hydoxy-1-(hydroxyl methyl)ethyl ester	354	$C_{21}H_{38}O_4$	20.66	5.15

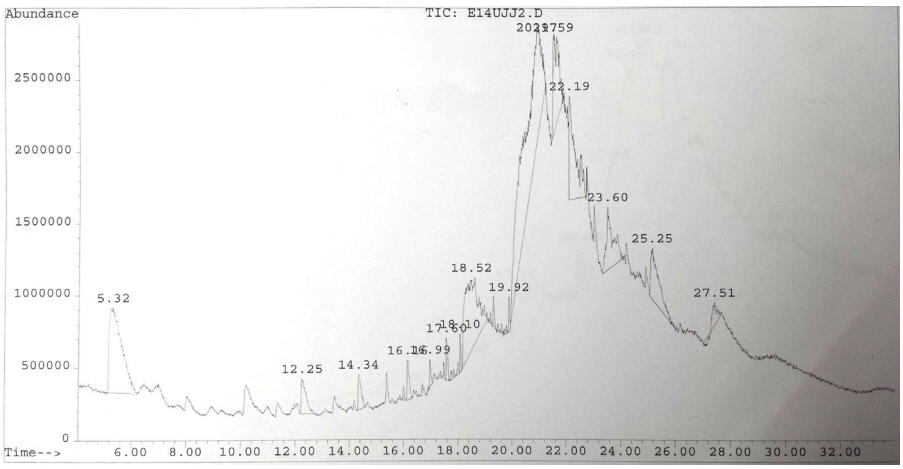

Fig. 1. GC–MS chromatogram of the *n*-hexane extract of the seeds of *C. frutescens*.

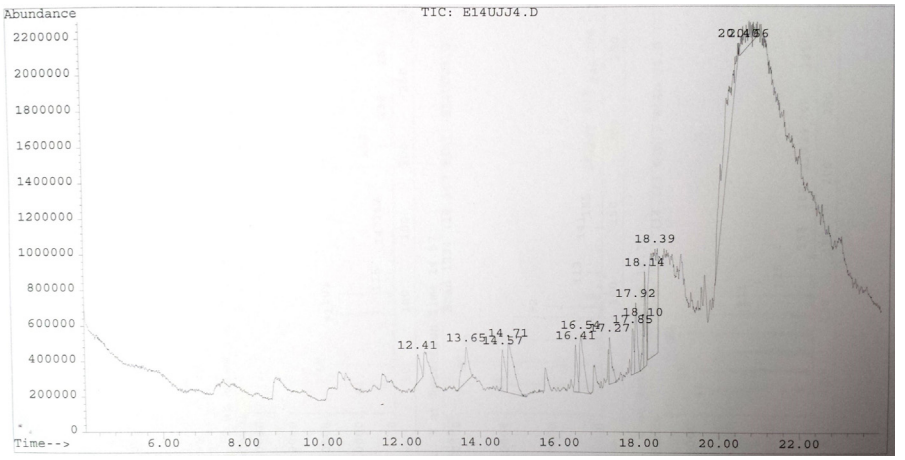

Fig. 2. GC–MS chromatogram of the chloroform extract of the seeds of *C. frutescens*.

role in the well-known pharmacological activities shown by red chillies.

3.2. Antimicrobial activity

The results of the antimicrobial activity of *n*-Hexane and chloroform extracts of the seeds of *C. frutescens* examined against different pathogenic microorganisms are presented in Table 2. *n*-Hexane extract showed significant activity (>10 mm) against *P. aeruginosa* (14 mm), *K. pneumonae* (12 mm), *S. aureus* (14 mm), *C. albicans* (13 mm), *C. krusei* (12 mm), *A. alternata* (11 mm) and *A. niger* (16 mm). Although the inhibitory activity exhibited by the chloroform extract was significant against *P. aeruginosa* (13 mm), *K. pneumonae* (13 mm), *S. aureus* (15 mm), *C. albicans* (20 mm) and *C. krusei* (14 mm), this extract could not inhibit the growth of the tested yeasts. Both the extracts were less active against *S. typhi*, *P. vulgaris* and *S. aureus* MRSA. Furthermore, he antibacterial and antifungal activities of plant extracts were compared with standard antibiotics ciprofloxacin and nystatin, respectively (Table 2). Against *E. coli*, *P. aeruginosa*, *K. pneumonae*, *S. aureus* and *C. krusei*, extracts of both *n*-hexane and chloroform showed better activity than standard drugs, while against *C. albicans*, the chloroform extract produced better results.

In recent years, research has moved in the direction of replacing toxic synthetic food preservatives with natural alternatives. Red pepper can play this role very well, as this spice is part of most cuisines worldwide. In previous studies, the antimicrobial activity of highly polar ethanol, methanol and aqueous extracts of *C. frutescens* pepper has already been reported against a number of microorganisms [31–33]. Even the methanolic extract of *C. frutescens* leaves showed dose-dependent antibacterial activity against *S. aureus*, *K. pneumoniae* and *P. aeruginosa* [34]. Aqueous extracts of the leaf and fruit of *C. frutescens* exhibited potential to prevent growth of seed-borne fungi [35], while chilli juice and oleoresins inhibited the growth of wood discolouring fungi [36].

In the present study, broad spectrum activity exhibited by low polar *n*-hexane and chloroform extracts confirmed the presence of even less polar antimicrobial compounds in chilli seeds. This significant antimicrobial activity may be attributed to the compounds identified in the GC–MS analysis. Isolation of these antimicrobial agents from capsicum fruit can lead to an important change in the area of food safety and can be used in the prevention of food-borne diseases.

3.3. Percentage yield and total phenolic content and total flavonoid content

Comparing both of the extracts of *C. frutescens* seeds, the extraction yield was almost double with *n*-hexane than with chloroform (Table 3). The amount of the total phenolic content and total flavonoid content ranged 7.95–26.15 gallic acid equivalents (GAE mg/g) and 4.64–12.84 rutin equivalents (RU mg/g) of dry weight of extract, respectively (Table 3). The efficiency of the solvents to extract phenolic and flavonoid compounds was in the order of *n*-hexane > chloroform. Total phenolic content measured in the *n*-hexane extract in the present study was higher than the value (15.57–16.22 GAE mg/g) reported for the ethanolic extract of the seeds of *C. frutescens* grown in India [37].

Phenolic compounds are secondary metabolites that can act as antioxidants due to their ability to donate

Table 2
Antimicrobial activity of crude extracts of *C. frutescens* seeds (inhibition zone in mm, excluding well/disc diameter).

Name of microorganism	*n*-Hexane extract	Chloroform extract	Positive control
Escherichia coli	10 ± 0.52^{a}	08 ± 1.32^{ac}	06 ± 0.34^{bc}
Salmonella typhi	5 ± 0.81^{a}	06 ± 0.95^{a}	18 ± 1.3^{b}
Proteus vulgaris	5.5 ± 1.0^{a}	7 ± 0.5^{a}	16 ± 1.5^{b}
Pseudomonas aeruginosa	14 ± 1.3^{a}	13 ± 1.7^{ac}	10 ± 0.86^{bc}
Klebsilla pneumonae	12 ± 0.5^{a}	13 ± 2.0^{a}	10 ± 2.59^{a}
Bacillus cereus	10 ± 1.0^{a}	10 ± 1.8^{ac}	19 ± 1.5^{bd}
Staphylococcus aureus	14 ± 0.5^{a}	15 ± 1.32^{ac}	8 ± 0.62^{bd}
Staphylococcus aureus MRSA	5 ± 0.2^{a}	0	13 ± 0.5^{b}
Candida albicans	13 ± 1.74^{a}	20 ± 0.43^{b}	18 ± 0.90^{b}
Candida krusei	12 ± 1.03^{a}	14 ± 1.51^{ab}	11 ± 0.86^{ac}
Alternaria alternata	11 ± 0.62^{a}	0	14 ± 0.45^{b}
Aspergillus flavus	0	0	15 ± 0.33
Aspergillus niger	16 ± 1.0^{a}	0	17 ± 0.50^{a}

Data are expressed as the mean ± standard deviation of three separate experiments ($p < 0.05$); Different letters in superscript indicate significant differences among different extracts and control according to Tukey HSD test. Positive control for antibacterial activity ciprofloxacin (5 μg/mL) and for antifungal activity nystatin (100 units/disc).

Table 3
Percentage yield, total phenolic content (TPC) and total flavonoid content (TFC) of crude extracts of *C. frutescens* seeds.

Extract	% Yield	TPC (GAE mg/g) of dry weight of extract	TFC (RU mg/g) of dry weight of extract
n-Hexane	11.2	26.15 ± 0.76	12.84 ± 0.22
Chloroform	5.4	7.95 ± 0.35	4.64 ± 0.54

TPC and TFC values are mean ± standard deviation of three separate experiments ($p < 0.05$).

hydrogen, quench singlet oxygen and act as metal chelators [38]. Flavonoids consist of a large group of polyphenolic compounds, have a benzo-γ-pyrone structure, and provide benefits in multiple ways to the plant producing them [29]. Flavonoids are found to be very useful as an antimicrobial agent, a mitochondrial adhesion inhibitor, an antiulcer agent, an antiarthritic agent, an antiangiogenic agent, and an anticancer agent [39]. It has been confirmed that consumption of phenolic rich foods or beverages prevent diseases, such as cancer, heart disease, inflammation, arthritis, immune related diseases, neurodegenerative diseases and diabetes [40]. In the present study, the presence of phenolic and flavonoid compounds in red chilli seeds confirm the health benefits associated with it.

3.4. Antioxidant activity by DPPH assay

The *n*-hexane extract exhibited 26.97% DPPH radical scavenging activity at a concentration of 1 mg/mL, while for the chloroform extract at the same concentration, 30.9% DPPH radical scavenging activity was observed (Fig. 3).

The DPPH assay is a very common spectrophotometric method to determine the activity of any antioxidant. The advantage of this method is that the antioxidant activity is measured at ambient temperature, and thus, the risk of the thermal degradation of the molecule tested is eliminated [41]. Free-radical scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation [42]. Although, in the literature, a very high DPPH radical scavenging ability has been reported in capsicum fruit, that report was for highly polar acetonitrile and methanol extracts [43,44]; in the present study, the antioxidant activity determined is of moderate type in comparison to standard ascorbic acid and also exhibits poor correlation with the total

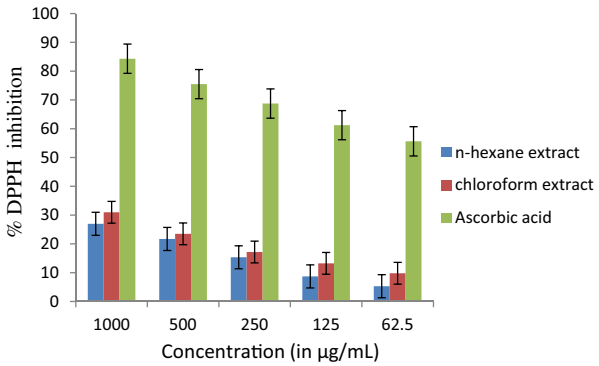

Fig. 3. DPPH radical scavenging ability of the crude extracts of *C. frutescens* seeds.

phenolic content. The results showed the possibility of the presence of non-phenolic antioxidant molecules in chilli seeds and the better ability of polar solvents to extract them.

4. Conclusion

The identification of pharmacologically active compounds and remarkable antimicrobial activity along with moderate antioxidant activity of low polar extracts of seeds of red chillies in the present study justifies the traditional use of red pepper as food preservatives in hot climates and also indicates the tremendous nutraceutical potential of red pepper. In further studies, bioactivity guided isolation from these crude extracts may provide fractions or constituents with high antimicrobial and antioxidant calibres, which can substitute synthetics of equal efficacies.

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