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Antibacterial and Antioxidant Activity of Different Staged Ripened Fruit of *Capsicum annuum* and Its Green Synthesized Silver Nanoparticles

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Abstract
Functional metabolites are believed to possess different bioactivities; thus, three different stages of ripened fruit (green, yellow, red) of *Capsicum annuum* were collected from Chennai, Tamil Nadu, India, and were used in this study. The aqueous extract of *Capsicum annuum* was screened for various phytochemical compounds and subjected to TLC bioautography and antibacterial and antioxidant activities. The aqueous extract of yellow-colored *Capsicum annuum* showed the highest antibacterial activity against *Pseudomonas aeruginosa* and also showed the highest antioxidant activity. Furthermore, silver nanoparticles were synthesized using the aqueous extract and characterized by UV-Vis, FTIR, SEM, and AFM analysis. The silver nanoparticles were investigated for its bactericidal activity. The silver nanoparticles produced using the extract of green-colored *Capsicum annuum* showed the highest bactericidal activity which was evidenced by protein leakage assay.

Keywords *Capsicum annuum* · Bioactivity · Functional metabolites · Silver nanoparticles

1 Introduction

The plants or the plant parts which are used in day-to-day life typically contain bioactive compounds that may be pharmacologically active and exhibit varied biological activities like anti-inflammatory, antifungal, antibacterial, antioxidant, anticancer, and several other pharmacological actions [1, 2]. Phytocompounds are the basic chemical substances in plants which hold the medicinal value. The secondary metabolites of plants produce definite physiological action on the human body. These bioactive molecules include alkaloids, flavonoids, anthocyanin, coumarins, carbohydrates, saponins, triterpenoids, and essential oils [3].

Capsicum annuum is a member of *Solanaceae* family and is believed to be originated from Central and South America [4]. It is the most commonly cultivated dicotyledonous plant [5]. These are available in different colors such as green, yellow, orange, and red as it ripens. These have various pigments in the pericarp [6], such as xanthophylls along with β-carotene, zeaxanthin, violaxanthin, and β cryptoxanthin in yellow-colored variety whereas carotenoids along with Keto carotenoids, capsanthin, capsorubin, and cryptocapsin are responsible for the red color in the ripened pods [7–9]. These pigments can be extracted and used in cosmetics and as non-toxic dyes [10]. It has several medicinal and nutritional values as they are an excellent treasure of natural colors and antioxidant compounds which react with the singlet oxygen and other free radicals, thereby suppressing peroxidation [11]. *Capsicum* sp. contains several phytochemical compounds such as phenolics (flavonoids), carotenoids, alkaloids, vitamin A, vitamin C, and vitamin E [12, 13]. Capsaicin isolated from *Capsicum* has disease preventive properties [14]. Red *Capsicum* sp. is a natural hair growth stimulator and helps in curing hair loss where it improves blood flow which is vital for the proper growth of hair and protection of hair follicles from the effects of dihydrotestosterone (DHT). It can be used to reduce pain [15] and inflammations [16] and treat gastrointestinal problems, skin diseases, and even arthritis [17, 18].

In this study, different stages of ripened fruit, i.e., red-, green-, and yellow-colored *C. annuum*, were taken and their aqueous extracts were screened for phytochemical

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compounds qualitatively and GC-MS was done to detect them. The extracts were also exploited for TLC bioautography analysis, antioxidant assay, and antibacterial activity. Further, the extracts were utilized to produce silver nanoparticles and were characterized using UV-Vis, FTIR, SEM, and AFM. The silver nanoparticles were also checked for their antibacterial activity against *Pseudomonas aeruginosa*.

2 Materials and Methods

2.1 Collection and Extraction of Plant Material

Different stages of ripened fruit, i.e., green, yellow, and red fruits of *C. annuum*, were collected from local markets in Chennai, Tamil Nadu, India. They were washed under running tap water and cut into small pieces. Thirty grams of each colored *Capsicum* sp. pieces was added to 120 ml of distilled water boiled for 45 min at 100 °C and was concentrated to obtain approximately 30 ml of extract. The obtained extracts were stored in airtight tubes at 4 °C till use [19, 20].

2.2 Qualitative Phytochemical Analysis

Qualitative phytochemical analysis for various secondary metabolites such as alkaloids, carbohydrates, glycosides, saponins, phytosterols, phenols, tannins, flavonoids, proteins and amino acids, and diterpenes was done according to the standard protocol by Jamal et al. [21] and Haque et al. [22].

2.3 GC-MS

The bioactive components present in the aqueous extract of different colored *C. annuum* were analyzed by gas chromatography as the condition prescribed earlier [23].

2.4 Separation of Bioactive Compounds by Thin-layer Chromatography

The aqueous extracts of three different *C. annuum* were subjected to thin-layer chromatography by using TLC silica plate (Merck, F245) having acetone and petroleum ether as the mobile phase. TLC plates were exposed to iodine-saturated chamber. Retention factor (Rf) value of separated components was recorded [24].

2.5 TLC Bioautography for Antioxidant Activity

The extracts were subjected for TLC on silica plates having acetone and petroleum ether as the mobile phase. Plates were then dried, sprayed with DPPH (0.004% *w/v* in 95% methanol) [24–26]. The Rf value was determined.

Table 1 Qualitative phytochemical screening of aqueous extracts of *C. annuum*

Phytochemical	Green	Yellow	Red
Alkaloids	++	++	++
Carbohydrates	++	++	++
Glycosides	+	+++	+
Saponins	+	−	−
Phytosterols	++	+	++
Phenols	−	−	−
Tannins	+	−	−
Flavonoids	++	++	++
Proteins and amino acids	++	++	++
Diterpenes	++	++	++

+++ indicates immediate change, ++ indicates change which occurred within 5 min, + indicates change that occurs after 5 min, − indicates no such change

2.6 Antibacterial Activity by Agar Well Diffusion Method

The bactericidal activities of three different extracts were tested against three different micro-organisms namely *Brevibacillus brevis*, *Pseudomonas aeruginosa*, and *Bacillus subtilis* by performing the agar well diffusion assay [27, 28]. After 24 h of incubation, inhibitory zones formed around each disk were measured (cm) and recorded [29]. Water and ciprofloxacin (8 μg) were used as negative and positive control, respectively.

2.7 FRAP Assay

The reducing power of all the three samples was determined by FRAP assay [30].

2.8 Synthesis of Silver Nanoparticles

Silver nitrate was used as a precursor in the synthesis of silver nanoparticles. Five milliliters of aqueous extract was mixed with 25 ml of 3 mM silver nitrate and kept in dark for the synthesis of silver nanoparticles at room temperature for 24 h [31]. After 24 h, the solution was centrifuged at 6000×*g* and

Table 2 Rf value of components separated using different mobile phases

Solvent	Retention factor (Rf)		
	Green	Yellow	Red
Acetone	0.73	0.83	0.76
Petroleum ether	0.09	0.18	0.15

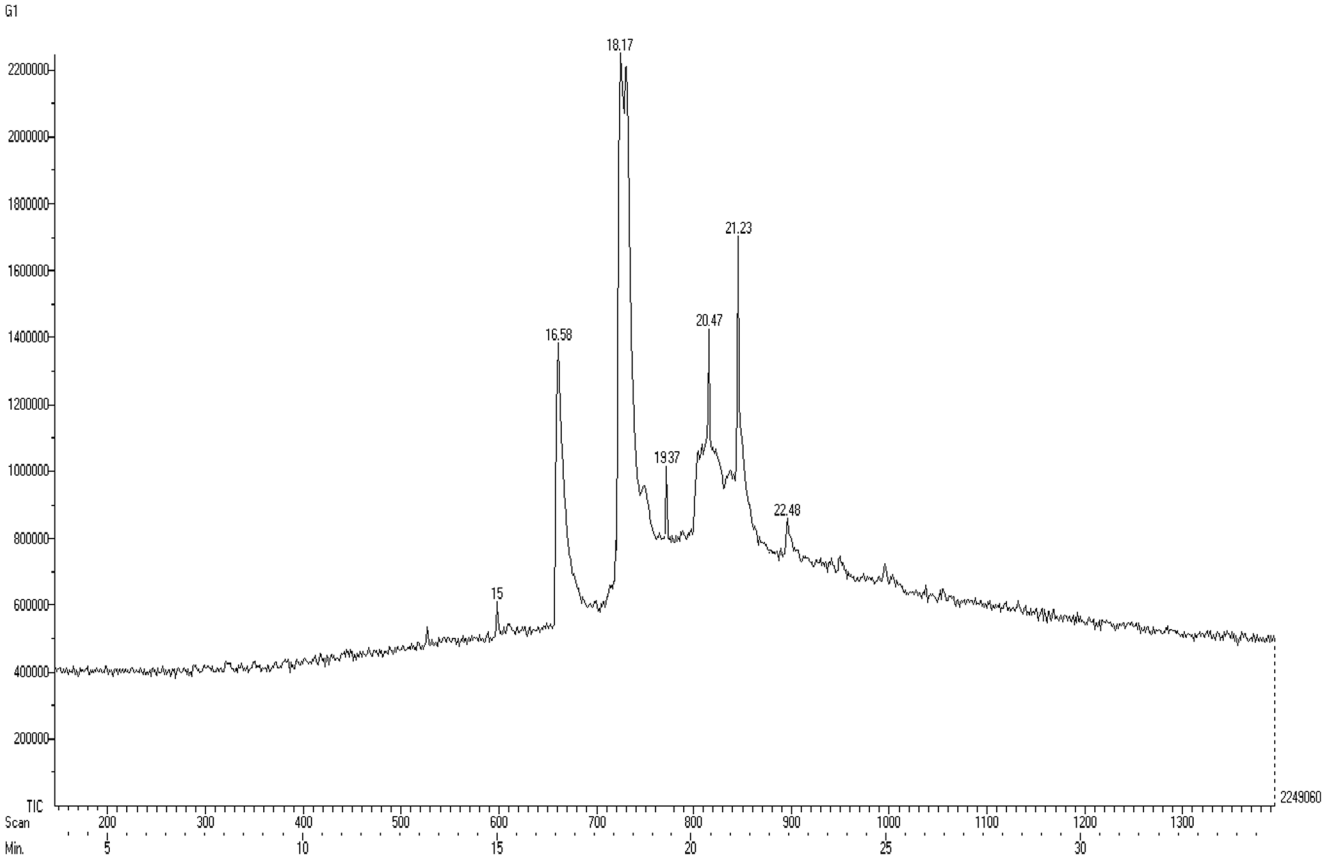

Fig. 1 GC-MS chromatogram of aqueous extracts of green-colored *C. annuum*

the pellet was collected, rewashed with distilled water, lyophilized, and stored in a black container at 4 °C.

2.9 Characterization of Silver Nanoparticles

The reduction of silver to silver nanoparticles was monitored by measuring its absorbance at various wavelengths using UV-Vis spectroscopy (Shimadzu, Japan). The FTIR spectra of synthesized nanoparticles were measured at the wave number region of 400–4000 cm^{-1} [32]. Scanning electron microscopy (SEM) study was done using Zeiss Ultra Plus, Germany, by preparing the sample as a thin film on a carbon-coated copper grid [33]. Atomic force microscopy (AFM) (Bruker, Germany) was also performed.

2.10 Antibacterial Activity of Silver Nanoparticles by Agar Well Diffusion Assay

Antibacterial activity was evaluated using agar well diffusion method [34]. The antibacterial activity of different concentrations of silver nanoparticles (2, 4, 6, and 8 μg/ml) was performed against gram-negative bacteria—*Pseudomonas*

Table 3 Phytoconstituents detected by GC-MS in aqueous extracts of green-colored *C. annuum*

S. No.	R.T	Compound name	Molecular weight (Da)	Molecular formula
1	15	1H pyrazole, 4, 5 dihydro 1 phenyl	146.1891	$C_9H_{10}N_2$
2	16.58	Cyclopentadecanone, 2-hydroxy	240.3816	$C_{15}H_{28}O_2$
3	18.17	Z, E-2-methyl-3, 13-octadecadien-1-ol	280.488	$C_{19}H_{36}O$
4	19.37	4H-1-benzopyran-4-one, 5, 7-dimethoxy-2-phenyl	298.294	$C_{17}H_{14}O_5$
5	20.47	Cyclopropaneoctanoic acid 2-octyl- methyl ester, trans	310.522	$C_{20}H_{38}O_2$
6	21.23	1-Tetradecene, 2-decyl	336.6379	$C_{24}H_{48}$
7	22.48	Flavone	222.239	$C_{15}H_{10}O_2$

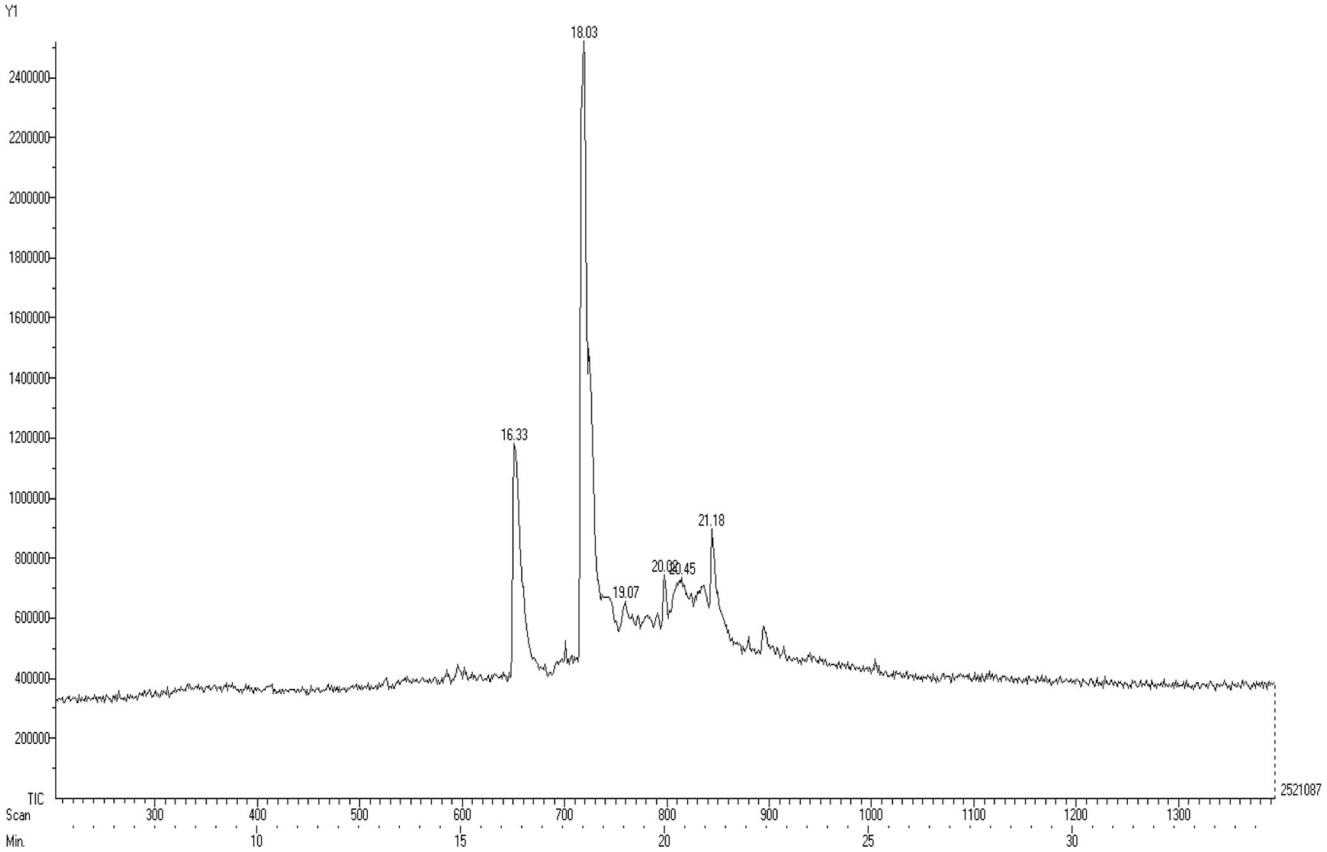

Fig. 2 GC-MS chromatogram of aqueous extracts of yellow-colored *C. annuum*

aeruginosa. Water was used as negative control and ciprofloxacin (8 μg) was used as positive control.

2.11 Minimum Bactericidal Concentration

The lowest concentration of silver nanoparticles that results in a 99.9% reduction in the bacterial cells was determined using broth microdilution method in 96-well plate using the modified protocol followed by Ali et al. [35]. Seventy-two microliters of sterile media was added to each well. Silver nanoparticles in gradient concentration (1, 2, 3, 4, 5, 6, 7, 8 μg/ml) were added in triplicate and made up to 80 μl with sterile distilled water. The standardized inoculum (20 μl) was added in each well to give a final concentration of 100 μl and then incubated at 37 °C for 24 h [36, 37].

2.12 Protein Leakage Assay

The intracellular protein leakage of cells treated with silver nanoparticles was estimated by Bradford's assay. Cells of *Pseudomonas aeruginosa* were treated with silver nanoparticles and then incubated for 24 h. After incubation, supernatant was collected by centrifugation at 6000 rpm for 15 min and protein was estimated by Bradford's assay. The optical density was measured at 620 nm [38–40].

Table 4 Phytoconstituents detected by GC-MS in aqueous extracts of yellow-colored *C. annuum*

S. No.	R.T	Compound name	Molecular weight (Da)	Molecular formula
1	16.33	Methyl dihydrohydnocarpate	268.441	$C_{17}H_{32}O_2$
2	18.03	13, 16-octadecadienoic acid methyl ester	294.479	$C_{19}H_{34}O_2$
3	19.07	4H 1 benzopyran 4 one, 5-hydroxy-7-methoxy-2 (4-methoxyphenyl)-	298.2901	$C_{17}H_{14}O_5$
4	20.02	16-Octadecenoic acid methyl ester	296.4879	$C_{19}H_{36}O_2$
5	20.45	5-Methoxycarbonyltubercidin	324.293	$C_{13}H_{16}N_4O_6$
6	21.18	13-Docosenoic acid methyl ester	352.5943	$C_{23}H_{44}O_2$

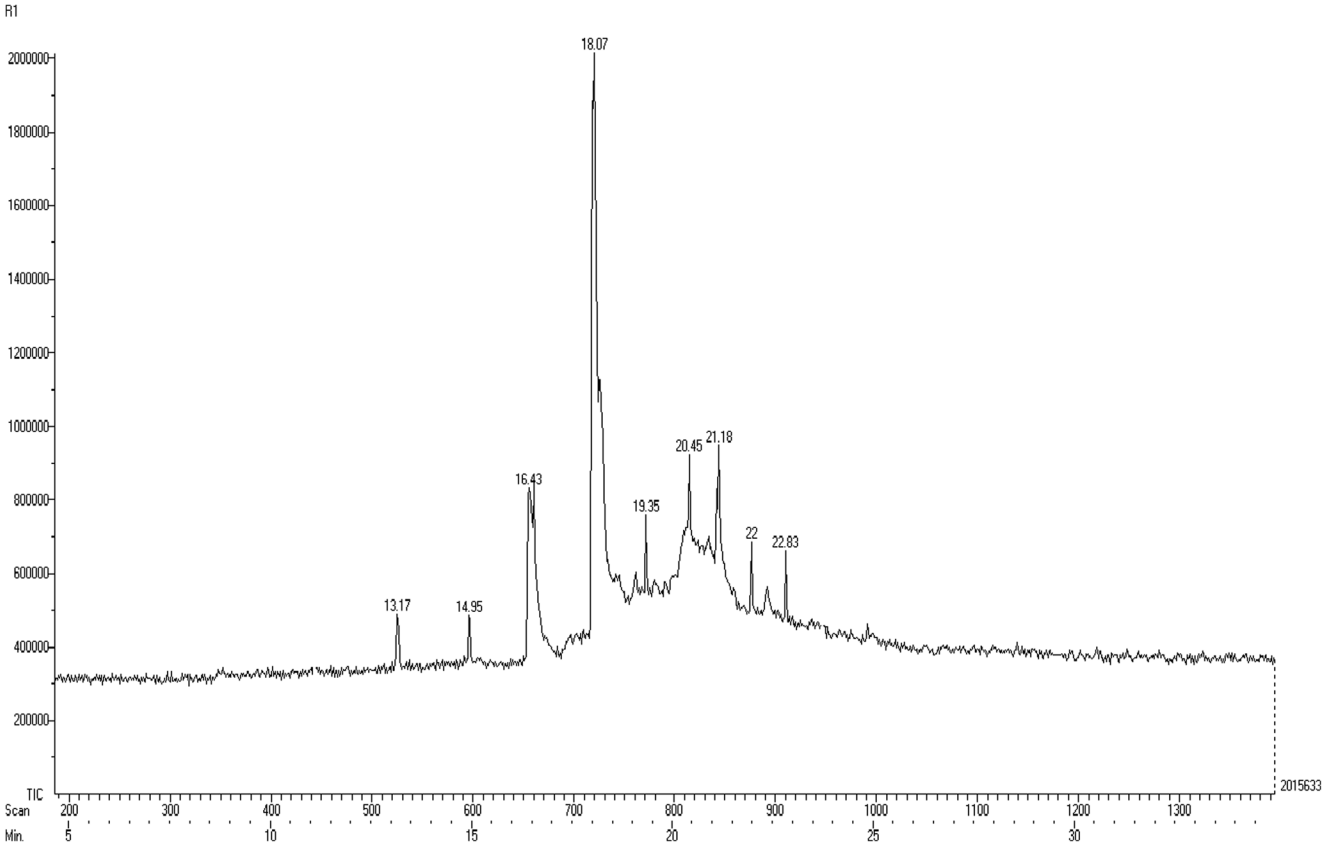

Fig. 3 GC-MS chromatogram of aqueous extracts of red-colored *C. annuum*

3 Results and Discussion

3.1 Qualitative Phytochemical Analysis

A qualitative analysis was used to screen phytochemical compounds from the crude aqueous extract (Table 1). The results were found to be similar to those of Gayathri et al. [41], but few other phytochemicals were also there; they were alkaloids, carbohydrates, and glycosides. Saponins along with tannins have been observed only in green-colored extract. Phytosterols, flavonoids, proteins, amino acids, and diterpenes were present in all extracts.

3.2 Separation of Bioactive Compounds by Thin-layer Chromatography

TLC analysis of the different capsicum extracts namely green, yellow, and red revealed the presence of compounds that were visualized in iodine chamber. The retention factor (Rf) of green, yellow, and red extracts was recorded as 0.73, 0.83, and 0.76, respectively (Table 2) in TLC run with acetone as mobile phase whereas the Rf values were noted as 0.09, 0.18, and 0.15 respectively while petroleum ether was used as mobile phase (Table 2).

Table 5 Phytoconstituents detected by GC-MS in aqueous extracts of red-colored *C. annuum*

S. No.	R.T	Compound name	Molecular weight (Da)	Molecular formula
1	13.17	Cyclopentaneundecanoic acid methyl ester	268.4348	$C_{17}H_{32}O_2$
2	14.95	Ergosta-5, 22-dien-3-ol (Brassicasterol)	398.664	$C_{28}H_{46}O$
3	16.43	Flavones	222.239	$C_{15}H_{10}O_2$
4	18.07	Piperidine, 4-(4-methylphenyl)-	175.275	$C_{12}H_{17}N$
5	19.35	Phytol	296.539	$C_{20}H_{40}O$
6	20.45	Z, E-2-methyl-3, 13-octadecadien-1-ol	280.488	$C_{19}H_{36}$
7	21.18	9-octadecenamide (oleamide)	281.484	$C_{18}H_{35}NO$
8	22	1 H-imidazole-1-ethanol, 2-heptadecyl-4,5-dihydro	350.58168	$C_{22}H_{42}N_2O$

Table 6 Antibacterial activity of aqueous extracts of *C. annuum* against *Brevibacillus brevis*

Extract	Zone of inhibition (cm)					
	Positive control	Negative control	5 (μg/ml)	10 (μg/ml)	15 (μg/ml)	20 (μg/ml)
Green	2.2	–	–	–	–	–
Yellow	3	–	–	–	–	–
Red	3.5	–	–	–	–	–

3.3 TLC Bioautography for Antioxidant Activity

TLC bioautography for the samples by antioxidant capacity was performed and the retention factor values were noted. The formation of fluorescent color indicates the presence of antioxidant activity in the sample. The green-, yellow-, and red-colored aqueous extract with acetone as mobile phase showed a single fluorescent band denoting the effect of antioxidant activity (figure not shown—refer to supplementary file). The components with Rf in green, yellow, and red *C. annuum* were found to be 0.73, 0.83, and 0.76 respectively while acetone was used as mobile phase (Table 2); when petroleum ether was used as mobile phase, the Rf obtained was 0.09, 0.18, and 0.15 for green, yellow, and red respectively (Table 2).

3.4 GC-MS Analysis

The phytoconstituents present in the aqueous extract of *C. annuum* were investigated using gas chromatography-mass spectrometry. Seven compounds were identified in green (Fig. 1, Table 3), six in yellow (Fig. 2, Table 4), and nine in red (Fig. 3, Table 5) aqueous extracts of *C. annuum*. Cyclopentadecanone was found in green- and red-colored *C. annuum*, which has been reported in ethyl acetate extract of *Goniothalamus umbrosus* [42] and also in *Pongamia pinnata* [43]. Flavones were found in green and red *C. annuum* (Tables 4 and 6 and Figs. 1 and 3) which are reported to have antioxidant properties [44, 45]. Ergosta-5, 22-dien-3-ol (brassicasterol) was seen in red-colored *C. annuum*, which is responsible for red color; it also has been reported in plants [46, 47]. 9-Octadecenamide was found in red-colored *C. annuum*, which is used in treatment of mood and sleep disorders and cannabinoid-regulated depression [48, 49]. Phytol, a diterpene, was also found in red-colored *C. annuum*, which is used for treatment of rheumatoid arthritis and chronic inflammatory diseases [50, 51]. It has antioxidant, antimicrobial, diuretic, anti-inflammatory, and antinociceptive effects and is a precursor for synthetic vitamin E and vitamin

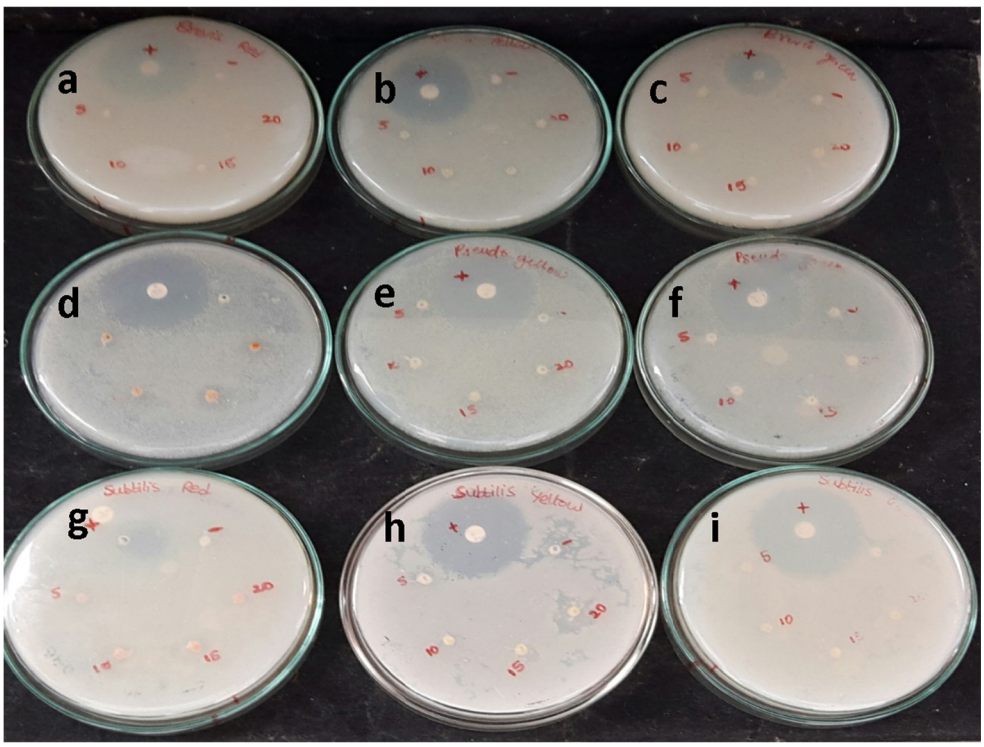

Fig. 4 Antimicrobial activity of *Capsicum annuum*. (a) Aqueous extract of red capsicum against *Brevibacillus brevis*. (b) Aqueous extract of yellow capsicum against *Brevibacillus brevis*. (c) Aqueous extract of green capsicum against *Brevibacillus brevis*. (d) Aqueous extract of red capsicum against *Pseudomonas aeruginosa*. (e) Aqueous extract of yellow capsicum against *Pseudomonas aeruginosa*. (f) Aqueous extract of green capsicum against *Pseudomonas aeruginosa*. (g) Aqueous extract of red capsicum against *Bacillus subtilis*. (h) Aqueous extract of yellow capsicum against *Bacillus subtilis*. (i) Aqueous extract of green capsicum against *Bacillus subtilis*

Table 7 Antibacterial activity of aqueous extracts of *Capsicum annuum* against *Pseudomonas aeruginosa*

Extract	Zone of inhibition (cm)					
	Positive control	Negative control	5 (μg/ml)	10 (μg/ml)	15 (μg/ml)	20 (μg/ml)
Green	2.9	–	–	–	–	–
Yellow	3	–	–	–	–	0.7
Red	3.5	–	0.4	0.5	0.8	1.3

Table 8 Antibacterial activity of aqueous extracts of *Capsicum annuum* against *Bacillus subtilis*

Extract	Zone of inhibition (cm)					
	Positive control	Negative control	5 (μg/ml)	10 (μg/ml)	15 (μg/ml)	20 (μg/ml)
Green	3.5	–	–	–	–	–
Yellow	3.5	–	0.3	0.5	0.6	1.3
Red	2	–	–	–	–	–

Table 9 Antibacterial activity of silver nanoparticles synthesized using different colored aqueous extracts of *Capsicum annuum* against *Pseudomonas aeruginosa*

	Zone of inhibition (cm)					
Silver nanoparticles	Positive control	Negative control	2 (μg/ml)	4 (μg/ml)	6 (μg/ml)	8 (μg/ml)
Green	3.5	–	0.7	0.8	0.9	0.9
Yellow	3.3	–	0.6	0.7	0.7	0.8
Red	3	–	0.5	0.6	0.7	0.7

K, which was found to be cytotoxic against breast cancer cell lines (MCF7) [52]. 4H 1 benzopyran 4 one was seen on yellow-colored and green *C. annuum* (Tables 4 and 5 and Figs. 1 and 2), which has been utilized as eukaryotic DNA polymerase inhibitor [53, 54]. 13-Docosenoic acid methyl ester was seen in yellow-colored *C. annuum*, where its bioactivity has been described earlier [55, 56].

3.5 Antibacterial Activity by Agar Well Diffusion Method

Antibacterial activity of different colored aqueous extracts of *C. annuum* was performed against three different microorganisms namely *Brevibacillus brevis*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The bacterial species showed differential sensitivity to the different aqueous extracts as indicated by their zone of inhibition (Fig. 4). From the results, it was observed that none of the extracts showed antibacterial activity against *Brevibacillus brevis* (Table 7). Yellow- and red-colored extracts showed activity against *Pseudomonas aeruginosa* with zone of inhibition of 0.7 cm for yellow extract at 15-μg concentration, whereas it was 1.3 cm for red-colored extract at 20-μg concentration (Table 8). The yellow-colored extract showed activity against *Bacillus subtilis* (Table 9). The present study indicated that the yellow-colored extract of capsicum was highly effective against *Pseudomonas aeruginosa* and *Bacillus subtilis*. Antimicrobial effect of *Capsicum* sp. extract has also been demonstrated by Careaga et al. [57] against *P. aeruginosa*.

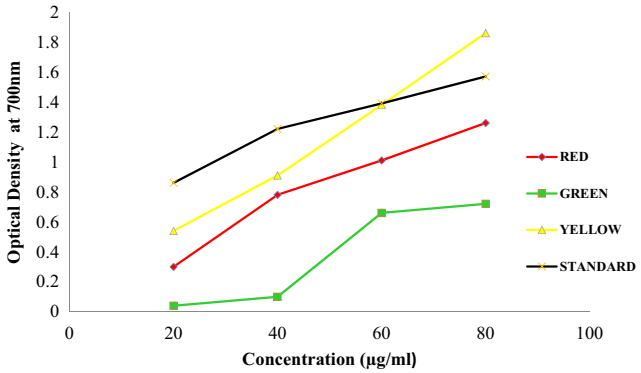

Fig. 5 FRAP assay for aqueous extracts of *C. annuum*

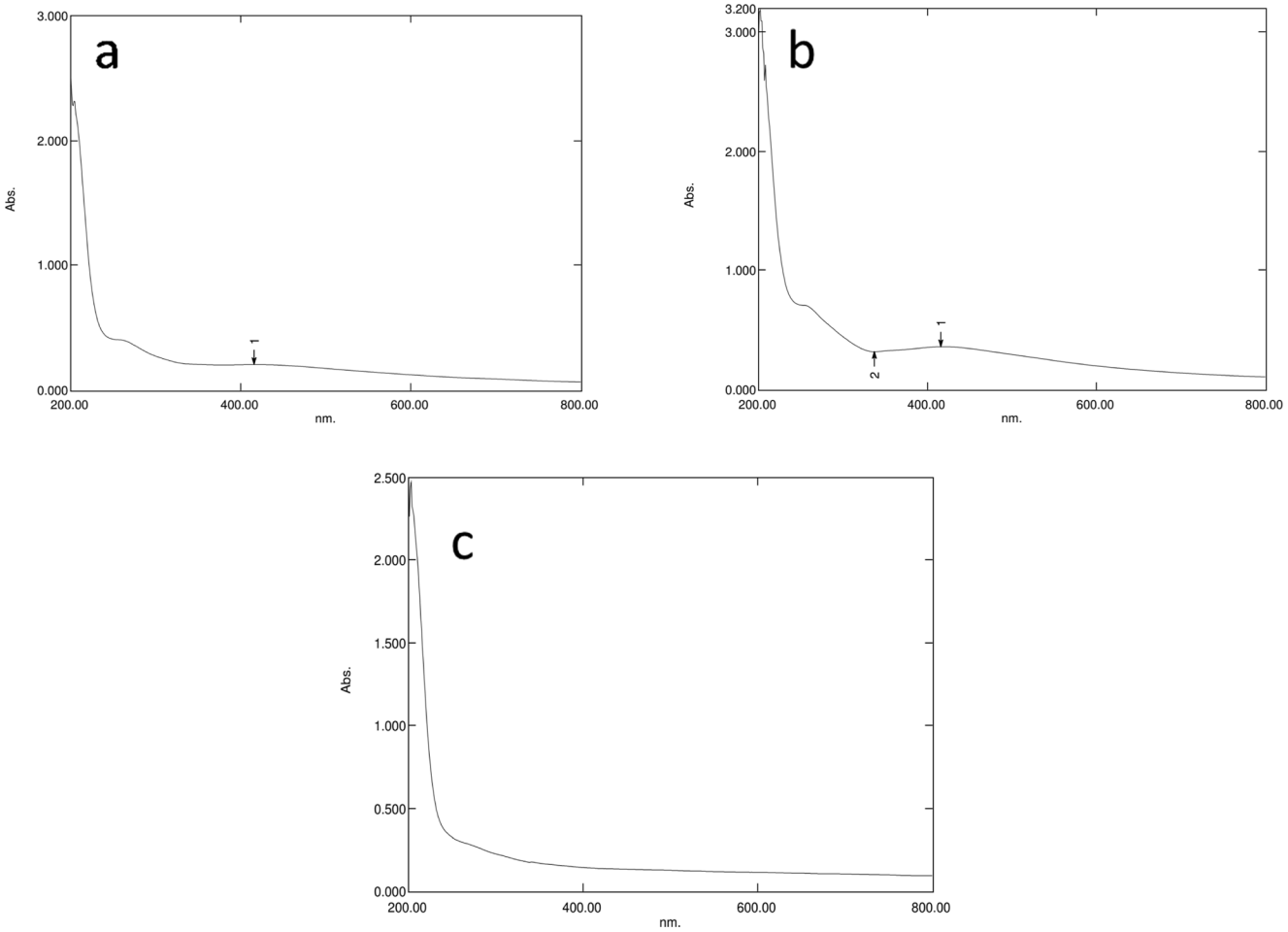

Fig. 6 UV-Vis spectroscopy for the synthesized silver nanoparticles. **a** Green. **b** Red. **c** Yellow

Capsaicin present in chili pepper is said to have antibacterial activity against *Bacillus subtilis* [58].

3.6 FRAP Assay

The highest antioxidant activity was exhibited by the yellow-colored aqueous extract followed by red and then by green. At 80 μg, maximum absorbance value was recorded for the yellow-colored extract when compared to the standard absorbance (Fig. 5) [59]. The antioxidant property of *Capsicum* sp. extracts is said to increase with its maturity [60].

3.7 UV-Visible Spectroscopy

The UV absorption spectrometric analysis of aqueous extract of different colored capsicum-AgNPs showed absorbance spectra at 416 nm, suggesting the bio reduction of silver nitrate into silver nanoparticles. Surface plasmon resonance of metal UV–Visible spectrometry showed a peak at 416 nm, confirming the formation of silver nanoparticles. The UV-Vis results obtained are given in Fig. 6a–c. Many researchers have reported the confirmation of silver nanoparticles at 410 to 430 nm [34, 61]. Phenolic acids present in the phytochemical compounds serve as reducing and stabilizing agent for the fabrication of silver nanoparticles [62, 63].

3.8 Fourier Transform Infrared Spectroscopy

The peaks at a range between 3385, 3293, and 3284 cm^{-1} for green, red, and yellow, respectively, indicated OH− stretching of alcohols. C–H bending was observed at a range between 1390 and 1365 cm^{-1} (Fig. 7a–c). All the silver nanoparticles showed peaks of C=C stretching of alkenes at 1629, 1637, and 1637 cm^{-1}. Peaks around

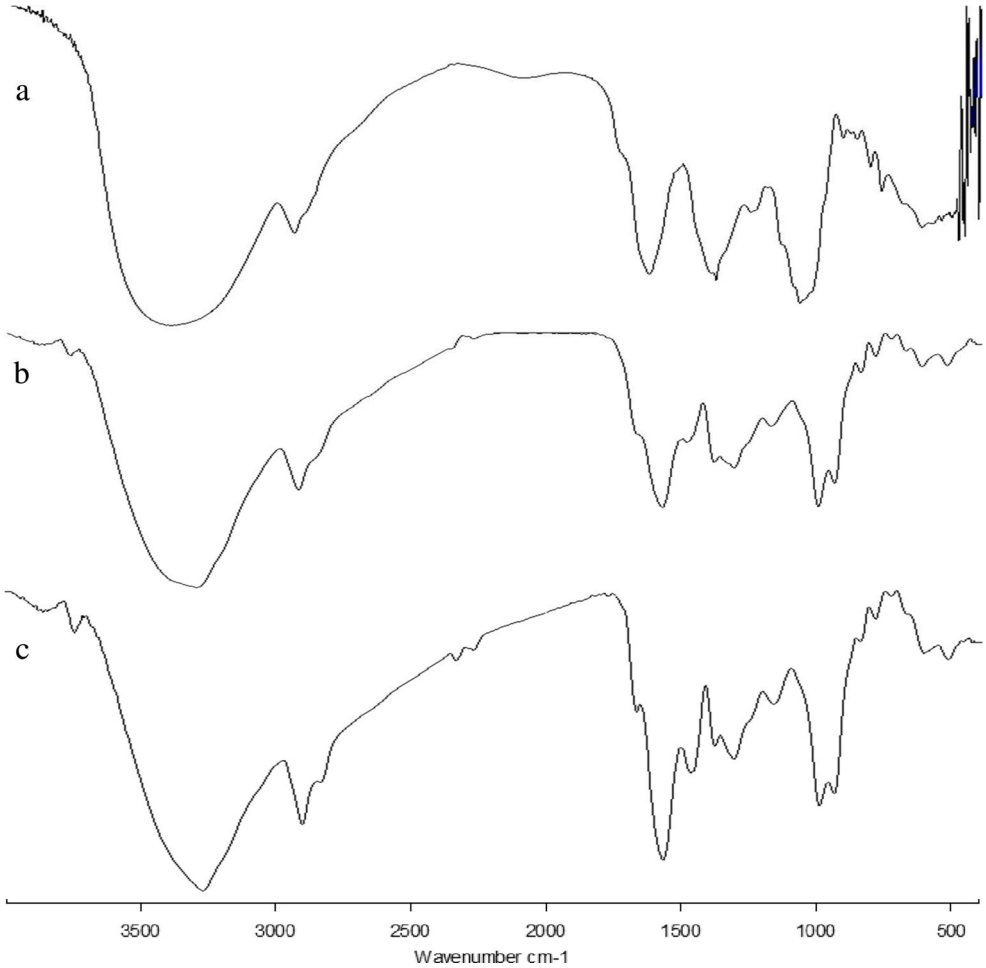

Fig. 7 Fourier transform infrared spectroscopy for the synthesized silver nanoparticles using aqueous extracts of different colored *Capsicum annuum.* **a** Green. **b** Red. **c** Yellow

2930 cm^{-1} revealed the presence of C–H stretching. Silver nanoparticle with similar groups has been earlier described by Rajathi and Sridhar [32] and Singh et al. [61].

3.9 Scanning Electron Microscopy

The SEM analysis showed the formation of spherical silver nanoparticles with slight aggregations. The size ranged between 30 and 80 nm (Fig. 8). Spherical-shaped silver nanoparticle synthesis using aqueous callus extract of *Capsicum annuum* has been reported to be in the range of 50–70 nm by Agarwal et al. [64].

3.10 Atomic Force Microscopy

The synthesized silver nanoparticles were subjected to AFM to check its size and morphology. The topography indicated an uneven size distribution and they were spherical to irregular (Fig. 9). Highly irregular shaped silver nanoparticles have been observed using AFM and have been reported by Logeswari et al. [34].

3.11 Antibacterial Activity of Silver Nanoparticles by Agar Well Diffusion Assay

The antibacterial activity of synthesized silver nanoparticles was evaluated by agar well diffusion method against gram-negative *Pseudomonas aeruginosa*. The highest zone of inhibition was found with lower concentration of silver nanoparticles (Fig. 10, Table 9). It has been reported earlier that gram-negative bacteria are highly susceptible to silver nanoparticles due to the fact that it has thinner peptidoglycan layer [65, 66]. These attach to the surface of the cell membrane and also penetrate inside causing damage by interacting with sulfur- and phosphorus-containing compounds such as DNA [67].

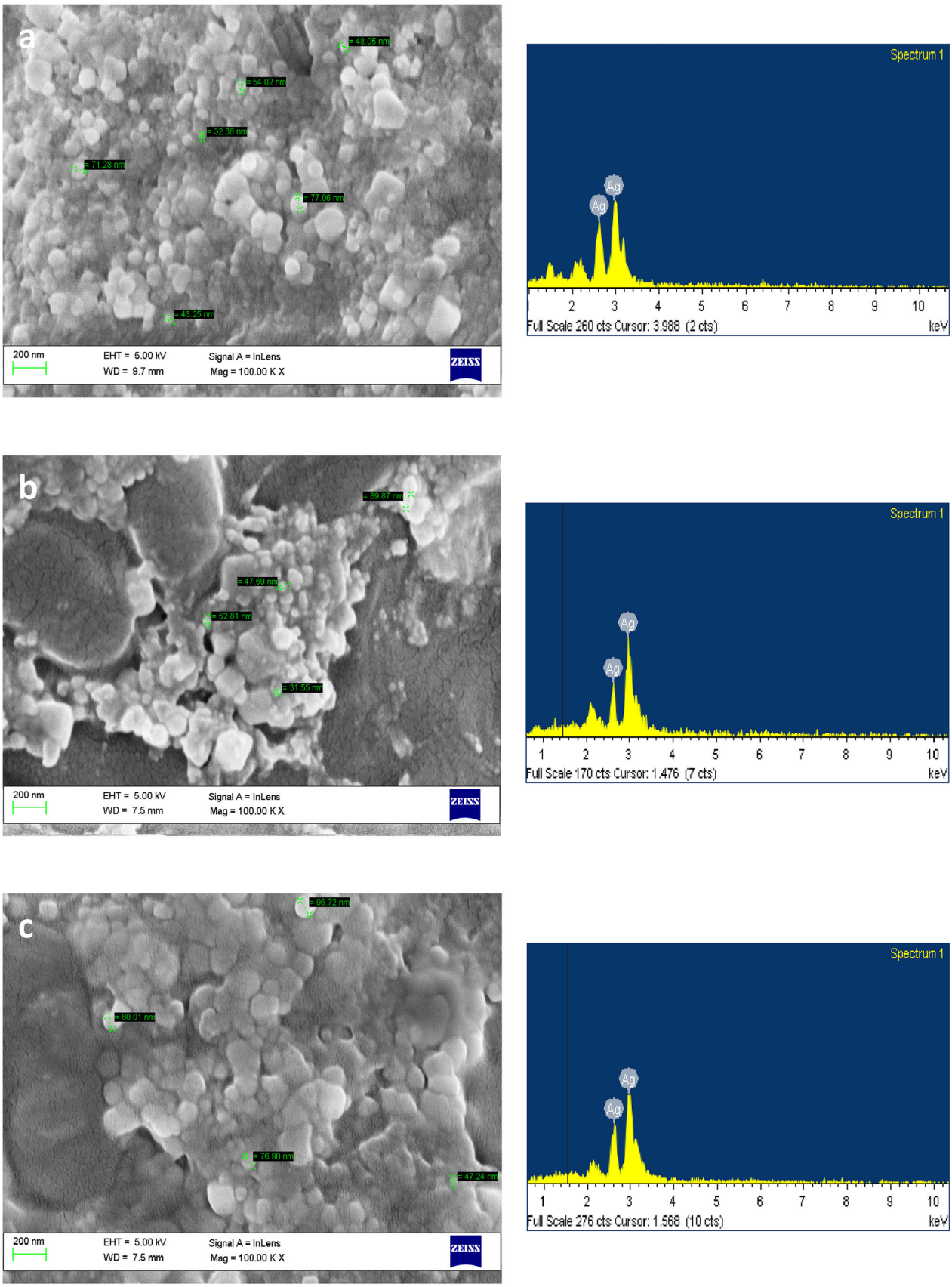

Fig. 8 SEM and EDX of silver nanoparticles prepared using aqueous extracts of *C. annuum.* **a** Green. **b** Red. **c** Yellow

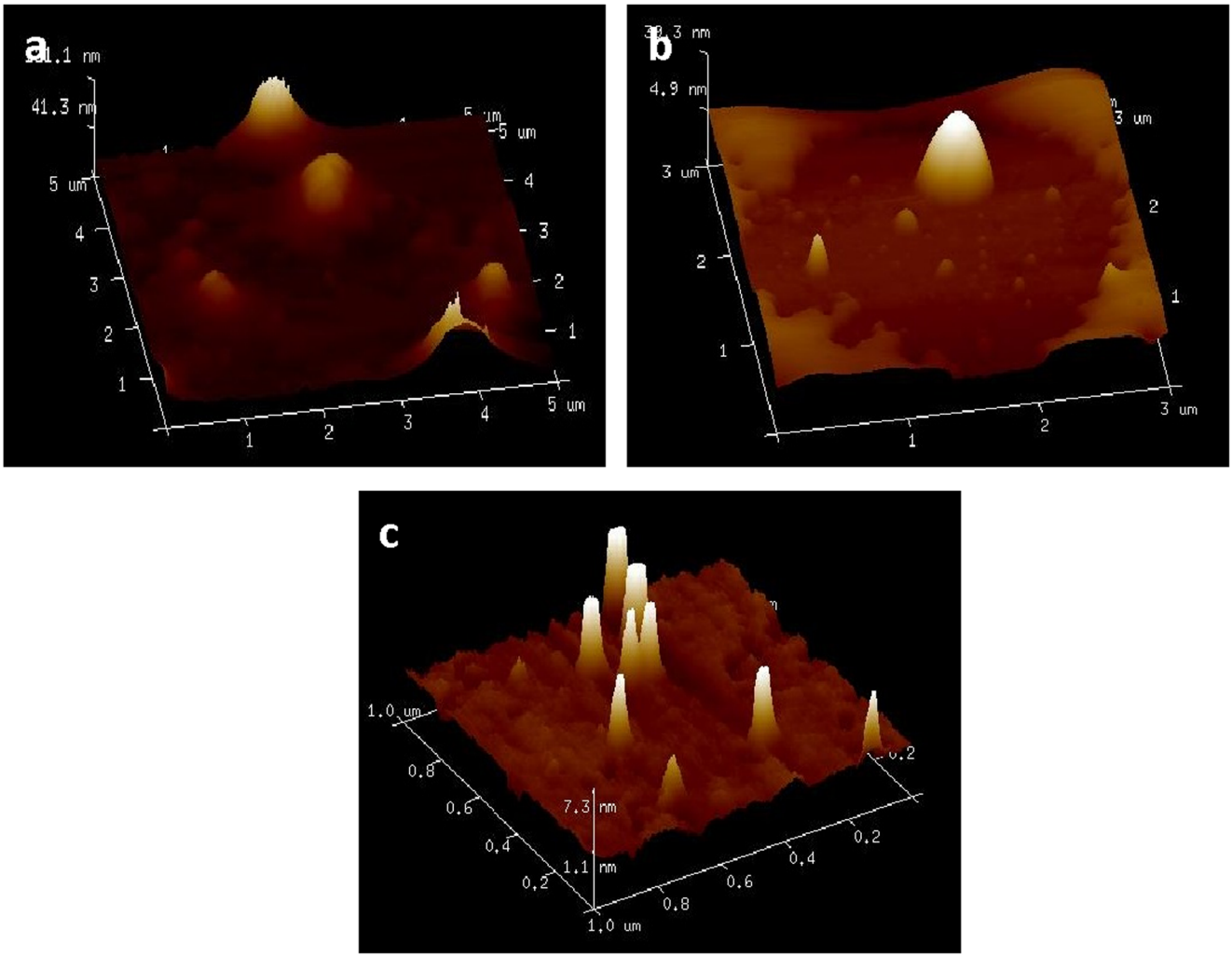

Fig. 9 AFM of silver nanoparticles prepared using aqueous extracts of *C. annuum.* **a** Green. **b** Red. **c** Yellow

3.12 Minimum Inhibitory Concentration

The results of the minimum inhibitory concentration (MIC) test showed that the synthesized silver nanoparticles from different colored extracts were inhibiting the growth of *Pseudomonas aeruginosa* at different concentrations. MICs ranged from 1 to 3 μg/ml for silver nanoparticles synthesized using green-, yellow-, and red-colored extract of *C. annuum*, respectively, thereby demonstrating the potential of these substances as antibacterial agents (Table 10).

3.13 Protein Leakage Assay

Size is said to have an influence on the silver nanoparticle's mechanism to enter the cell membrane leading to its death [65]. Cells of *Pseudomonas aeruginosa* experienced a huge leakage of proteins after incubation with different concentrations of silver nanoparticles (Fig. 11). It was also observed that there was an increase in protein leakage as the concentration of silver nanoparticles increased. Similar results were described by Ghosh and Ramamoorthy [68]. Twenty nanograms per milliliter of silver nanoparticles has made *E. coli* and *Enterobacter* sp. to leak protein [39].

4 Summary and Conclusion

Different stages of ripened, i.e., green-, yellow-, and red-colored *C.annuum*, were taken and their aqueous extracts were screened for phytochemical compounds qualitatively and also by GC-MS analysis. Aqueous extracts of yellow and red *C. annuum* showed antimicrobial activity against *P. aeruginosa*. Silver nanoparticles were synthesized using aqueous extracts of different colored *C. annuum*. The SEM analysis indicated the size of the nanoparticles to be the range between 31 and 80 nm. Silver nanoparticles synthesized using green *C. annuum* exhibited maximum zone of inhibition against *Pseudomonas aeruginosa* which was further

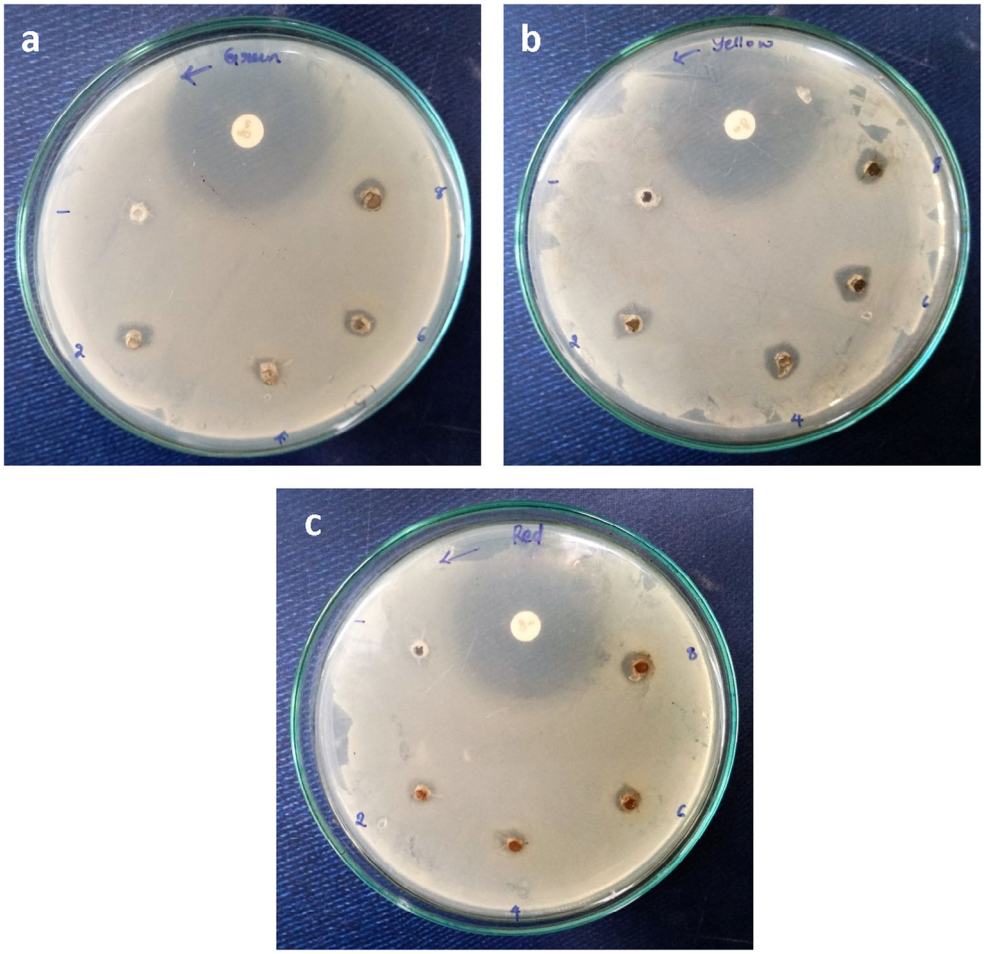

Fig. 10 Antibacterial activity for silver nanoparticles synthesized using aqueous extracts of *C. annuum* against *Pseudomonas aeruginosa.* **a** Green, **b** yellow, and **c** red

Table 10 Determination of minimum inhibitory concentration for *Pseudomonas aeruginosa* cells treated with silver nanoparticles synthesized using aqueous extracts of *C. annuum*

	1 μg/ml	2 μg/ml	3 μg/ml	4 μg/ml	5 μg/ml	6 μg/ml	7 μg/ml	8 μg/ml
Green	+	+	–	–	–	–	–	–
Yellow	–	–	–	–	–	–	–	–
Red	–	–	–	–	–	–	–	–

+ presence of growth, − absence of growth

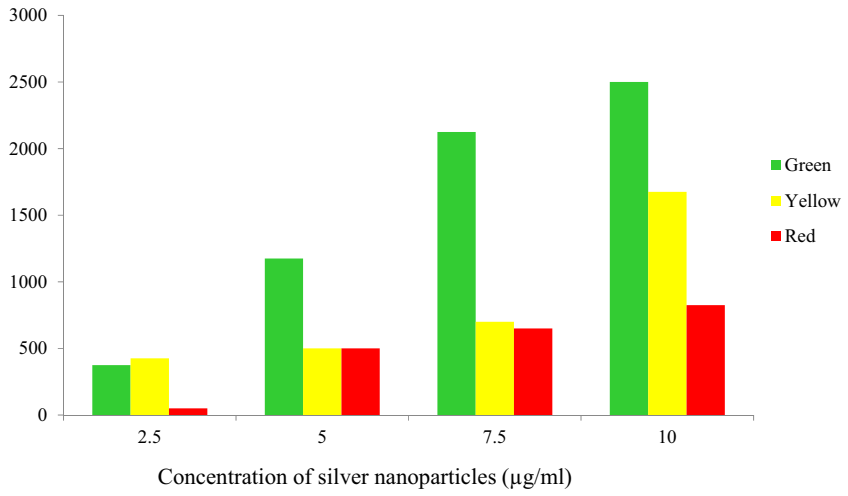

Fig. 11 Total protein leakage by silver nanoparticles synthesized using aqueous extracts of *C. annuum* against *Pseudomonas aeruginosa*

confirmed by highest protein leakage. Therefore, this approach of synthesizing silver nanoparticles from plant source will afford towards the growth of nanomedicines and other biomedical applications.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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