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GREEN FRUIT OF CHILI (*Capsicum annum* L.) SYNTHESIZES NANO SILVER!

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A rapid way of synthesizing silver nanoparticles by treating silver ions with a green *Capsicum annum* L. alcoholic fruit extract containing principally polyphenols, pigments, capsaicin and ascorbic acid is reported. The reaction protocol was monitored using UV-visible spectroscopy. Accordingly, the entire span of experimentation has been proposed in the form of a mathematical model in the light optical dependence of maturing Ag nanoparticles. The crystalline morphology and phase of the nanoparticles were determined with the help of transmission electron microscopy, selected area electron diffraction and X-ray diffraction studies. Nanoparticles almost spherical in shape having a size of 2-6 nm are found. The results indicated that the extract, which has capsaicin containing amine groups along with other candidate polyphenols and ascorbic acid, played a reducing and controlling role during the formation of silver nanoparticles in the solutions of green *Capsicum annuum* L. fruit.

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1. Introduction

Chili (*Capsicum annum* L.) has been a part of human lives as common spice and folk medicine in many parts of the world since yore. It is since ages, preparations of capsicum fruits were used as counter-irritant in lumbago, neuralgia and rheumatic disorders. Its pharmacological promises are extensively studied and it has been shown that the presence and position of hydroxyl group (-OH) in the aromatic ring and the amide moiety and its length decides the degree of pungency of chili fruits [1]. Along with this, it is an important agricultural crop, not only because of its economic importance, but also for the nutritional value of its fruits, mainly due to the fact that they are an excellent treasure of natural colours and antioxidant compounds [2,3]. The intake of these compounds in food is an important anti-aging factor. They have also been recognized to be quite promising for prevention of widespread human diseases, including cancer and cardiovascular diseases, when taken daily in adequate amounts [4,5]. A wide spectrum of antioxidant compounds is present in chili fruits. Phenolic compounds retard or inhibit lipid autoxidation by acting as radical scavengers [6] and consequently, are essential antioxidants that protect against propagation of the oxidative chain. It is also known that vitamin C, an important compound of chili fruits, chelates heavy metal ions [6], reacts with singlet oxygen and other free radicals, and suppresses peroxidation [7], reducing the risk of arteriosclerosis, cardiovascular diseases, and some forms of cancer [8]. Carotenoids play an important role in fruit colouring and act as antioxidants, reacting with free radicals, mainly peroxide radicals and singlet molecular oxygen [6]. Lycopene is a powerful natural antioxidant that acts as the most efficient singlet

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oxygen quencher in vitro among common carotenoids [9] and as a determinant factor in reducing the mortality from several cancers [10-12]. For another major carotenoid in chili, β-carotene, there is much in vitro evidence of its interaction with free radicals, acting as a chain-breaking antioxidant and as a scavenger and quencher of singlet oxygen [13,14].

Synthesis of metallic and/or oxide nanoparticles taking assistance of plant extracts has been substantially reported in the past [15-23]. Such studies could prove to have an enormous impact in immediate future if plant tissue culture and downstream processing procedures are applied in order to synthesize metallic as well as oxide nanoparticles on industrial scale. Present investigation is an effort in that direction. Synthesis of Ag nanoparticles taking use of *Capsicum annum* L. plant extract has been reported in the immediate past [22]. The background work [1] suggests that the non-fleshy fruit like chili more treasure of candidate metabolites than the vegetative part of the chili plant as reported earlier [22]. Along with this, an extensive literature survey suggested that no attempt, to best of the authors' knowledge, has so far been made to employ matured and green *Capsicum annum* fruit extract negotiated synthesis of silver nanoparticles. Accordingly, the present work reports the structural, microstructural and UV-vis spectroscopy studies on silver nanoparticles. An effort has also been made to understand the mechanism of nano transformation in the light of available literature and the entire span of experimentation has been proposed in the form of a mathematical model in the light optical dependence of burgeoning/maturing silver nanoparticles.

2. Experimental

Known weight (5 g) of freshly collected, taxonomically authenticated healthy green fruits of *Capsicum annum* L. (Figure 1 a) were taken and washed thoroughly in flush of tap water in the laboratory for 10 min in order to remove the dust particles, were cut in to small pieces and ringed briefly in sterile distilled water. Now it was taken in 250 mL capacity beaker having 200 mL of 50% Et-OH and was placed on boiling steam bath for 15 to 20 min till colour of the solvent changes to light yellowish-green. The extract was cooled to room temperature, pressed and filtered firstly through sterile serene cloth. This solution was treated as source extract (containing crude capsaicin) and was utilized in subsequent procedures. Now, 40 mL of the source extract was doubled in volume by adding 40 mL of sterile distilled water. The extract solution was treated with 20 mL of 0.25(M) $AgNO_3$ solution and warmed again on the steam bath for 5 min until the colour of solution changes to deep reddish-brown and was allowed to cool and incubate in the laboratory ambience. Concurrently, UV-vis spectrophotometric study was pursued in which 50% Et-OH extract of the fruit (containing crude capsaicin) was taken as blank. The deposition gets distinctly visible in the flask within 5 min which was left for 5 h (Figure 1b) and subsequently filtered.

The formation of single-phase compound was checked by X-ray diffraction (XRD) technique. The XRD pattern was taken with X-ray diffractometer (XPERT-PRO, PW3050/60) at room temperature, using CuK_α radiation $\lambda = 1.5406$Å over a wide range of Bragg angles ($10° \leq 2\theta \leq 90°$). TEM micrograph of Ag NPs was obtained using Hitachi H-7500 transmission electron microscope. The specimen was suspended in distilled water, dispersed ultrasonically to separate individual particles, and one or two drop of the suspension deposited onto holey-carbon coated copper grids and dried under Infrared lamp. The absorption spectra of the sample were measured by a computer interfaced UV-visible spectrophotometer (UV160, Systronics, India).

3. Results

Fig. 1a shows the image recorded from drop-coated film of silver nanoparticles synthesized by treating $AgNO_3$ solution with *Capsicum* for 4 h. The micrograph clearly shows individual nanoparticles whose sizes ranging between 2-6 nm. The measurement of size was performed along the largest diameter of the particles. Inset Fig. 1b shows the selected area electron diffraction (SAED) pattern obtained from Ag NPs shown in Fig. 1a. The Scherrer rings, characteristic of fcc silver is clearly observed, showing that the structure seen in the TEM image

are nanocrystalline in nature. The particle size was calculated with the help of UTHSCA IMAGE TOOL considering 50 particles. The particles are found almost spherical in shape having a size of the order of 2-6 nm. The particle size histogram of Ag NPs (Fig. 1c) shows a broad distribution of particle sizes which ranged from 2-6 nm. The average particle size comes out to be 3.62 ± 0.013 nm. Also, it is observed that the silver nanoparticles are scattered over the surface and no aggregates are noticed under TEM. The difference in size is possibly due to the fact that the nanoparticles are being formed at different times.

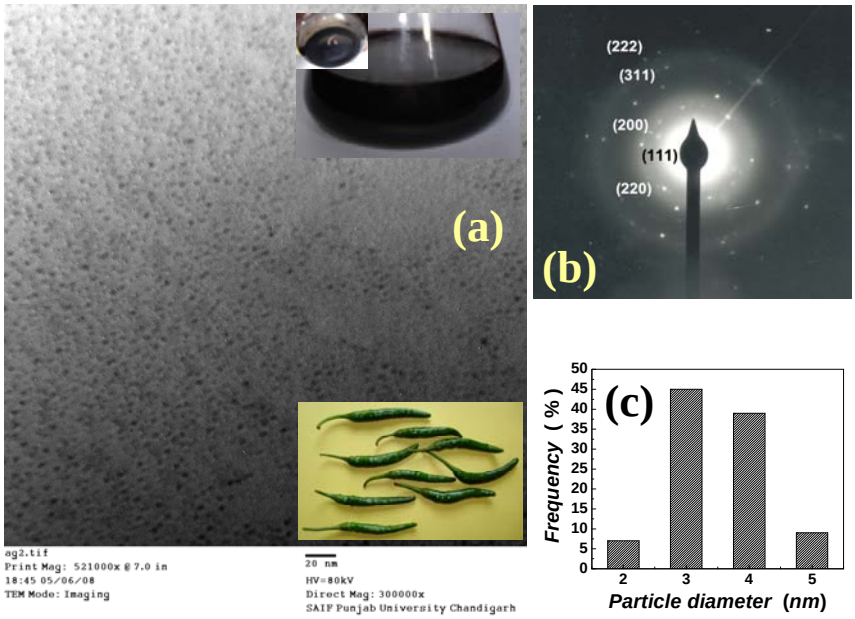

Fig. 1. (a) TEM image of Ag NPs synthesized by reduction of Ag^+ ions using Capsicum fruits extract, Insets: Ag NPs in medium, deposition of Ag NPs (bottom view) and Capsicum fruits, (b) SAED pattern and (c) particle size distribution (%) of Ag NPs.

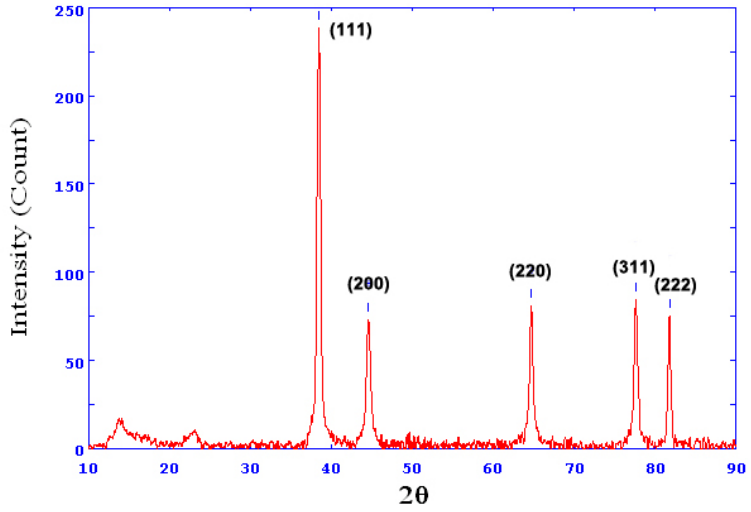

Fig. 2. X-ray diffraction pattern of Ag NPs at room temperature synthesized by Capsicum fruits extract with $AgNO_3$ solution.

Fig. 2 shows the X-ray diffraction pattern obtained for Ag NPs synthesized by *Capsicum* fruits extract The XRD clearly indicates that the silver nanoparticles formed by reduction of Ag^+ ions by *Capsicum* are crystalline in nature. A number of Bragg reflections corresponding to the (111), (200), (220), (311) and (222) sets of lattice planes of face-centered cubic (fcc) silver were indexed using a software "PowderX". Also, the broadening of X-ray peaks is primarily due to the small particle size. The unit cell parameter was estimated using experimental 2θ-values of peaks to be 4.077 Å which is in excellent agreement with the literature report (PDF # 870720, ICSD #: 064997). The unit cell volume was estimated to be 67.77 $Å^3$. Also, the structure of Ag NPs as observed in SAED pattern resembles with XRD result. These results are in consistent with the earlier report [24,25]. Considering the nanoparticles to be spherical and is made up of *n* number of unit cells having the edge length *a*, then $\frac{4}{3}\pi < R >^3 = nV$, where <*R*> is the mean radius of nanoparticle and $V = a^3$ (for fcc structure) is the volume of the unit cell. Hence, $n = \frac{4}{3}\pi < R >^3 / V$. The value of *n* estimated to be 366 for *Capsicum annum* assisted Ag NPs.

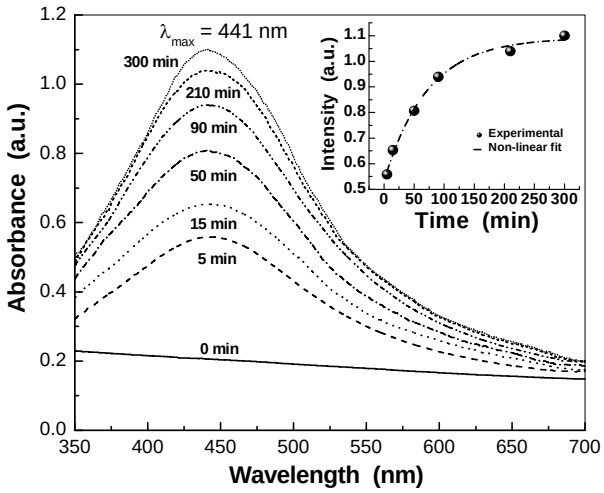

Fig. 3. UV-vis spectra for Ag NPs recorded as function of time of reaction of 0.25M aqueous solution of $AgNO_3$ with Capsicum fruits extract. Inset: Absorbance at λ_{max} (ξ) as a function of time with theoretical fit Eq.(1).

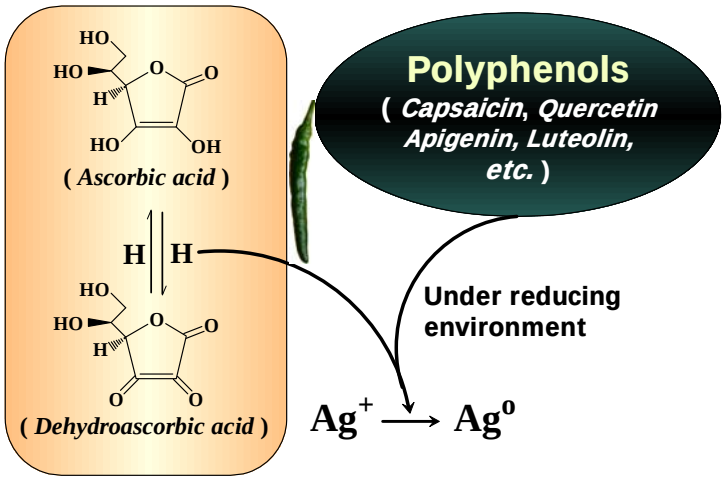

Fig. 4. Schematics for the biosynthesis of Ag NPs using Capsicum fruits extract.

Fig. 3 shows the UV-vis spectra recorded from aqueous *Capsicum* fruits extract-$AgNO_3$ (0.25M) solution as a function of time of reaction. Immediately after addition of $AgNO_3$ solution to the extract, the colour of extract changes to reddish brown. At this stage, formation of metal nanoparticles due to reduction was followed by UV-vis spectroscopy. The generation of colour is due to excitation of surface plasmons in metal nanoparticles [26]. The silver surface plasmon resonance was observed at 441 nm which steadily increases in intensity as a function of time of reaction (ranging from 5 min to 300 min) (inset Fig. 3) without showing any shift of the wavelength maximum. This simply indicates longitudinal plasmon vibrations. Also, the plasmon bands are broadened with an absorption tail in the longer wavelengths, which may be due to the size distribution of the particles [27]. The inset of Fig. 4 depicts the reduction of the silver ions taking place at a faster rate and that saturation of data occurs at 300 min which clearly indicates the completion of reaction. The absorbance at $\lambda_{max}(\xi)$ – time data were modeled with the function of type:

$$\xi(t) = \xi_s - \kappa.r^t \tag{1}$$

where the parameters ξ_s and r ($0 < r \leq 1$) are respectively perceived as the saturation value of absorbance and the rate of reaction. κ is the material dependent constant, which may be reckoned as the response range of the reaction. It is observed that the experimental data fits excellently well ($R^2 = 0.99458$) with the proposed theoretical model (inset Fig. 3). The computer fitting of $\xi(t)$ data with Eq.(1) provided the model parameters: $\xi_s = 1.09214$, $\kappa = 0.55975$ and $r = 0.98616$. The inset of Fig. 4 illustrates the reduction of the silver ions taking place at a faster rate and that saturation of data occurs at 300 min which clearly indicates the completion of reaction. It seems that the present procedure of biosynthesis of Ag NPs proceeds with a drastically faster pace and bioreduction of silver ions is complete within five hours.

4. Discussion

In earlier studies on synthesis of silver nanoparticles employing bacteria [28,29], fungi [27,30,31] or plant extracts [15-23] the time required for completion (*i.e.* complete reduction of Ag-ions) ranged from 24 h-120 h, and are rather slow. Reduction accomplished due to capsaicin and ascorbic acid present in *Capsicum* fruits may be considered as a significant advance in this direction. Earlier, a good number of works has been done with regard to the plant assisted reduction of metallic nanoparticles [15-23] and the responsible candidate phytochemicals were broadly been ascertained to be terpenoids (citronellol and geraniol), flavones, ketones, aldehydes, amides and carboxylic acid in the light of exhaustive IR studies.

Studies with chili fruits have shown that although capsaicinoids increase with development, the maximal levels of free phenolics and lignin are observed during the early stages of development (*i.e.* maturation). A decrease of peroxidase activity was observed during maturation, and this was related with a decrease in other physiological parameters studied, namely chlorophylls and pH [32].

Chili fruits (*Capsicum annuum*) contain a wide array of phytochemicals with well-known antioxidant properties [33]. In a study involving different cultivars (*Bronowicka Ostra, Cyklon, Tornado, and Tajfun*) for phenolics contents and antioxidant activity, two fractions of phenolics, flavonoids (with phenolic acids) and capsaicinoids, were isolated from the pepper fruit at two growth stages (green and red) and were studied for their antioxidant capacity. A comparison of the capsaicinoid fraction with the flavonoid and phenolic acid fraction from red fruit with respect to their antioxidant activity gave similar results. Phenolic compounds were separated and quantified by LC and HPLC. Contents of nine compounds were determined in the flavonoid and phenolic acid fraction: *trans*-*p*-feruloyl-β-d-glucopyranoside, *trans*-*p*-sinapoyl-β-d-glucopyranoside, quercetin 3-*O*-α-l-rhamnopyranoside-7-*O*-β-d-glucopyranoside, *trans*-*p*-ferulyl alcohol-4-*O*-[6-(2-methyl-3-hydroxypropionyl] glucopyranoside, luteolin 6-*C*-β-d-glucopyranoside-8-*C*-α-l-arabinopyranoside, apigenin 6-*C*-β-d-glucopyranoside-8-*C*-α-l-arabinopyranoside, lutoeolin 7-*O*-[2-(β-d-apiofuranosyl)-β-d-glucopyranoside], quercetin 3-*O*-α-l-rhamnopyranoside, and luteolin 7-

O-[2-(β-d-apiofuranosyl)-4-(β-d-glucopyranosyl)-6-malonyl]-β-d-glucopyranoside. The main compounds of this fraction isolated from red chili were sinapoyl and feruloyl glycosides, and the main compound from green pepper was quercetin-3-*O*-l-rhamnoside. Capsaicin and dihydrocapsaicin were the main components of the capsaicinoid fraction. A high correlation was found between the content of these compounds and the antioxidant activity of both fractions. Their antioxidant activities were elucidated by heat-induced oxidation in the β-carotene−linoleic acid system and the antiradical activity by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) discoloration test. The highest antioxidant activity in the β-carotene−linoleic acid system was found for *trans*-*p*-sinapoyl-β-d-glucopyranoside, which was lower than the activity of free sinapic acid. Quercetin 3-*O*-α-l-rhamnopyranoside had the highest antiradical activity in the DPPH system, which was comparable to the activity of quercetin. The activities of capsaicin and dihydrocapsaicin were similar to that of *trans*-*p*-feruloyl-β-d-glucopyranoside in the DPPH model system [34].

Recent progress is being made on the biosynthetic pathway, and several of the genes coding for biosynthetic enzymes have been cloned and expression studies performed. With regard to catabolism, cumulative evidence supports that capsaicinoids are oxidized in the pepper by peroxidases. Peroxidases are efficient in catalyzing *in vitro* oxidation of both capsaicin and dihydrocapsaicin. These enzymes are mainly located in placental and the outermost epidermal cell layers of pepper fruits, as occurs with capsaicinoids, and some peroxidases are present in the organelle of capsaicinoid accumulation, that is, the vacuole. Hence, peroxidases are in the right place for this function. The products of capsaicin oxidation by peroxidases have been characterized *in vitro*, and some of them have been found to appear *in vivo* in the *Capsicum* fruit [35].

The above mentioned factors might cumulatively have culminated in to an appreciably fast pace of synthesis of the Ag NPs during the present investigation, with a major role being played by the polyphenols, ascorbic acid and capsaicinoids which are shown in the schematics (Fig. 4).

5. Conclusion

In conclusion, the present method is capable of producing Ag NPs. It is a green, high yield, fast and low cost approach. Reduction accomplished principally due to different phytochemicals like polyphenols, ascorbic acid and capsaicinoids which might have played the pivotal role in fabrication of Ag NPs.

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