

Anti-acne, anti-dandruff and anti-breast cancer efficacy of green synthesised silver nanoparticles using *Coriandrum sativum* leaf extract



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ABSTRACT

In this present investigation, AgNPs were green synthesised using *Coriandrum sativum* leaf extract. The physico-chemical properties of AgNPs were characterised using UV–visible spectrophotometer, field emission scanning microscopy/energy dispersive X-ray (FESEM/EDX), Fourier transformed infrared spectroscopy (FT-IR), X-ray diffraction (XRD) and Brunauer-Emmett-Teller (BET) analysis. Further, *in vitro* anti-acne, anti-dandruff and anti-breast cancer efficacy of green synthesised AgNPs were assessed against *Propionibacterium acnes* MTCC 1951, *Malassezia furfur* MTCC 1374 and human breast adenocarcinoma (MCF-7) cell line, respectively. The flavonoids present in the plant extract were responsible for the AgNPs synthesis. The green synthesised nanoparticles size was found to be ≈ 37 nm. The BET analysis result shows that the surface area of the synthesised AgNPs was found to be $33.72 \text{ m}^2 \text{ g}^{-1}$. The minimal inhibitory concentration (MIC) of AgNPs for acne causative agent *P. acnes* and dandruff causative agent *M. furfur* was found to be at 3.1 and $25 \mu\text{g mL}^{-1}$, respectively. The half maximal inhibitory concentration (IC_{50}) value of the AgNPs for MCF-7 cells was calculated as $30.5 \mu\text{g mL}^{-1}$ and complete inhibition was observed at a concentration of $100 \mu\text{g mL}^{-1}$. Finally, our results proved that green synthesised AgNPs using *C. sativum* have great potential in biomedical applications such as anti-acne, anti-dandruff and anti-breast cancer treatment.

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1. Introduction

Silver nanoparticles (AgNPs) are finding rich applications in various area including nanodevices, nanoelectronics, nanosensors, information storage, biomedical treatments and water purification [1–3]. The reduction of various complexes with silver (Ag^+) ions in aqueous solution leads to the formation of silver (Ag^0) atoms, which is followed by agglomeration into oligomeric clusters. These clusters eventually lead to the formation of colloidal AgNPs [4]. Several biological, chemical and physical methods are proposed to generate the nanoparticles [5,6]. However, most of physicochemical strategies are suffering in the application process due to the utilization of high energy, hazardous nature,

difficulty in separation and time consuming steps. Interestingly, AgNPs synthesised using enzymes, proteins, microorganisms and plant materials would offer a more biocompatible, eco-friendly, cost-effective, and easy biological approach for large scale production [7,8]. Particularly, plant mediated green synthesis of nanoparticles is an efficacious approach which finds immense application in the field of many modern medicines [9,10]. In addition, plant extracts may act both as reducing agents and stabilizing agents in the synthesis of nanoparticles.

Coriandrum sativum commonly known as coriander is a medicinally important herb of the Apiaceae family. The plant parts of *C. sativum* are highly rich in aromatic flavor and are commonly used for soups in Asian countries. Traditionally, this plant has been used to cure alleviate spasms, gastric complaints, bronchitis, gout and giddiness [11]. The available literatures on this herb confirms their biomedical properties such as antidiabetic, antioxidant, hypocholesterolemic, antihelminthic, antibacterial, appetizer, hepatoprotective, anticancer and anxiolytic activities [12–14]. Phytochemicals present in the plant materials are involved in the prevention of different chronic and degenerative diseases [15]. Flavonoids are a secondary metabolites that occupy an

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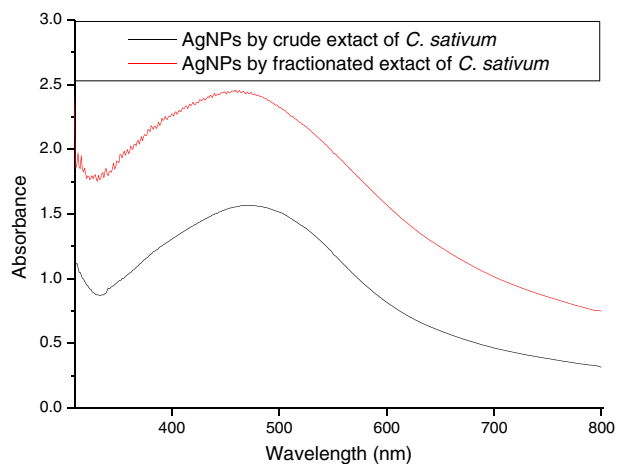


Fig. 1. UV-vis spectrum of green synthesised AgNPs by crude and fractionated flavonoids of *C. sativum* leaf extract.

important position among the phytochemicals, which have proven pharmacological activities such as antiallergic, antibacterial, anti-inflammatory, antiviral and anticancer [16–18]. In addition, flavonoids are considered as free radical scavengers, cyclooxygenase inhibitors and tumor suppressors [19,20].

Acne is a chronic inflammatory disease of the pilosebaceous units, which is characterised by seborrhea, the formation of open and closed comedones, erythematous papules, nodules, deep pustules and pseudocysts [21]. Three major factors are involved in the pathogenic mechanisms for increased sebum production viz. hypercornification of the pilosebaceous duct, an abnormality of the microbial flora (particularly, colonization of the duct with anaerobic diphtheroids *Propionibacterium acnes*) and inflammation. The sebum produced by acne patients are shown to be deficient in linoleic acid, which is directly associated with retention hyperkeratosis of the pilosebaceous follicle. Once the follicles occluded, *P. acnes* inhabit the follicles at puberty, and produce lipases, which hydrolyze sebaceous gland triglycerides into free fatty acids. Further, these acids in combination with bacterial proteins and keratin are extruded through the dilated follicular wall into the dermis, producing a neutrophilic inflammatory response [22,23]. However, there is no ideal treatment for acne, although a suitable regimen for reducing lesions can be found for most patients [24]. Moreover, the treatment of acne with antimicrobial agents has been found to be associated with the development of resistance to these agents by *P. acnes*, leading to treatment failure. Thus, alternative approaches with new biologically active principles for the antimicrobial treatment of acne are needed.

Dandruff is one of the serious troubles in human beings worldwide, characterised by scaling of the scalp and skin. Persistence of dandruff may lead to itching and hair loss [25]. *Malassezia* species such as *M. furfur*, *M. sympodialis*, *M. sloofia*, *M. pachydermatis*, *M. globosa* and *M. restricta* are well recognized as a causative organism for dandruff [26, 27]. Association between *M. furfur* and dandruff, and their pathogenic effects in human beings is well documented. In spite of several commercially available ketoconazole based anti-dandruff shampoo, dandruff recurrence is more frequent. Furthermore, resistance of dandruff to antifungal agent is also of immense interest due to the development of resistant strains. Thus, the formulation of an efficient anti-dandruff agent to prevent recurrence is essential.

Breast cancer is the development of cancer in breast tissues. Signs of breast cancer are a lump in the breast, a change in breast shape, dimpling of the skin, fluid leakage from the nipple, or a red scaly patch of skin. Breast cancer is more common in developed countries and leading cancer in women, accounting for 25% of all cases [28]. Thus, there is a need to give much more attention to develop more potent anti-cancer drugs so as to effectively check the increasing prevalence of this cancer.

In this study, AgNPs were green synthesised using *C. sativum* leaf extract and the active phytochemicals responsible for the AgNPs synthesis was assessed. Furthermore, in order to know their anti-acne, anti-dandruff and anti-breast cancer properties, *in vitro* microbial and cytotoxicity studies were performed against *P. acnes* MTCC 1951, *M. furfur* MTCC 1374 and human breast adenocarcinoma (MCF-7) cell line. This is the first report for anti-acne, anti-dandruff and anti-breast cancer efficacy of green synthesised AgNPs using *C. sativum* leaf extract.

2. Materials and Methods

2.1. Chemicals, Medium, Microorganisms and Cell Line

Nutrient agar, brain-heart infusion agar (BHIA), Leeming Notman agar (LNA) and Mueller-Hinton agar (MHA) were obtained from HiMedia (Mumbai, India). Silver nitrate was procured from Merck (Darmstadt, Germany). Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), ficoll histopaque, potassium bromide and Roswell Park Memorial Institute (RPMI) 1640 medium were purchased from Sigma-Aldrich (St. Louis, MO, USA). *P. acnes* MTCC 1951 and *M. furfur* MTCC 1374 were obtained from the Microbial Type Culture Centre (MTCC, Chandigarh, India). The MCF-7 cell line was received from American Type Culture Collection (ATCC, Manassas, USA). All other reagents used in this study were of analytical grade.

2.2. Plant Material

The well grown plant *C. sativum* was collected from agricultural field near to Periyar University, Tamil Nadu, India. The voucher specimen was identified by Botanical Survey of India, Southern Regional Centre, Coimbatore, Tamil Nadu, India. The reference number of the identified plant is BSI/SRC/5/23/2014-15/Tech-341. The fresh leaves of *C. sativum* were washed thoroughly with double distilled water in order to remove the surface contaminants. The leaf extract of *C. sativum* was prepared from 10 g of fresh leaf boiled in 500 mL distilled water for 5 h. Then, the boiled extract was filtered with Whatman No. 1 filter paper and used for nanoparticles synthesis.

2.3. Green Synthesis of AgNPs

A 50 mL of AgNO_3 (1 mM) aqueous solution was mixed with the same volume of *C. sativum* aqueous leaf extract and incubated at room temperature until the brownish color was formed, which indicated the synthesis of AgNPs. The green synthesis of AgNPs in the colloidal solution was confirmed by UV-visible spectrophotometer (PerkinElmer, USA) with distilled water as a reference. The green synthesised AgNPs was centrifuged and washed with Milli-Q water in order to remove the excess Ag^+ ions.

Table 1

Phytochemicals screening of *C. sativum* leaf extract and active fraction involved in the AgNPs green synthesis.

S. no.	Phytochemicals	Crude leaf extract of <i>C. sativum</i>	Active fraction involved in AgNPs green synthesis
1	Alkaloids	–	–
2	Carbohydrates	+	–
3	Flavonoids	+	+
4	Glycosides	+	–
5	Phenols	+	–
6	Saponins	+	–
7	Steroids	+	–
8	Tannins	+	–
9	Terpenoids	+	–

(+) denotes the presence of phytoconstituents.

(–) denotes the absence of phytoconstituents.

Table 2
Flavonoids content in crude and fractionated aqueous *C. sativum* leaf extract.

Samples	Flavonoids (mg g ⁻¹)	Yield (%)
Crude leaf extract of <i>C. sativum</i>	27.25	100
Fractionated leaf extract of <i>C. sativum</i>	19.48	71.49

2.4. Identification of Phytochemicals Responsible for AgNPs Synthesis

The leaf extract of *C. sativum* was further fractionated by column chromatography with methanol solvent system, and the fractions were used for nanoparticles synthesis as described in Section 2.3. Phytochemicals screening of the crude and fractionated samples were performed to find out the presence of alkaloids, carbohydrates, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids using standard protocol as follows.

2.4.1. Alkaloids

A 2 mL sample was mixed slowly with equal volume of concentrated HCl (1:1 ratio (v/v)). Then a few drops of Mayer's reagent were added to the mixture. The formation of green color or white precipitate indicated the presence of alkaloids.

2.4.2. Carbohydrates

A 2 mL of sample was mixed with 1 mL of Molisch's reagent (2:1 ratio (v/v)) and a few drops of concentrated H₂SO₄ were added. The appearance of purple or reddish color indicated the presence of carbohydrates.

2.4.3. Flavonoids

A 2 mL of sample was mixed with 1 mL of 2 N NaOH (2:1 ratio (v/v)). The appearance of yellow color indicated the presence of flavonoids.

2.4.4. Glycosides

A 2 mL of sample was added with 3 mL of chloroform and 10% ammonia solution. The formation of pink color indicated the presence of glycosides.

2.4.5. Phenols

A 1 mL of sample was mixed with 2 mL of distilled water followed by a few drops of 10% FeCl₃ were added. The formation of blue or green color indicated the presence of phenols.

2.4.6. Saponins

A 2 mL of sample was mixed with equal volume of distilled water (1:1 ratio (v/v)) and the mixture shaken in a graduated cylinder for 15 min. The formation of 1 cm layer of foam indicated the presence of saponins.

2.4.7. Steroids

A 2 mL of sample was mixed with equal volume of chloroform (1:1 ratio (v/v)) and subjected with a few drops of concentrated H₂SO₄. The appearance of brown ring indicated the presence of steroids.

2.4.8. Tannins

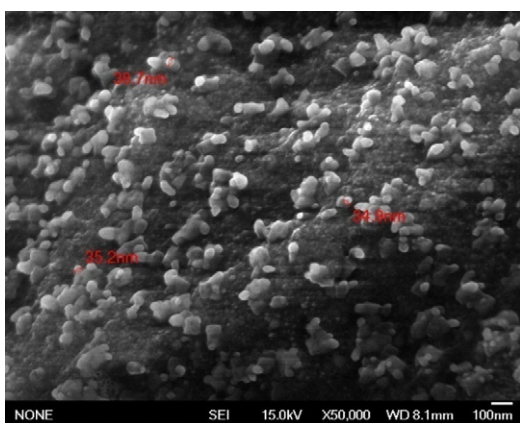
A 2 mL of sample was mixed with equal volume of 5% FeCl₃ (1:1 ratio (v/v)) and shaken well. The formation of dark blue or greenish black indicated the presence of tannins.

2.4.9. Terpenoids

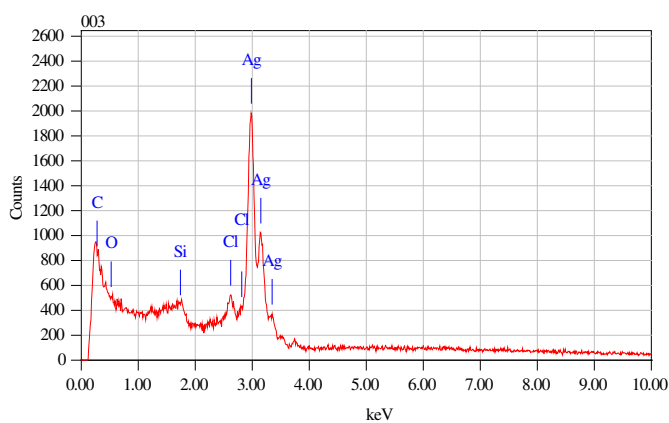
A 2 mL of the sample was carefully mixed with 2 mL of solution A (chloroform and concentrated H₂SO₄ (2:3 ratio)) to form a layer. The appearance of reddish brown color in the inner face was indicated the presence of terpenoids.

2.5. Physicochemical Characterisation of AgNPs

The AgNPs were examined using field emission scanning microscopy/energy dispersive X-ray (FESEM/EDX, JSM-6701F, USA) in order to check their surface morphology and chemical properties. The nanoparticles were coated with gold using a gold sputter at 10⁻¹ Mbar and the instrument was operated under the accelerating voltage of 2500 for FESEM/EDX analysis. Fourier transformed infrared spectroscopy (FT-IR, Perkin Elmer, USA) was used to determine the surface functional groups of the nanoparticles by vibrational frequency changes in the functional groups, which were obtained by averaging the results of thirty-two scans in the range of 4000 to 400 cm⁻¹. In order to avoid co-adsorbed water, the nanoparticles were dried under vacuum with KBr (1:100 (w/w)) before the FT-IR spectrum was recorded. The X-ray diffraction (XRD) analysis of the samples AgNPs was carried out on an XRD instrument (Bruker axs System, D8, Germany) with the scanning range between 10° and 90°. The surface area of nanoparticles were assessed by the method of Brunauer-Emmett-Teller (BET) using metrometrics ASAP 2010 surface area analyzer (USA) with N₂ adsorption-desorption isotherms at degassing temperature of 110 °C.



(a)



(b)

Fig. 2. (a) FESEM image and (b) EDX micrograph of green synthesised AgNPs by fractionated flavonoids of *C. sativum* leaf extract.

2.6. Anti-acne Activity

P. acnes was grown in BHIA at 37 °C for 48 h under anaerobic condition. BHIA plates were spread with 100 µL of 0.5 McFarland standard equal *P. acnes* suspension. The sterile disc (6 mm) was loaded with 10 µL of AgNPs at various concentrations dissolved in BHI broth. Further, the AgNPs loaded discs were placed on the plates spread with *P. acnes* and were incubated at 37 °C for 48 h. Finally, the zone of incubation (ZOI) (mm in diameter) was measured. Tetracycline (1 µg disc) was used in this study as a control drug to compare the anti-acne efficiency of the test sample.

In 96 wells plate, 100 µL of BHI broth was added into each well. A 100 µL of AgNPs was dissolved in BHI broth was added to first well and the drug suspension was serially diluted up to 10th well. *P. acnes* culture turbidity was adjusted to 0.5 McFarland standard ($\approx 1.5 \times 10^8$). Further, the culture was diluted to 100-fold with sterile BHI broth. Finally, 100 µL of *P. acnes* suspension was added to each well, except sterility control. Then, the plate was incubated at 37 °C under anaerobic condition. After 72 h incubation, the minimal inhibitory concentration (MIC) was determined by adding Alamar blue dye.

2.7. Anti-dandruff Activity

M. furfur was grown in LNA at 32 °C for 48 h. The AgNPs were dissolved in sterile distilled water. LNA plates were spread with 100 µL of 0.5 McFarland equal *M. furfur* suspensions grown at 32 °C for 48 h. Then, LNA plates were subjected to well cutting (6 mm) and wells were loaded with various concentrations of AgNPs solution and the plates were incubated at 32 °C. After 48 h, the ZOI was measured.

In 96 wells plate, 100 µL of LN broth was added into all wells. Then, 100 µL of AgNPs was dissolved into broth and added to first well of the plate and then serially diluted up to 10th well. *M. furfur* culture turbidity was adjusted to 0.5 McFarland standard ($\approx 1.5 \times 10^6$). Finally, 100 µL of *M. furfur* MTCC 1374 suspension was added to each well, except sterility control. Then, the plate was incubated at 32 °C for 72 h. After incubation, the MIC was determined by adding Alamar blue dye.

2.8. Anti-breast Cancer Activity

The anti-breast cancer activity of green synthesised AgNPs was assessed against MCF-7 cells. In brief, MCF-7 cells were maintained in DMEM supplemented with FBS (10%), L-glutamine (1%) and 1% of penicillin-streptomycin. The MCF-7 cells were cultured in a humidified CO₂ incubator at 37 °C for 24 h. The grown cells were treated with AgNPs (ranging from 25 to 100 µg mL⁻¹) and incubation continued for another 24 h at same conditions. Then, 20 µL of MTT was added to each well and incubated for 3 h. After the incubation, culture was removed and the formazan crystals formed were dissolved by adding 100 µL of DMSO. Finally, the plate was read in a microplate reader (BioTek, Germany) at 590 nm and the percentage of cell inhibition was calculated by Eq. (1).

$$\text{Cell inhibition (\%)} = \frac{\text{Absorption of the control} - \text{Absorption of the test}}{\text{Absorption of the control}} \times 100 \quad (1)$$

3. Results and Discussion

3.1. Green Synthesis of AgNPs

In order to check the green synthesis of AgNPs, AgNO₃ treated with *C. sativum* leaf extract was examined after 3 h incubation at 25 °C. The result demonstrates that the reaction mixture was turned into brownish color, which was the specific indication of AgNO₃ reduction (data not show). The UV-vis spectrum in Fig. 1 shows the absorption peak around 430 nm, which confirms the silver surface plasmon resonance [29].

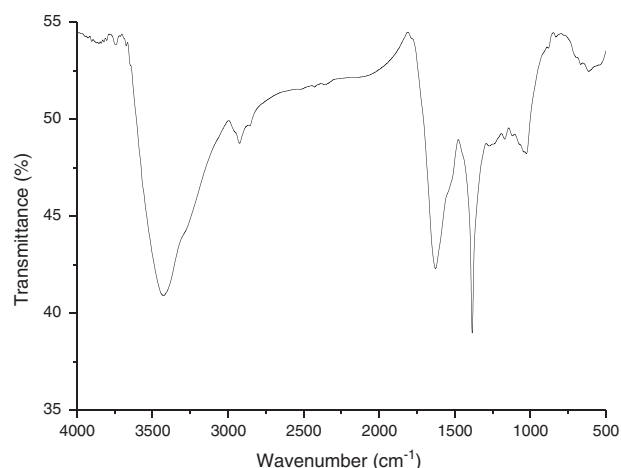


Fig. 3. FT-IR spectrum of AgNPs of green synthesised AgNPs by fractionated flavonoids of *C. sativum* leaf extract.

These results proved that the phytochemicals were present in the aqueous leaf extract of *C. sativum* might play a critical role in the reduction of AgNO₃ to form AgNPs.

3.2. Identification of Active Phytochemicals Involved in AgNPs Synthesis

The aqueous leaf extract of *C. sativum* was fractionated by column chromatography and used for nanoparticles synthesis to find out the type of active phytochemicals involved in the silver ion reduction process. Table 1 shows the phytochemicals present in the leaf extract of *C. sativum* and the active phytochemicals present in the fraction which are involved in the nanoparticles formation. The results clearly revealed carbohydrates, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids were present in the *C. sativum* leaf extract. Interestingly, the fraction which involved in the nanoparticles synthesis contains flavonoids. These results show that flavonoids may be responsible for the nanoparticles synthesis. This might be due to the hydroxyl and carbonyl groups of flavonoids acting as a reducing agent for the reduction of silver ion to AgNPs and also capping agent to prevent agglomeration [30–33]. The total flavonoids content of *C. sativum* crude leaf aqueous extract was found to be 27.25 mg g⁻¹; whereas, after purification 19.48 mg g⁻¹ was obtained with 71.49% of yield (Table 2).

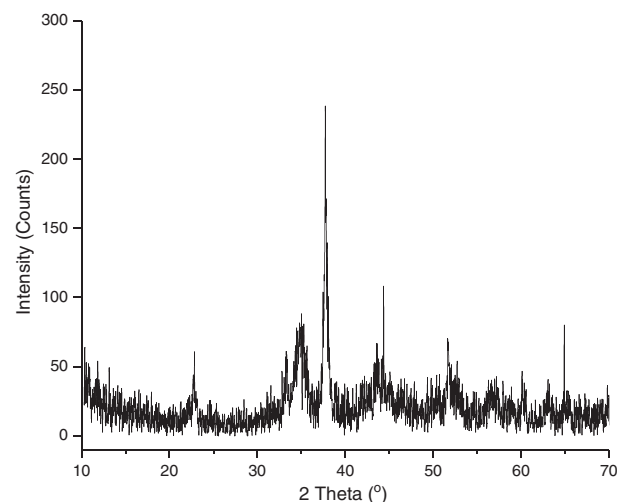


Fig. 4. XRD pattern of AgNPs of green synthesised AgNPs by fractionated flavonoids of *C. sativum* leaf extract.

Table 3
Anti-acne, anti-dandruff activities of green synthesised AgNPs.

Organisms	Zone of inhibition (mm in diameter)						Tetracycline ($\mu\text{g mL}^{-1}$)	Ketoconazole ($\mu\text{g mL}^{-1}$)	Minimal inhibitory concentration ($\mu\text{g mL}^{-1}$)
	Different concentration of green synthesised AgNPs ($\mu\text{g mL}^{-1}$)								
	4	8	12	20	40	60	1	50	
<i>P. acnes</i>	11.3 \pm 0.3	13.7 \pm 0.4	17.5 \pm 0.2	–	–	–	13.4 \pm 0.5	–	3.1
<i>M. furfur</i>	–	–	–	25.7 \pm 0.3	29.2 \pm 0.5	32.4 \pm 0.7	–	27.1 \pm 0.6	25

Tetracycline is a control drug for *P. acnes*.

Ketoconazole is a control drug for *M. furfur*.

In previous reports, Mariselvam et al. [34] observed alkaloids, carbohydrates, terpenoids, tannins, saponins and phenolic compounds containing fractionated extract of *Cocos nucifera* to synthesis nanoparticles. Recently, Sathishkumar et al. [10] found flavonoids present in the fractionated leaf extract of *Alternanthera tenella* induced AgNPs synthesis and as well as acted as a capping agent to prevent agglomeration. Sahu et al. [35] successfully synthesised AgNPs using three different flavonoids such as hesperidin, naringin and diosmin, and the size of the synthesised nanoparticles were found in the range of 5–80 nm. The presence of phytochemicals such as tannins, flavonoids and glycosides in *Chrysanthemum indicum* may be responsible for the formation of AgNPs by AgNO_3 reduction [36]. Mittal et al. [37] noticed the flavonoids present in *Potentilla fulgens* mediated nanoparticles synthesis was four-fold higher than phenolic compound, which indicated the significant role of flavonoids in the nanoparticles stabilization. Mittal et al. [38] successfully synthesised AgNPs using two different flavonoids such as quercetin and gallic acid. Moldovan et al. [33] reported that the hydroxyl and carbonyl groups of flavonoids derivatives present in the European black elderberry fruit extract may be involved in the reduction of silver ions to metallic silver. These previous literatures clearly indicates that flavonoids and few more phytochemicals present in the plant materials are involved in the AgNPs synthesis. Similarly, the present result also confirms that *C. sativum* flavonoids might be themselves involved in the green synthesis of AgNPs.

3.3. Physicochemical Characterisation of AgNPs

Fig. 2a illustrates the FESEM image of AgNPs green synthesised using fractionated flavonoids of *C. sativum*. The result shows that the green synthesised AgNPs was found to be spherical shape with average particle size of ≈ 37 nm. These nanoposts were subsequently identified using EDX as shown in Fig. 2b. The EDX shows strong signals for the silver

atoms; whereas, weaker signals for carbon, oxygen, potassium and chloride. The surface functional groups of green synthesised AgNPs were recorded by FT-IR analysis and the results are shown in Fig. 3. The spectrum shows intense and broad absorption band between 3500 and 3400 cm^{-1} , which denotes N—H stretching. A medium peak was found at 2925 cm^{-1} corresponds to C—H stretching, which might arise from alkyl group. The absorption bands appearing in 1638 cm^{-1} and 1100–1000 cm^{-1} , which denote the C=O and C—X stretching vibrations, respectively. According to Mariselvam et al. [34] absorption band at 1638 cm^{-1} in the spectrum indicating the formation of AgNPs and capped with the bio moieties. This result proved that the water soluble flavonoids present in the *C. sativum* leaf extract played an important role in AgNO_3 reduction.

The crystalline nature of the green synthesised AgNPs using fractionated leaf extract of *C. sativum* was studied by XRD and the results are shown in Fig. 4. Three major peaks was observed at 2θ values of 38.1°, 44.3° and 64.6°, which corresponds to the (111), (200) and (220) reflection planes of face-centered cubic structure Ag. These results confirms the synthesis of AgNPs with the sharp bands due to the stabilization of the synthesised nanoparticles by the reducing agent present in the leaf extract of *C. sativum*; thus, providing for the crystallinity of the AgNPs. The BET analysis result shows that the surface area of the synthesised AgNPs was found to be 33.72 $\text{m}^2 \text{g}^{-1}$.

3.4. Anti-acne, Anti-dandruff and Anti-breast Cancer Activities of AgNPs

From ancient period onwards, elemental silver and silver compounds have been used in traditional medicine to cure different kind of diseases. In this study, in order to know the anti-acne, anti-dandruff and anti-breast cancer efficiency of green synthesised AgNPs using fractionated leaf extract of *C. sativum*; *in vitro* antimicrobial and cytotoxicity studies were performed against *P. acnes*, *M. furfur* and MCF-7 cells.

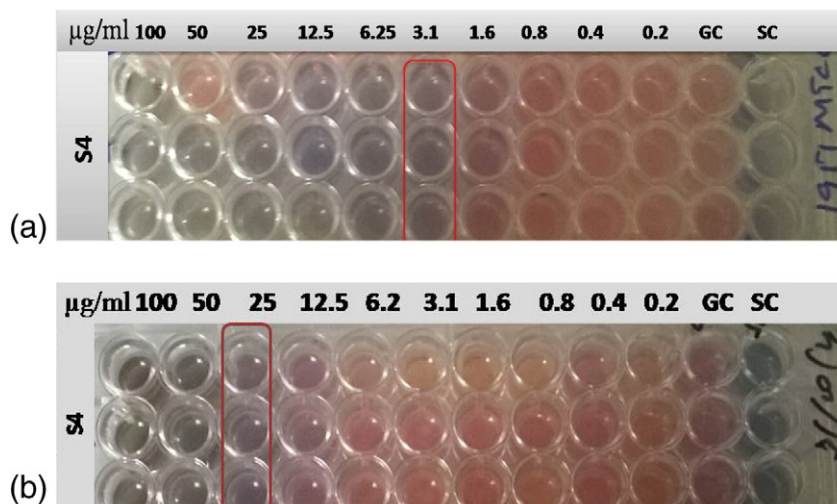


Fig. 5. The MIC determination of AgNPs for (a) *P. acnes* MTCC 1951 and (b) *M. furfur* MTCC 1374 in 96 well plate, respectively. (The experiment was run in triplicate.)

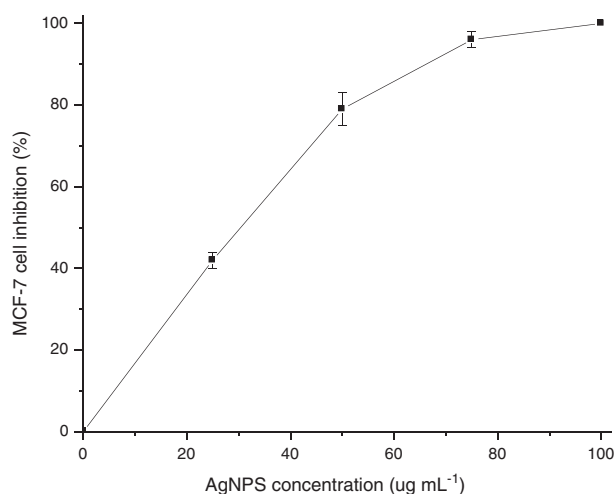


Fig. 6. Anti-breast cancer activity of AgNPs against MCF-7 cell line.

3.4.1. Anti-acne Activity

Silver nanoparticles have been reported for the antibacterial activity against various pathogens; however, there are very few reports on anti-acne activity. The plate culture of acne causative agent *P. acnes* showed apparent inhibition zones around the tested samples such as green synthesised AgNPs and tetracycline (control drug). As shown in Table 3, the antimicrobial activity result demonstrates that the ZOI of AgNPs against *P. acnes* were found to be 11.3 ± 0.3 , 13.7 ± 0.4 and 17.5 ± 0.2 mm by disc diffusion method for 4, 8 and 12 µg, respectively. Further, for more quantitative examination and also in order to know the antibacterial efficacy of AgNPs, MIC assay was performed. The MIC result clearly indicates that the MIC of AgNPs was found to be $3.1 \mu\text{g mL}^{-1}$ against *P. acnes* (Fig. 5a and Table 3). The mechanism of the bactericidal effect of silver colloid particles against bacteria is not completely proven. However, previous literatures survey establishes that the antibacterial property of AgNPs was maybe due to the adsorption of Ag to cell walls [39], membranous enzyme deactivation [40] and interaction of released ions with cell wall proteins leading to protein denaturation and formation of porous structures [41]. Plant secondary metabolites exhibit anti-acne properties with limited success and at higher concentrations than the present study using the green synthesised AgNPs using fractionated

leaf extract of *C. sativum* [42]. Recently, Gupta et al. [43] observed that the antimicrobial efficiency of *Lawsonia inermis* mediated synthesis of AgNPs against *P. acne* that causes acne. Paralikar [44] noticed the anti-acne activity of *Epiphyllum oxypetalum* leaf extract mediated AgNPs with a ZOI of 12.66 mm in diameter against *P. acne*. Pandit [45] reported the anti-acne activity of AgNPs synthesised by *Brassica nigra* seed extract, and their ZOI was found to be 8 mm in diameter. However, Paralikar [44] and Pandit [45] didn't mention the concentration of AgNPs used to measure the ZOI in their studies. Thus, it is difficult to compare the anti-acne efficacy of green synthesised AgNPs by *C. sativum* leaf extract with previous reports.

3.4.2. Anti-dandruff Activity

The antifungal activity of nanoparticles against dandruff causative agent *M. furfur* is illustrated in Fig. 5b and Table 3. The green synthesised AgNPs using fractionated leaf extract of *C. sativum* showed a maximum ZOI of 25.7 ± 0.3 , 29.2 ± 0.5 and 32.4 ± 0.7 for 20, 40 and 60 µg against *M. furfur* by well diffusion method, respectively (Table 3). It confirms the ZOI was increased along with increasing concentration of AgNPs. As evident in Fig. 5b, AgNPs showed MIC of $25 \mu\text{g mL}^{-1}$ against *M. furfur* by micro broth dilution method. Recently, AgNPs synthesised by *Macrophomina phaseolina* showed maximum antifungal activity against *M. furfur* [46]. Recently, Rao and Paria [47] observed the antifungal activity of *Aegle marmelos* leaf extract mediated AgNPs against *M. furfur* with a ZOI of 20.5 mm in diameter at 6.8 µg concentration. However, there is only limited report on anti-dandruff activity of green synthesised AgNPs in the literature. The present result confirms green synthesised AgNPs might be a better nanomedicine due to their biogenic nature and also it will affect the drug-resistant strains.

3.4.3. Anti-breast Cancer Activity

Fig. 6 illustrates the anti-breast cancer activity of green synthesised AgNPs using fractionated leaf extract of *C. sativum* on MCF-7 cells. The MCF-7 cells treated with AgNPs showed a dose dependent cell inhibition. The half maximal inhibitory concentration (IC_{50}) value of the AgNPs was calculated to $30.5 \mu\text{g mL}^{-1}$ and complete cell inhibition was found at $100 \mu\text{g mL}^{-1}$ of AgNPs. The mechanisms for AgNPs induced cytotoxicity might be due to the disruption of mitochondrial respiratory chain, which leads to the production of reactive oxygen species (ROS) and interruption of adenosine triphosphate (ATP) synthesis, thereby causing and DNA damage [48]. Recently, Jang et al. [49] noticed AgNPs inhibits the MCF-7 cells by the up-regulation of the p53

Table 4

Comparison of green synthesised AgNPs for anti-acne, anti-dandruff and anti-cancer activities with previous reports.

S. no.	Medicinal properties of AgNPs	Plant materials used for AgNPs synthesis	MIC ($\mu\text{g mL}^{-1}$)	ZOI (mm in diameter)	LD ₅₀ ($\mu\text{g mL}^{-1}$)	Reference
(i)	Anti-acne activity <i>P. acnes</i>	<i>E. oxypetalum</i> (leaf)	–	12.66 ^a		[44]
		<i>B. nigra</i> (seed)	–	8 ^a		[45]
		<i>C. sativum</i> (leaf)	3.1	17.5 (12 µg)		This study
(ii)	Anti-dandruff activity <i>M. furfur</i>	<i>A. marmelos</i> (leaf)	–	20.5 (6.8 µg)		[47]
		<i>C. sativum</i> (leaf)	25	25.7 (20 µg)		This study
(iii)	Anti-cancer activity	a Human liver cancer cell line	<i>M. pubescens</i> (leaf)		93.8	[9]
			<i>A. calamus</i> (rhizome)		69.4	[50]
		b Human cervical cancer cell line	<i>M. oleifera</i> (stem)		132.9	[51]
			<i>A. calamus</i> (rhizome)		32.1	[50]
		c Human lung cancer cell line	<i>G. hirsutum</i> (cotton)		40	[52]
			<i>O. vulgare</i> (leaf)		100	[54]
		d Human breast cancer cell line	<i>A. tenella</i> (leaf)		42.5	[10]
			<i>M. dubia</i> (leaf)		31.2	[53]
			<i>P. longum</i> (fruit)		67	[58]
			<i>C. sativum</i> (leaf)		31.5	This study

^a Unknown concentration of AgNPs used to determine ZOI.

tumor suppressor gene expression and the subsequent increase in the expressions of pro-apoptotic proteins like, Bax, caspase-3 and caspase-9. In addition, AgNPs down-regulates the mRNA levels of anti-apoptotic Bcl-2 and curtailed the JAK/STAT signaling in MCF-7 cancer cells.

Many studies have been published on the anticancer activity of AgNPs synthesised using various plant materials on different cancer cells (Table 4). Certainly, AgNPs in different formulations may exhibit variable dose effects. Recently, Sathishkumar et al. [10] noticed that the *A. tenella* leaf extract green synthesised AgNPs showed a dose-dependent increase in MCF-7 cell inhibition and the IC₅₀ value was calculated to be 42.5 µg mL⁻¹. The AgNPs synthesised using *Acorous calamus* extract had anticancer activity against human cervical cancer (HeLa) and human lung adenocarcinoma (A549) cell lines with the IC₅₀ values of 69.44 and 32.1 µg mL⁻¹, respectively [50]. Vasanth et al. [51] observed *Moringa oleifera* mediated AgNPs had anticancer activity on HeLa cell line by apoptosis induction up to a maximum of 94% mortality rate at 250 µg mL⁻¹ after 24 h incubation. The anticancerous activity of *Gossypium hirsutum* mediated AgNPs was observed against A549 cells and their IC₅₀ value was found at 40 µg mL⁻¹ [52]. Kathiravan et al. [53] found that AgNPs synthesised from *Melia dubia* leaf extract acts against human breast cancer (KB) cells with the IC₅₀ value of 31.2 µg mL⁻¹. The AgNPs biosynthesised by *Origanum vulgare* showed dose dependent response against A549 cell line and the LD₅₀ value was found at 100 µg mL⁻¹ [54]. Rajkuberan et al. [55] noticed the cytotoxicity of AgNPs biosynthesised by *Calotropis gigantea* latex was found against HeLa cell line with the LC₅₀ value of 91.3 µg [55]. The AgNPs biosynthesised using *Andrographis echinoides* leaf extract inhibited the MCF-7 cell line with 31.5 µg mL⁻¹ at 24 h [56]. *Rosa indica* triggers ethanolic petals extract mediated AgNPs showed apoptosis of human colon cancer (HCT 15) cell line by activating the caspases 3 and 9 [57]. The quercetin and gallic acid mediated synthesis of bimetallic (Ag–Se) nanoparticles had anticancer against dalton lymphoma (DL) cells and 80% of its viability was reduced at 50 µg mL⁻¹ [38]. Reddy et al. [58] reported that the AgNPs green synthesised using *Piper longum* fruit showed an effective cytotoxicity against MCF-7 cancer cell line with an IC₅₀ value of 67 µg mL⁻¹ at 24 h. Chanthini et al. [59] found the anti-cancer activity of *Cymodocea serrulata* mediated synthesis of AgNPs on HeLa and African green monkey kidney (Vero) cell line with the concentration of 34.5 and 61.24 µg mL⁻¹, respectively. Interestingly, this study confirmed that the AgNPs green synthesised using *C. sativum* leaf extract had better anticancer activity compared to most of the reports.

4. Conclusions

In this study, AgNPs were green synthesised using *C. sativum* leaf extract. The phytochemicals screening results proved flavonoids are mainly responsible in the reduction process of Ag⁺ to Ag⁰. The green synthesised AgNPs showed excellent anti-acne, anti-dandruff and anti-breast cancer activities against *P. acnes*, *M. furfur* and MCF-7 cells, respectively. The method used in this study is very simple, eco-friendly and economically viable, making it amenable to large-scale industrial production of AgNPs. However, further investigation of this green synthesised AgNPs for its biocompatibility will bring it into effective nano-drug for *in vivo* medical application.

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