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GREEN SYNTHESIS, CHARACTERIZATION AND APPLICATIONS OF SILVER NANOSTRUCTURES USING LEAF EXTRACT OF *ACACIA PENNATA*

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ABSTRACT

The Green synthesis of silver nanoparticles (AgNPs) was done by using the aqueous solution of *Acacia pennata* leaf extract and $AgNO_3$. Silver was of a particular interest for this process due to its evocative physical and chemical properties. A fixed ratio of plant extract to metal ion was prepared and the color change was observed which proved the formation of nanoparticles. The nanoparticles were characterized by UV-VIS Spectrophotometer, FTIR, DLS, Zeta Analysis and SEM. The nanoparticles were found have the size ranges from 160-180 nm. In antimicrobial activity, the test sample was most effective against certain bacterial strains whereas it was not showed good activity against fungal strains. This plant mediated Ag nanoparticles has the potential for the development of drugs for various diseases.

INTRODUCTION

In recent science, nanotechnology is a burning field for the researchers. Nanotechnology deals with the Nanoparticles having a size of 1-100 nm in one dimension used significantly concerning medical chemistry, atomic physics and all other known fields. Nanoparticles are used immensely due to its small size, orientation, physical properties, which are reportedly shown to change the performance of any other material which is in contact with these tiny particles. These particles can be prepared easily by different chemical, physical, and biological approaches. But, the biological approach is the most emerging approach of preparation, because, this method is easier than the other methods, eco-friendly and less time consuming. Due to swift industrialization and urbanization, our environment is undergo huge smash up and a large amount of perilous and superfluous chemical, gases or substances are released, and so now it is our need to learn about the secrets that are present in the Nature and its products which leads to the growth of advancements in the synthesis processes of nanoparticles. Nanotechnology applications are highly suitable for biological molecules, because of their exclusive properties. The biological molecules undergo highly controlled assembly for making them suitable for the metal nanoparticles synthesis which was found to be reliable and eco friendly (Harekrishna bar et al., 2009). The synthesis of metal and semiconductor nanoparticles is a vast area of research due to its potential applications which was implemented in the development of novel technologies (Ahmed John and Koperuncholan, 2012) the field of nanotechnology is one of the upcoming areas of research in the modern field of material science. Nanoparticles show completely new or improved properties, such as size, distribution and morphology of the particles etc. Novel applications of nanoparticles and nanomaterials are emerging rapidly on various fields (Kaviya and Viswanathan, 2011). Metal nanoparticles have a high specific surface area and a high fraction of surface atoms. Because of the unique physicochemical characteristics of nanoparticles, including catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties (Catauro et al., 2005) they are gaining the interest of scientist for their novel methods of synthesis.

MATERIALS AND METHODS

Preparation of plant extract

Fresh leaves of *A. pennata* were collected from Kolli hills in Namakkal district, Tamil Nadu and washed several times with water to remove the dust particles. The shade dried to remove the residual moisture and grinded to form powder. Then plant extract was prepared by mixing

1% of plant extract with deionized water in a 250 mL of (Borosil, India) conical flask. Then the solution was incubated for 30 min. and then subjected to centrifuge for 30 min. at room temperature with 5000 rpm (Koperuncholan and Ahmed John, 2011). The supernatant was separated and filtered with (mm filter paper pore size) filter paper with the help of vacuum filter. Then the solution was used for the reduction of silver ions (Ag^{+}) to silver nanoparticles.

Synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, silver nitrate was prepared at the concentration of 10^{-3} M with pre-sterilized Milli Q water. A quantity of 1.5 mL of each extract was mixed with 30 mL of 10^{-3} M of silver nitrate for the synthesis of silver nanoparticles. Silver nitrate was taken in similar quantities of 1.5 mL each without adding plant extracts to main respective controls (Koperuncholan and Ahmed John, 2011). The saline bottles were tightly covered with aluminium foil in order to avoid photo reduction of silver ions, incubated at room temperature under dark condition and observations were recorded at 15 m, 30 m, 1 and 2h.

Characterization of silver nanoparticles

UV-VIS analysis

The optical property of AgNPs was determined by UV-Vis spectrophotometer (Perkin-Elmer, Lamda 35, Germany). After the addition of $AgNO_3$ to the plant extract, the spectra's were taken in different time intervals up to 24 hrs between 350 nm to 500 nm. Then the spectrum was taken after 24 hrs of $AgNO_3$ addition.

FTIR analysis

The chemical composition of the synthesized silver nanoparticles was studied by using FTIR spectrometer (perkin-Elmer LS-55- Luminescence spectrometer). The solutions were dried at 75^{o} C and the dried powders were characterized in the range 4000–400 cm^{-1} using KBr pellet method.

SEM analysis

The morphological features of synthesized silver nanoparticles from *A. pennata* plant extract were studied by Scanning Electron Microscope (JSM-6480 LV). After 24Hrs of the addition of $AgNO_3$ the SEM slides were prepared by making a smear of the solutions on slides. A thin layer of platinum was coated to make the samples conductive. Then the samples were characterized in the SEM at an accelerating voltage of 20 KV.

DLS and zeta-potential analysis

Dynamic light scattering (DLS) which is based on the laser diffraction method with multiple scattering techniques was employed to study the average particle size of silver nanoparticles. The prepared sample was dispersed in deionized water followed by ultra-sonication. Then solution was filtered and centrifuged for 15 min. at 25 ^{0}C with 5000 rpm and the supernatant was collected. The supernatant was diluted for 4 to 5 times and then the particle distribution in liquid was studied in a computer controlled particle size analyzer (ZETA sizer Nanoseries,Malvern instrument Nano Zs).

Testing of antimicrobial activity

Microbial strains were tested for antimicrobial sensitivity using the well diffusion method. This method was used to evaluate in vitro antibacterial and antifungal activity of test sample against certain human pathogenic microorganisms on muller hinton agar (MHA) and potato dextrose agar (PDA), respectively (Ahmed John and Koperuncholan, 2012; Anitha et al., 2011; Beevi et al., 2012). A sterile cotton swab was used to inoculate the standardized bacterial suspension on surface of agar plate for even growth (Vignesh et al., 2012a; Pandiyarajan et al., 2013). The 2.5, 5 and 10 µL of test solutions (prepared with 100% of DMSO) were poured in each well (6 mm diameter), separately. One separate well was used for control study by taking 100% of DMSO (without test sample). The plates were incubated at 37±1°C for 24–48 h (for bacteria) and 25 ±1°C for 48-72 h (for fungus) (Lakshmi praba et al., 2013; Vignesh et al., 2013). After incubation, the zone of inhibition was measured with ruler/HiAntibiotic ZoneScale-C (Vignesh et al., 2012b). The assays were performed in triplicate and the average values are presented. Ampicillin (A); Erythromycin(E); Kanamycin (K); Methicillin (M); Nalidixic acid (Na); Trimethoprien (Tr); Tetracycline (T); Gendamicin (G). (for bacteria) and Ketoconazole(K) = fungus; Chloromphenicol (Ch) (for fungus) was used as positive control whereas DMSO (100%) used as a negative control. All the media, standard discs and HiAntibiotic ZoneScale-C were purchased from Hi-Media (Mumbai, India).

RESULT AND DISCUSSION

UV-Vis spectrophotometer analysis

Reduction of silver ions into silver nanoparticles during exposure to plant extracts was observed as a result of the color change. The color change is due to the Surface Plasmon Resonance (SPR) phenomenon. The metal nanoparticles have free electrons, which give the SPR absorption band, due to the combined vibration of electrons of metal nanoparticles in

resonance with light wave. The sharp bands of silver nanoparticles were observed around 421 nm in case of *A. pennata.* From different literatures it was found that the silver nanoparticles show SPR peak at around 420 nm. From our studies we found the SPR peak for *A. pennata* at 421 nm. So we confirmed that *A. pennata* leaf extract has more potential to reduce Ag ions into Ag nanoparticles, which lead us for further research on synthesis of silver nanoparticles from *A. pennata* leaf extracts (Figure 1). The intensity of absorption peak increases with increasing time period. This characteristic color variation is due to the excitation of the SPR in the metal nanoparticles. The reduction of the metal ions occurs fairly rapidly; more than 90% of reduction of Ag+ ions is complete within 2 Hrs. after addition of the metal ions to the plant extract. The metal particles were observed to be stable in solution even 4 weeks after their synthesis. By stability, we mean that there was no observable variation in the optical properties of the nanoparticles solutions with time. On the behalf of UV-vis data it was cleared that reduces metal ions (Ahmed John and Koperuncholan, 2012). So the further characterizations were carried out with *A. pennata.* The UV-Vis absorption spectroscopy is one of the main techniques followed to examine size and shape of the nanoparticles in the aqueous suspensions (Koperuncholan et al., 2010). Ramesh et al. (2014) reported formation of silver nanoparticles when constant aqueous $AgNO_3$ at 50 mL, 1 mM with 0.1 g bio-mass produced silver nanoparticles as indicated by sharp absorbance at around 440 nm in *Myristica dactyloides*.

FTIR analysis

FTIR measurements were carried out to identify the biomolecules for capping and efficient stabilization of the metal nanoparticles synthesized. The FTIR spectrum of silver nanoparticles (Figure 2) wherein some pronounced absorbance was recorded in the region between 4000 and 400 cm^{-1}. They include 3435 (secondary amine, free, N-H asymmetric stretching), 2076 (Diazo, RCH=N=N Stretching), 1638 (Nitrate, O-NO_2 Stretching asymm), 1371 (Alkanes, CH_3 symmetric bending, R-CH_3), and 695 (C-S, R-C-CH_3 stretching for sulphur compounds), cm^{-1}. Therefore the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids having functional groups. From the analysis of FTIR studies we confirmed that the carbonyl groups from the amino acid residues and proteins has the stronger ability to bind metal indicating that the proteins could possibly from the metal nanoparticles (i.e.; capping of silver nanoparticles) to prevent agglomeration and thereby stabilize the medium. This suggests that the biological molecules could possibly perform dual functions of formation and stabilization of silver nanoparticles in the aqueous

medium. Carbonyl groups proved that flavanones or terpenoids absorbed on the surface of metal nanoparticles. Flavanones or terpenoids could be adsorbed on the surface of metal nanoparticles, possibly by interaction through carbonyl groups or π-electrons in the absence of other strong ligating agents in sufficient concentration. The presence of reducing sugars in the solution could be responsible for the reduction of metal ions and formation of the corresponding metal nanoparticles. It is also possible that the terpenoids play a role in reduction of metal ions by oxidation of aldehydic groups in the molecules to carboxylic acids. These issues can be addressed once the various fractions of the plant extract are separated, identified and individually assayed for reduction of the metal ions. This rather elaborate study is currently underway (Figure 2).

SEM analysis

SEM provided further insight into the morphology and size details of the silver nanoparticles. Comparison of experimental results showed that the diameters of prepared nanoparticles in the solution have sizes several nm. i.e. between 1-100 nm (Figure 3). The size was more than the desired size as a result of the proteins which were bound in the surface of the nanoparticles (Koperuncholan and Ahamed John, 2011).

DLS analysis

The particle size distribution (PSD) of synthesized silver nanoparticles, it was found that Ag nanoparticles size was in the range of 80-120 nm. However, beyond 100 nm range the percentage of nanoparticles present is very less. The highest fraction of AgNPs present in the solution was of 73nm is very appropriate since it gives lowest average size of nanoparticles (Figure 4).

Zeta Potential Analysis

The Figure 4 shows the zeta potential (ζ) is a measure of the electrostatic potential on the surface of the nanoparticles and is related to the electrophoretic mobility and stability of the suspension of nanoparticles of the nano-silver. The overall absorbance of Zeta Potential revealed the energetically very unstable. Therefore the particles undergo agglomeration/aggregation to stabilize themselves. So there were some potential charges on the surface of the nanoparticles which makes them stable. These charge potential we got from this analysis. Zeta potential (Surface potential) has direct relation with the stability of a form/structure as mentioned below (Table 1 and Figure 5)

Antimicrobial activity of nanoparticles

Silver nanoparticles were tested in triplicates for antimicrobial activity. The mean values were recorded and presented (Tables 2). *A. pennata has* tested and recorded the results for the gram-positive, gram-negative bacteria and fungus. The gram-positive were highly sensitive than gram-negative bacteria. Selected microorganisms were showed significant sensitivity against the biosynthesized nanoparticles (Koperuncholan and Ahmed John, 2011). The antimicrobial activity of test sample was examined with various pathogenic microorganisms using the (measure the inhibition zone) well diffusion test. The results of the antimicrobial activities are summarized in Table 2. The three tested concentrations such as 2.5, 5 and 10 µL /well produce zone of inhibition on MHA and PDA plates for bacteria and fungi, respectively (5). In the present study, higher (10 µL/well) concentration of sample got greater sensitivity than (2.5 and 5 µL/well) lower concentration in all the tested microorganisms. In this study, all the pathogens were fairly affected and nil effect was not observed in the test samples. In bacteria, the test sample was most effective against B2, B3, B4, B5 & B9. While moderate effect was noticed from B1, B6, B7 & B8. In fungi, which was effective against F4 & F6 whereas average effect was observed in F1, F2, F3 & F5. All the microbial strains depict higher sensitivity to the higher concentration (10 µL) for the test samples. There is no antimicrobial activity in solution devoid of sample used as a vehicle control (100% DMSO), reflecting that antimicrobial activity was directly related to the sample. Overall good antimicrobial activity was observed.

CONCLUSION

The rapid biological synthesis of silver nanoparticles using *A. pennata* leaves extract provides environmental friendly, simple and efficient route for synthesis of benign nanoparticles. The synthesized nanoparticles were of spherical and sheet shaped and the estimated sizes were 160-180 nm. The sizes of the nanoparticles in different concentration were also different which depend on the reduction of metal ions. From the data of DLS it was found that the 30:1 ratio solution had sharp nanoparticles of around 5 nm and some has around 180 nm and they had the potential of around 15.5 mV. Anti microbial studies on some human pathogens, show the excellent results in the test sample and also give the good results. From the technological point of view these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as cost-effectiveness, compatibility for medical and pharmaceutical applications as well as large scale commercial production.

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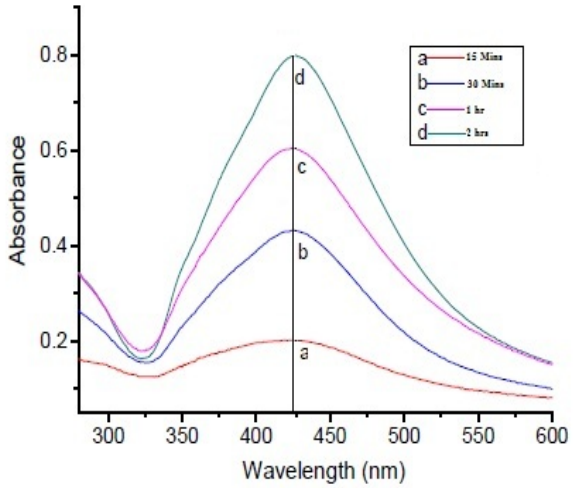


Figure 1. UV-VIS spectral analysis of Ag nanoparticles

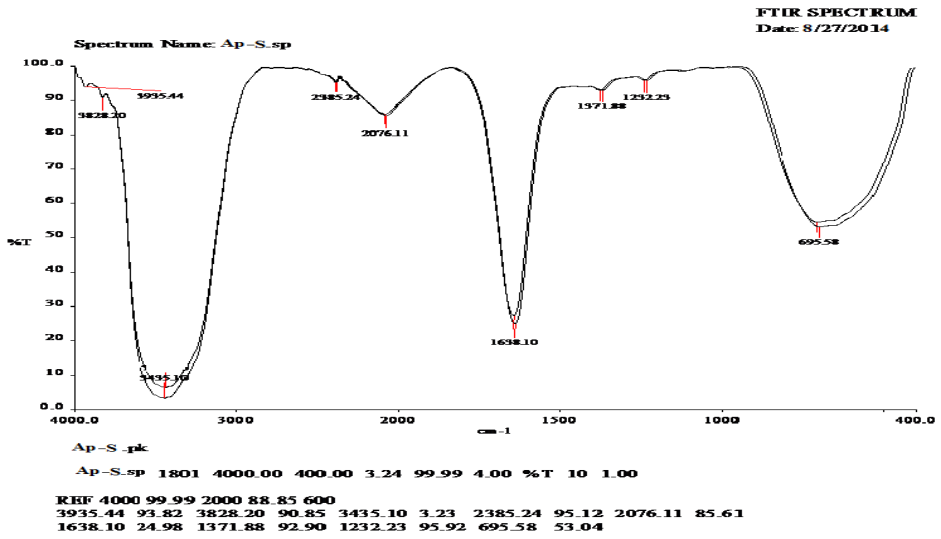


Figure 2. FTIR analysis of vibration modes and function groups of *A. pennata and* AgNPs

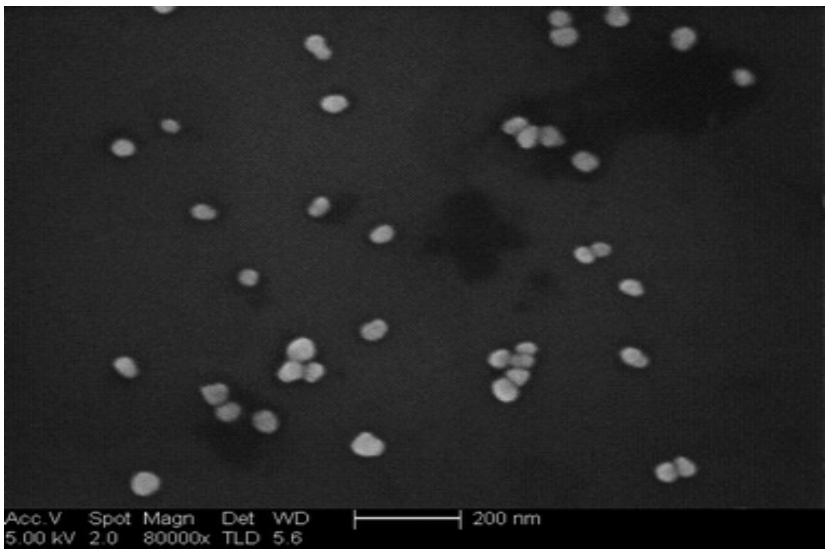
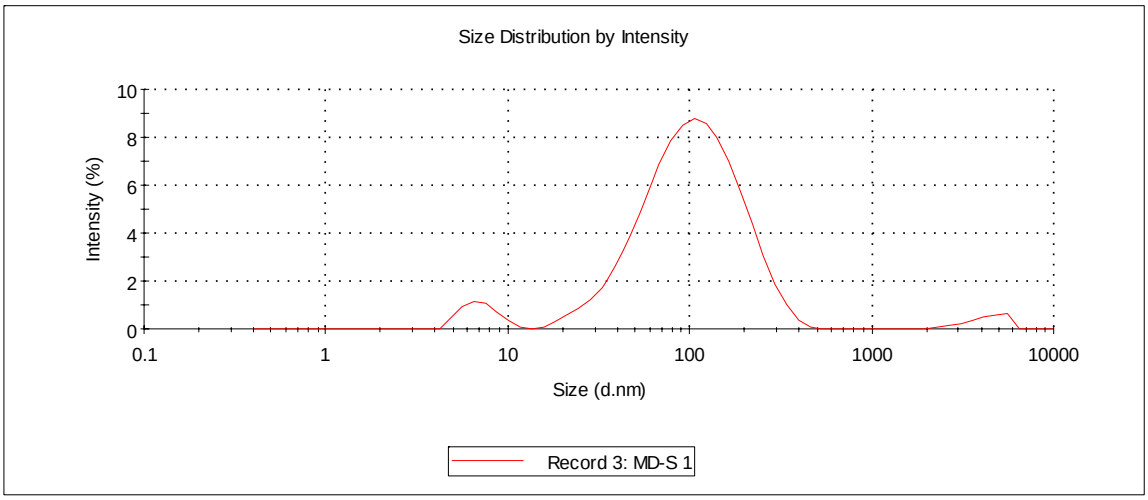


Figure 3. SEM –microscopic view of *A. pennata* reduced silver nano particles



A. pennata (Silver Nano particles); Z-Average (d.nm): 73.84

Figure 4. Dynamic light scattering of particle size analyzer of Ag nanoparticles

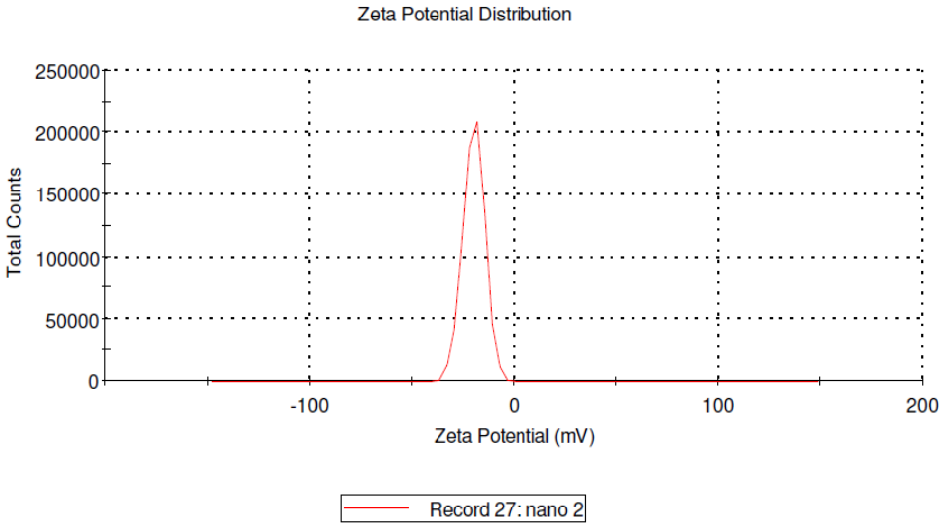


Figure 5. Zeta potential measurement of Ag nanoparticles

Results

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -19.7	Peak 1:	-19.7	100.0	5.29
Zeta Deviation (mV): 5.29	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.820	Peak 3:	0.00	0.0	0.00
Result quality : Good				

Zeta Potential (mV): from ±10 to ±30 = Incipient instability

Table 1. A table showing the stability of the NPs according to the potential charge

Zeta potential [mV]	Stability behavior of the colloid
from 0 to ±5	Rapid coagulation or flocculation
from ±10 to ±30	Incipient instability
from ±30 to ±40	Moderate stability
from ±40 to ±60	Good stability
more than ±61	Excellent stability

Table 2. Antimicrobial activity of Silver nanopartilces

Microorganisms	Silver nanoparticles (mg/mL)			Standard
	2.5	5	10	
Bacillus cereus (B1)	9	13	16	33 (T)
Bacillus subtilis (B2)	9	18	23	26 (A)
Staphylococcus aureus (B3)	12	16	23	45 (M)
Staphylococcus epidermidis (B4)	9	16	23	29 (T)
Gram-negative				
Aeromonas hydrophila (B5)	11	16	23	20 (Tr)
Escherichia coli (B6)	16	18	19	30 (K)
Klebsiella pneumoniae (B7)	10	11	11	30 (K)
Proteus mirabilis (B8)	12	19	20	25 (E)
Proteus vulgaris (B9)	11	19	23	30 (T)
Pseudomonas aeruginosa (B10)	12	17	23	20 (K)
Salmonella paratyphi(A) (B11)	9	12	15	30 (G)
Salmonella typhi (B12)	10	17	20	20 (Na)
Fungus				
Aspergillus fumigatus (F1)	13	16	17	(30) K
Aspergillus niger (F2)	12	14	17	(12) K
Candida albicans (F3)	13	17	21	(24) K
Microsporum canis (F4)	14	15	23	(32) Ch
Microsporum gypseum (F5)	16	18	21	(30) Ch
Trichophyton rubrum (F6)	17	19	25	(32) K

S= Standard; - = No activity; Measurements are given in mm; Ampicillin (A);

Erythromycin(E); Kanamycin (K); Methicillin (M); Nalidixic acid (Na); Trimethoprien (Tr); Tetracycline (T); Gendamicin (G); Ketoconazole(K) = fungus; Chloromphenicol (Ch).

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