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ORIGINAL ARTICLE

Synthesis and characterisation of zinc oxide nanoparticles using terpenoid fractions of *Andrographis paniculata* leaves

S. Kavitha[1,4] · M. Dhamodaran[1,2] · Rajendra Prasad[3] · M. Ganesan[3]

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Abstract Zinc oxide (ZnO) nanoparticles have been widely employed for various pharmacological applications. Several approaches were tried to synthesize ZnO nanoparticles. In this study, ZnO nanoparticles were biosynthesized using terpenoid (TAP) fractions isolated from *Andrographis paniculata* leaves. Subsequently, the $ZnNO_3$ (0.1 N) is treated with the isolated TAP fractions to biosynthesize zinc oxide nanoparticles (Zn-TAP NPs). This nanoparticle preparation has been confirmed by the colour change from green to cloudy-white and the peak at 300 nm by UV–Visible spectra. FTIR analysis of Zn-TAP NPs showed the presence of functional group (i.e.) C=O which has further been confirmed by H^1-NMR studies. From SEM and XRD analysis, it has been found that the hexagonal nanorod particle is 20.23 nm in size and +17.6 mV of zeta potential. Hence, it can be easily absorbed by negatively charged cellular membrane to contribute for efficient intracellular distribution. Therefore, it is suggested that the synthesised Zn-TAP NPs are more suitable in drug delivery processes.

✉ S. Kavitha
coolkavesh@gmail.com

M. Dhamodaran
dhamu_762003@yahoo.co.in

[1] Department of Chemistry (R&DC), Bharathiar University, Coimbatore, Tamil Nadu, India

[2] Department of Chemistry, Perunthalaivar Kamarajar Institute of Engineering and Technology (PKIET) (Government of Puducherry Institution), Karaikal, Puducherry U.T., India

[3] Department of Biochemistry and Biotechnology, Annamalai University, Chidambaram, Tamil Nadu, India

[4] A.V.C. Polytechnic College, Mannampandal, Mayiladuthurai, Nagai (DT), Tamil Nadu 609305, India

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Introduction

Nanotechnology is an upcoming field of science which has its impact in various fields such as energy, environment, electronics, etc. The widespread practical applications of metal nanoparticles (particles less than 100 nm) are attributed to their unique properties [1]. Different physical and chemical processes are widely used to synthesize metal nanoparticles [2]. However, these production methods are usually expensive, labor-intensive, and are potentially hazardous to the environment and living organisms [3]. Thus, there is an obvious need for an alternative, cost-effective and at the same time, safe and environmentally sound method of nanoparticles production [4]. During the past decades, it has been demonstrated that many biological systems, including plants and bacteria [5] and fungi [6] can transform inorganic metal ions into metal nanoparticles via the reductive capacities of the proteins and metabolites present in these organisms.

The ability of plant extracts to reduce metal ions has been known since the early 1900s, although the nature of the reducing agents involved was not well understood. Because of its simplicity, the use of live plants or whole plant extracts and plant tissue for reducing metal salts to nanoparticles has attracted considerable attention within the last 30-years [7]. Plant extracts act both as reducing and stabilizing agents in the synthesis of nanoparticles [8]. The source of the plant extract is known to influence the characteristics of the nanoparticles [9]. This is because different extracts contain different concentrations and combinations of organic reducing agents [10]. Typically, a

plant extract-mediated bioreduction involves mixing aqueous extract with an aqueous solution of the relevant metal salt. The reaction occurs at room temperature and is generally completed within a few minutes. Due to the number of different chemicals involved, the bioreduction process is relatively complex.

Nanoparticles have gained importance due to the awareness in biological processes. The chemicals present in the plant with anti-oxidant property are the basis for the preparation of zinc oxide nanoparticle. It has become a necessity to develop nanoparticles which in turn can be targeted on different applications [11]. Nanoparticles of zinc oxide are under intensive study for their applications in the field of optical devices, catalysis, biotechnology, DNA labeling, drugs and medical and chemical sensors. Nanosized zinc oxide has found various applications (sunscreen coatings and paints) due to its high absorption in UV [12, 13].

Currently, researchers are focusing on the synthesis of nanoparticles using green methods. Synthesis of nanoparticles using green methods increases the biological effectiveness. Bio-nanoparticles have greater catalytic activity due to the increase in the surface area. The possibility of using plant materials as nano-precursors has also been studied. The plant species *A. paniculata*, commonly known as *Nilavembu* in India, belongs to the *Acanthaceae* family. It is found in a large extent throughout South China, Asian countries and Sri Lanka. It is also known as "King of bitters" [14, 15]. Despite its bitter taste, this species possesses pharmacological properties, i.e. antimicrobial, antioxidant, antiinflammatory, antiparasitic, antihyperglycemic, hypoglycemic and antiallergic [16]. *Andrographis paniculata* reduces oxidation level due to its steroidal characteristics and destroys infected somatic cells. It contains diterpenes, lactones and flavonoids. The leaf and stem extracts have glycosides, flavonoids, gums, steroids, terpenoids, tannins, saponins and phenolic compounds [17]. Compared to its other parts, the leaf, which has multiple clinical applications, has huge amounts of terpenoid (TAP) (2.39%) which accounts for the bitter taste in leaves. This reason has been an impetus to isolate the medically most active and major compound TAP from the leaf. Therefore, in this study TAPs were isolated from *A. paniculata* and used to prepare ZnO nanoparticles for possible pharmacological applications.

Materials and methods

Collection of plant, chemical materials and cancer cell lines

The plant *A. paniculata* was collected from the campus of A.V.C. Arts and Science College, Mayiladuthurai. The AR grade chemicals and solvents like zinc nitrate tetrahydrate, sodium hydroxide, ethanol, chloroform, silica gel and $CDCl_3$ were procured from Merck Chemicals, Pune, India.

Extract preparation

The collected *A. paniculata* leaves were cleaned with tap water then rinsed with distilled water, dried, cut into small parts and ground into fine powder. It was further stored at 37 °C.

Preparation of terpenoid fractions from *A. paniculata*

The terpenoid fractions were separated by column chromatography. In this method, 25 g silica gel powder was filled in column apparatus and mixed with ethanol up to slurry formation. Then the solvent was completely eluted, and added 50% mixture (65 mL $CHCl_3$ and 1 mL methanol). It was followed by adding 10 mL ethanolic sample. Finally, the remaining mixture was added. The solvent was eluted. Within 12 h terpenoid was collected in test tubes.

Phytochemical test for terpenoid (TAP) fractions from *A. paniculata*

Confirmative test for terpenoids

Salkowskis test TAP was mixed with a few drops of chloroform and concentrated sulphuric acid. The formation of yellow colour indicates the presence of terpenoids.

The extract was treated with 1 mL of $CHCl_3$, 1 mL CH_3-COOH and few drops of concentrated sulphuric acid. Appearance of brown ring indicates the presence of terpenoids.

Synthesis of zinc oxide nanoparticles (Zn-NPS)

Zinc oxide nanoparticles were prepared by green synthesis (co-precipitation) method. 0.1 N aqueous solution of zinc

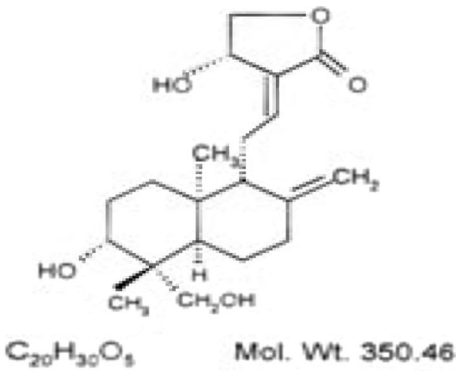

Fig. 1 TAP mediated Zn-TAP NPs synthesis

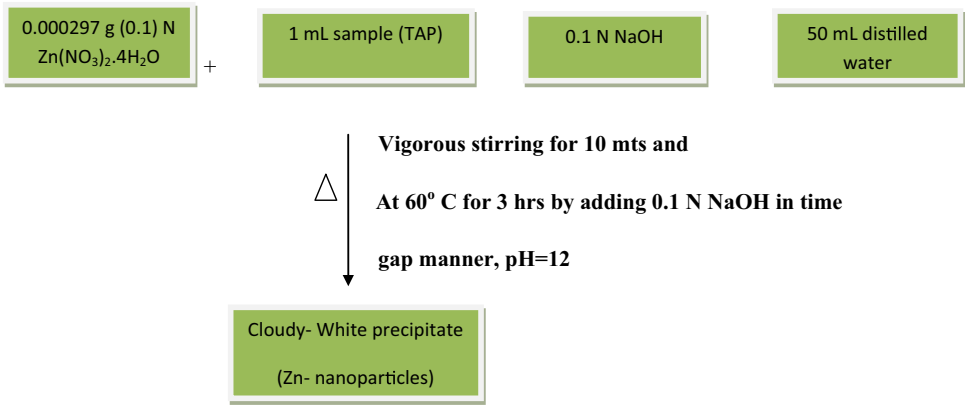

Fig. 2 Schematic diagram of Zn-TAP NPs synthesis

nitrate tetrahydrate ($Zn(NO_3)_2{\cdot}4H_2O$) was added to 50 mL distilled water by continuous shaking. Later 0.1 N NaOH was added in 10 m gap for an hour. Following that, the time gap was increased for adding the NaOH. The procedure was repeated for 2 h. The obtained white solution was stirred for 2 h at pH 12. The product was washed with distilled water and ethanol to get the final product. It is then dried overnight. The whole mode of proposed method for the synthesis of Zn-NPs mediated by the aqueous extract was illustrated.

Characterisation of terpenoid zinc oxide nanoparticles (Zn-TAP NPs) from *A. paniculata*

Particle size and zeta potential measurements

DLS and zeta potential were based on the direction and velocity of particles under the influence of known electric field. Malvern Zetasizer ZS (Malvern Instruments, Malvern, UK) instrument was used to measure particle size, size distribution and zeta potential of Zn-TAP NPs. To suit the above situation a homogenous suspension was created using the lyophilized nanoparticles in double distilled water and repeated thrice.

Scanning electron microscopy (SEM)

The samples were placed on a carbon plated platinum strip. Splash drops were wiped off. It was dried in mercury lamp for 5 m and examined under SEM (using JEOL JSM-6610 LV SEM machine).

X-ray diffraction (XRD) analysis

At $\lambda = 0.1546$ nm, running at 40 kV and 30 mA in X-ray diffractometer (X'Pert PRO-PANalytical Philips). Zinc oxide nanoparticles were recorded in the region from 10° to 80° at a scan speed of 2θ per minute.

UV spectroscopic analysis

The Zn-TAP NPs were dissolved in distilled water (1 mg/mL) and scanned in a Perkin Elmer Lambda 25 UV–Vis spectrometer at 25 °C in the range of 250–650 nm. The UV spectrum was repeated three times.

Fourier transform infrared spectroscopy (FTIR) analysis

Zn-TAP NPs and potassium bromide 10 and 100 mg were respectively mixed to form a salt plate. Spectra between 4000 and 400 cm^{-1} were noted in a Bio-Rad FTIR–40 (USA).

Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) analysis

An approximately 30 mg of Zn-TAP NPs was made soluble in 0.5 mL $CDCl_3$ (99.9%). At 27 °C ^{1}H-NMR spectra were recorded by Luo and Fan method (2011).

Results

Green synthesis of zinc oxide nanoparticles

The biosynthesised Zn-TAP NPs were isolated (Fig. 1) using 0.1 N Zinc nitrate with NaOH of co-precipitation method at pH 12 (Fig. 2).

Characterisation of Zn-TAP NPs from *A. paniculata*

Particle size and zeta potential of Zn-TAP NPs

Figure 3a shows the size distribution of the Zn-TAP NPs in aqueous medium. It was measured by DLS. The average particle size was 20.23 nm. Figure 3b reveals that the zeta potential of synthesized ZnO nanoparticles was 17.6 mV.

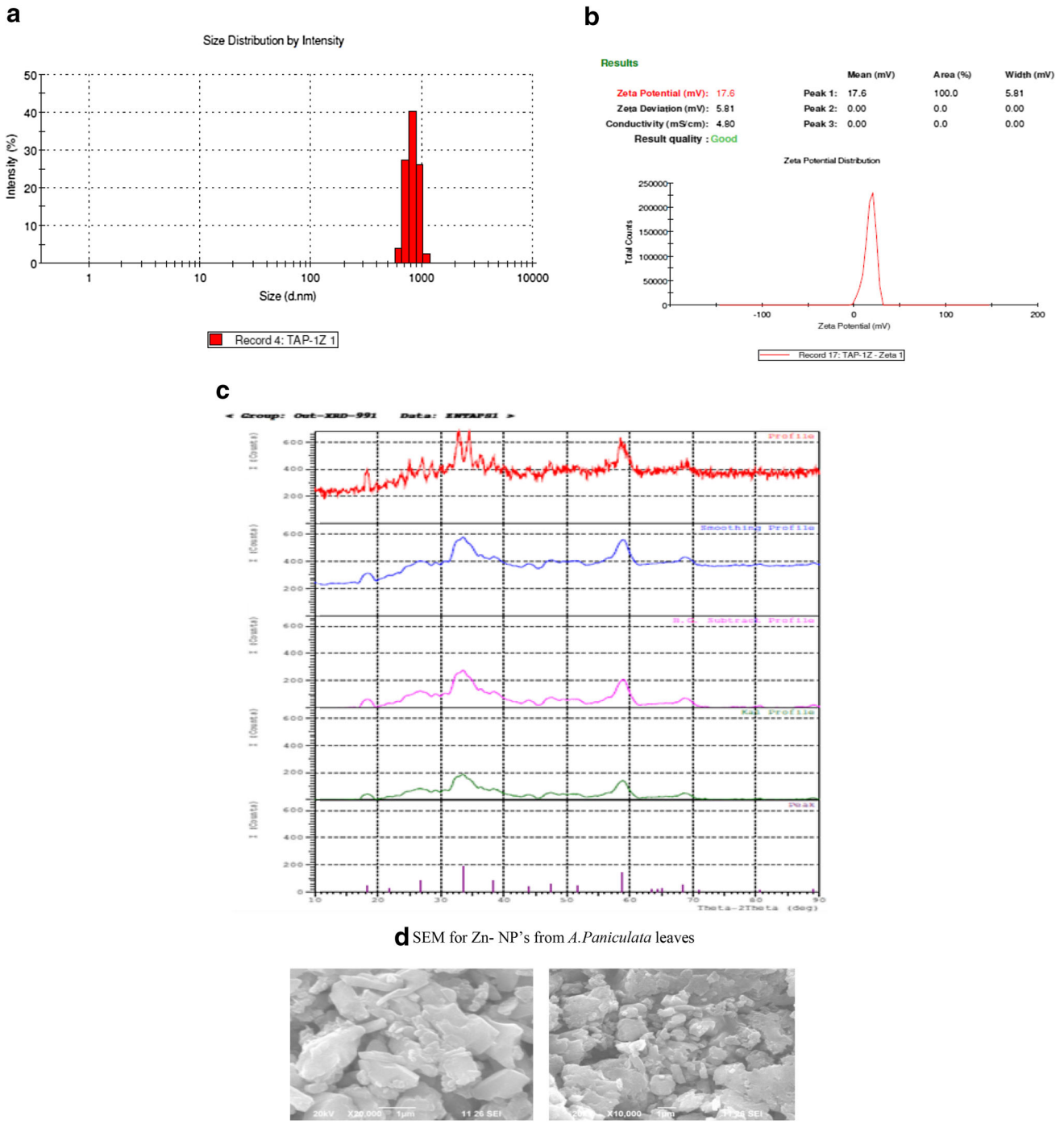

Fig. 3 **a** Particle size for Zn-TAP NPs from *A. paniculata* leaves. **b** Zeta potential of Zn-TAP NPs. **c** XRD images of Zn-TAP NPs. **d** SEM for Zn-TAP NPs

XRD analysis of Zn-TAP NPs

X-ray diffraction was used to confirm the crystalline nature of the particles by no discernible peak in the low range ($2\theta = 1°–10°$). Figure 3c shows a representative XRD pattern of the ZnO nanoparticles synthesized by the *A. paniculata* extract after the complete reduction of Zn^{2+} to Zn^0. A number of Bragg reflections were present which can be indexed on the basis of the hexagonal Wurtzite structure of ZnO. The diffraction peaks at (100), (002), (101), (102) and (110) were obtained with those reported values of standard card (JCPDS no: 36–1451) [18].

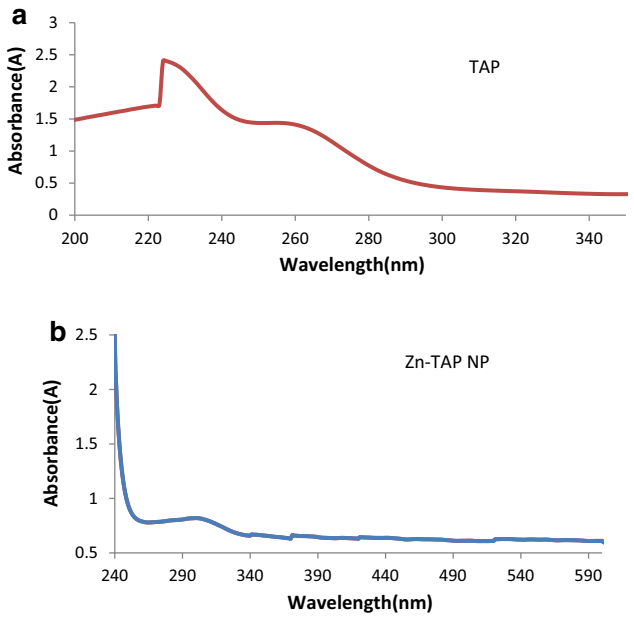

Fig. 4 **a** UV absorption of TAP isolated from *A. paniculata* leaves. **b** UV absorption of Zn-TAP NPs from *A. paniculata* leaves

From the XRD peaks, to estimate the average particle size was calculated by using Scherrer's equation as 22.23 nm. These values are merely similar to DLS size.

SEM analysis of Zn-TAP NPs

The synthesised Zn-TAP NPs morphology was examined by SEM in JEOL JSM-6610 LV instrument. When the nanoparticle was placed on carbon coated platinum grid, after completion of reaction, it showed a hexagonal shape as indicated in Fig. 3d.

UV spectroscopy studies

The formation of zinc oxide nanoparticles might be due to reduction of Zn^{2+} to Zn^{0} by terpenoids present in the leaf of *A. paniculata*. The colour transformation of *A. paniculata* extract treated with zinc nitrate might be due to vibrations in surface plasmon resonance of zinc [19]. Because it has free ¶-electrons (C=O). The broad band at 300 nm indicates the reduction of Zn^{2+} ions which further confirmed the formation of zinc oxide nanoparticles in Fig. 4b. Whereas, the isolated active compound TAP has no absorbance change in the range of 280–350 nm (Fig. 4a).

Fourier transform infrared spectroscopy (FTIR) studies

The FTIR spectroscopy measurements were studied to identify the possible bio-molecules responsible for reducing the ZNO-NPs synthesised through TAP. The IR spectra of TAP (Fig. 5a) exhibit strong absorption band at 3334.71 cm^{-1} for O–H stretching vibration. The C–O axial stretching band appears at 1656.36 cm^{-1}. The peak at 2852.85 cm^{-1} corresponds to C–H stretching vibration. Another report was assigned that the peak at 1656.36 cm^{-1} is due to –C=C– aromatic stretching.

The peak was observed for Zn-TAP NPs between 513 and 466 cm^{-1} (Fig. 5b). The bands appeared between 600 and 400 cm^{-1} which may be assigned to the metal oxide or metal chloride [20, 21], which confirm the formation of ZnO NPs at 466.77 cm^{-1}. The absorbance at 3479.58 cm^{-1} indicates the O–H stretching vibration in hydroxyl functional group of alcohols and phenol compounds of ZnO NPs. The absorption peak at 2929.87 cm^{-1} corresponds to –CH stretching and 2767.85 cm^{-1} for aldehydic –CH vibration mode. 1764.87 cm^{-1} is assigned

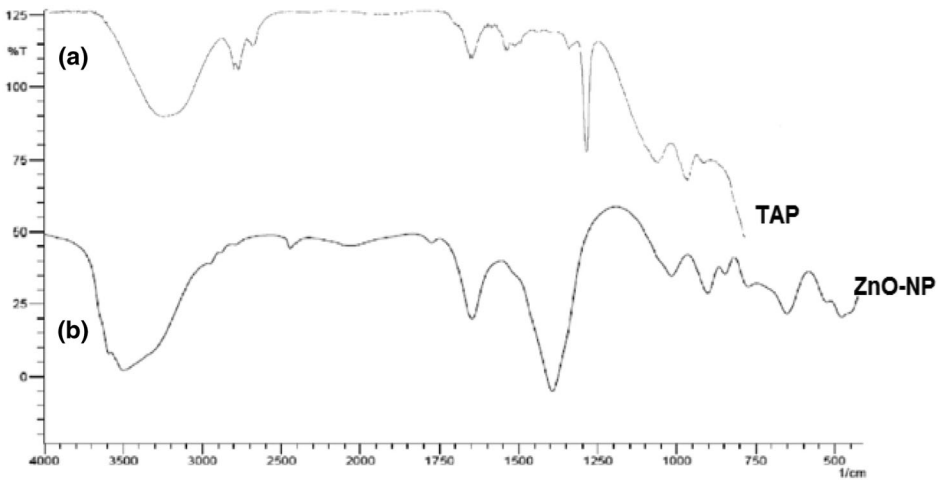

Fig. 5 **a** and **b** FTIR of TAP and Zn-TAP NPs from *A. paniculata* leaves

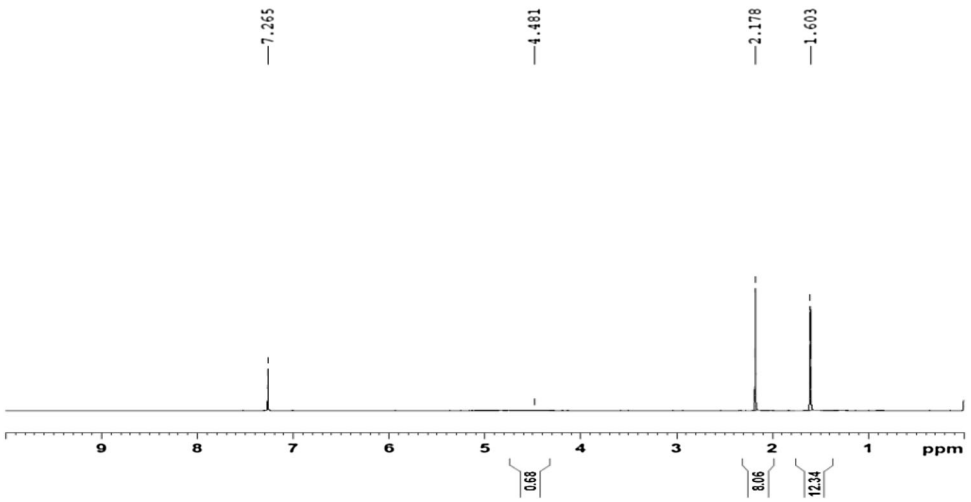

Fig. 6 H^1NMR for Zn-TAP NPs from *A. paniculata* leaves

to aldehydic carbonyl (–CH=O) group. Some other bands at 1637.56, 1382.96, 1004.91 and 640.37 cm^{-1} were also seen which correspond to aromatic –C=C–, C–H group, $–CH_2$ group and mono substituted ring which indicate that the TAP of *A. paniculata* reduced by ZnO NPs might be surrounded by aromatic ring polyphenol [22]. The band at 1637.56 cm^{-1} remained in ZnO NPs and this is due to groups with a benzene ring. Another report has assigned that the peak at 1637.56 cm^{-1} is due to –C=C– aromatic stretching [23].

Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) studies

In $CDCl_3$ solvent, H^1-NMR spectrum (Fig. 6) showed the 3H, s, $>C=CH_2$ group at δ 1.603. The peak at δ 2.178 assigned to phenyl group stretching. The absorption peak at δ 4.481 corresponds to $H_3C–O–CO–CH3$ stretching vibration. The band appears at δ 7.265 for aromatic compound. Another report was assigned that the peak at δ 2.178 and δ 4.481 corresponds to aliphatic –OH and aromatic –OH group respectively.

Conclusion

TAP could be adsorbed on the surface of nanoparticles possibly by interaction through carbonyl groups or ¶-electrons. The formation of ZnO NPs was confirmed by colour changes and was characterised by UV–Visible spectrophotometer. The broad band was observed at 300 nm. It was proved by IR spectra band at 466.77 cm^{-1}. The presence of functional groups in ZnO NPs was also confirmed by IR and H^1-NMR studies. The SEM analysis shows hexagonal shape particles with 20.23 nm size which was merely close to Scherrer's value (i.e.) 22.23 nm. Zn-TAP NPs has positive zeta potential value 17.6 mV. Hence, it was proposed that it can be easily absorbed by negatively charged cellular membrane and then contributes to efficient intracellular distribution of drug.

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