Supporting information

Biofilm impeding AgNPs target skin carcinoma by inducing mitochondrial membrane depolarization mediated through ROS production

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1. Methods

1.1 Preparation of methanolic extract

Both the powdered plant materials (5g) were extracted with 95% methanol for three days with occasional shaking. The extract was then filtered and dried in a rotary vacuum evaporator (IKA®RV10 rotary evaporator, China). The resultant extract was further lyophilized (LFD5508, Daihan labtech, India) and kept at 4° C for further characterization studies.

1.2 Qualitative and quantitative screening of phytochemicals

Qualitative screening of nanoparticles was done following the procedure standard protocol [1]. Quantitative screening of Total phenolic content (TPC) was determined by Folin-Ciocalteu's calorimetric method [2] and Total flavonoids content (TFC) was determined by spectrophotometrically by $AlCl_3$ method [3].

1.3 Analysis of total antioxidant activity

The free radical scavenging activity of methanolic extracts of *E. prostrata* and *A. sessilis* and their synthesized nanoparticles i.e. As-AgNPs and Ep-AgNPs were measured *in vitro* against the stable 2, 2, diphenyl-1-picryl hydrazyl (DPPH) [4].The hydrogen peroxide (H_2O_2) scavenging activities of the samples were determined by standard protocol [5]. The nitric oxide (NO) scavenging activity was quantified by Griess Illosvoy reaction [6]. The Ferric reducing antioxidant power activity (FARP) was measured by following standard protocol [7]. Ferrous ion (Fe^{2+}) chelating activity was estimated by the following the method of Dinis et al.[8].

2. Result

2.1 Qualitative and quantitative screening of phytochemicals

The phytochemical screening was done to detect the presence of diverse classes of secondary metabolites in the methanolic plant extracts. The test was based on the visual observation of the color change or formation of a precipitate after the addition of specific reagents. The results of the phytochemical screening are shown in Table 1. Phenolic compounds are the most abundantly found phytochemicals which as produced by the plants for their defence against various pathogens and predators. After qualitatively assessing the presence of phenolic group the total phenolic content (TCP) was calculated quantitatively. TPC of *A. sessilis* was calculated to be 241µg of GAE/mg and 139µg of GAE/mg for *E. prostrata*. Similarly the total flavonoid content (TFC) was calculated to be 266µg of RUE/mg and 171µg of RUE/mg for *A. sessilis* and *E. prostrata* respectively.

2.2 Antioxidant activity analysis

Various qualitative and quantitative assays are employed for determining the antioxidant properties of diverse samples based upon their rapid, cost effective and convenience. Among the various employed assays DPPH method is widely popular as it is independent of sample's polarity and its effectiveness in screening numerous samples at a given stretch of time. The DPPH scavenging activity of each samples and their synthesized AgNPs were investigated

for their antioxidative potentials. Among the plant extracts *A. sessilis* extract showed higher DPPH scavenging than *E. prostrata* extract (Fig 6a). Interestingly, in-case of their synthesized AgNPs, Ep-AgNPs showed higher scavenging potentials than As-AgNPs. The IC_{50} values were calculated to be 50.64±1.5 µg/mL, 48.26±2.2 µg/mL, 45.3±1.9 µg/mL and 44.35±3.9 µg/mL for As-PE, Ep-PE, As-AgNPs and Ep-AgNPs respectively. The lesser the IC_{50} value more is the antioxidant property. H_2O_2 is a weak oxidating agent which in itself is not very reactive but as it can rapidly cross the cell membrane it can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals and this may be the origin of its toxic effects. Therefore removing of H_2O_2 is very important for antioxidant defence in the cell organisation [9,10]. Among the four samples *E. prostrata* plant extract showed the highest H_2O_2 scavenging activity followed by *A. sessilis* plant extract, As-AgNPs and Ep-AgNPs (Fig 6b). The Fe^{2+} chelating activity is of great significance because it has been proposed that the transition metal ions contribute to the oxidative damage in various neurological disorders such as Alzheimer's and Parkinson's [11]. Therefore reduction of the formation of ROS can be achieved by the chelation of metal ions with chelating agents such as the plant extracts and nanoparticles. In the present study, *E. prostrata* plant extract showed the highest Fe^{2+} chelating activity followed by *A. sessilis* plant extract, As-AgNPs and Ep-AgNPs (Fig 6c).
Ferric reducing antioxidant power assay (FRAP) depicts the electron donating capability of the bioactive compound thereby reflecting their reducing power. Greater the absorbance at 700 nm greater is their FARP activity. Fig 6d shows the FARP activity of the plant extracts and their synthesized AgNPs where *E. prostrata* plant extract exhibited higher ferrous reducing activity followed by *A. sesillis* plant extract and in-case of their synthesized AgNPs Ep-AgNPs showed greater ferrous reducing potentials than As-AgNPs. The samples exhibited dose dependant NO scavenging activity where both the plant extracts of *A. sessilis* and *E. prostrata* having IC_{50} value of ~51.5±84 µg/mL whereas As-AgNPs had IC_{50} value of 57.97±0.92 µg/mL and Ep-AgNPs 48.07±0.44 µg/mL. NO is one of the components of the ROS family and is implicated in various inflammation reactions, cancer and other pathological conditions. Thus the plant products and their synthesized AgNPs might have some property to counter act the effect of NO formation which might help to prevent the ill effects of excessive NO generation in the body [12].

Supporting information

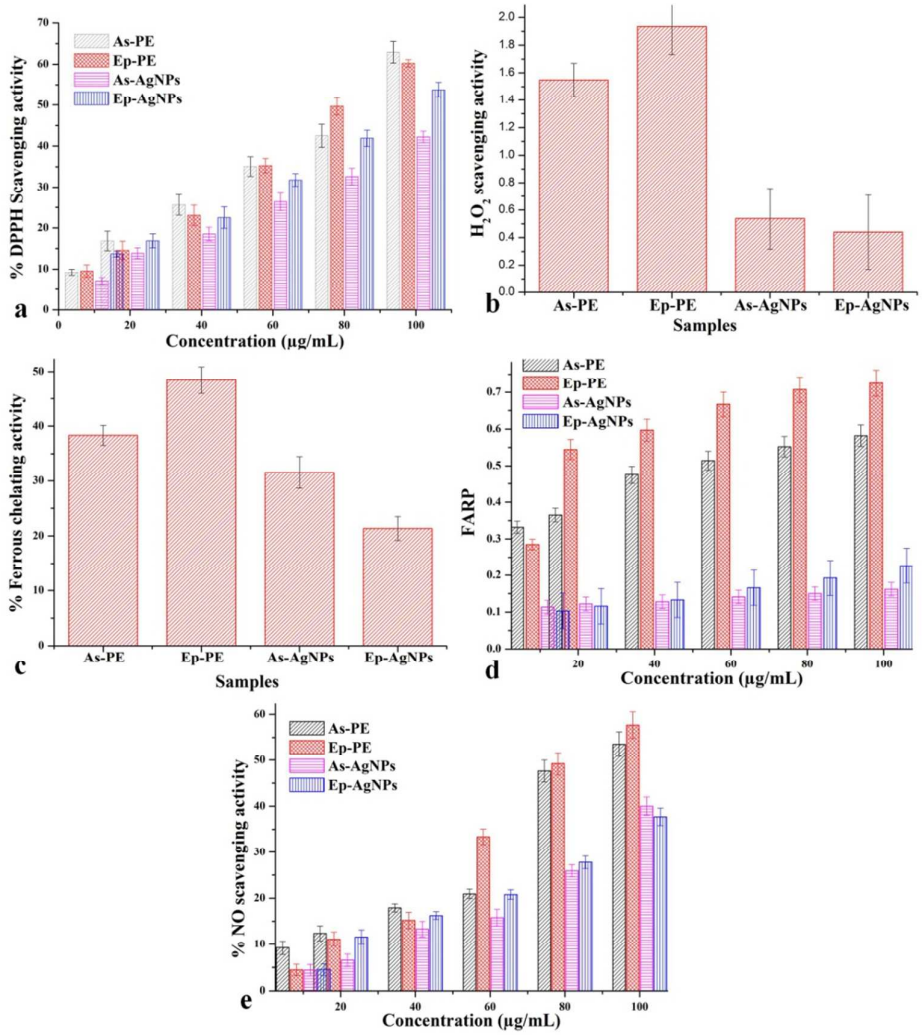

Figure S1: Screening of antioxidant activity of *A. sessilis* and *E. prostrata* plant extracts and their synthesized As-AgNPs and Ep-AgNPs through (a) DPPH scavenging activity; (b) H_2O_2 scavenging activity; (c) Ferrous chelating activity; (d) Ferrous reducing antioxidant power activity; (e) Nitric oxide scavenging activity.

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