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Green synthesis of silver nanoparticles using *Artemisia annua* and *Sida acuta* leaves extract and their antimicrobial, antioxidant and corrosion inhibition potentials

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Abstract
Silver nanoparticles were synthesized by a rapid, cost effective and environmentally benign technique using ground leaves extract of *Artemisia annua* and *Sida acuta* as reducing as well as capping agent. Silver nanoparticles (AgNps) were formed within 10-15 minutes by sunlight irradiation of aqueous solution (0.1M) of silver nitrate ($AgNO_3$) with leaves extract of *Artemisia annua* and *Sida acuta*. The synthesized AgNp's of both extracts were characterized using UV-visible spectrophotometer. The reaction mixtures showed absorbance at 450nm which is characteristic of silver nanoparticles due to the surface plasmon resonance absorption band. The antibacterial activity of these nanoparticles was studied against (*Staphylococcus aureus, Escherichia coli and Streptococcus faecalis*). Antifungal activity was studied against *Candida albicans.* The results of the antimicrobial studies showed good inhibitory effect against *Staphylococcus aureus, Escherichia coli and Candida albican*. Also, anti-corrosion activity of these silver nanoparticles on the corrosion of mild steel in 0.5M HCl solution was studied by potentiodynamic polarization curves and the results show that they are good inhibitors. The antioxidant activity of both the extracts and synthesized AgNP's was analyzed by 1,1-diphenyl-2-picryl-hydrazzyl (DPPH) radical scavenging assay using ascorbic acid as control and they were all found to exhibit good antioxidant activity especially at lower concentrations.

Keywords: Silver nanoparticles, Antioxidant activity, Antimicrobial activity, Corrosion inhibition.

1. Introduction
The field of nanotechnology is one of the most active areas of research in modern materials science. Nanotechnology is concerned with the development of experimental processes for the synthesis of nanoparticles of different sizes, shapes and controlled disparity [1]. This provides an efficient control over many of the physical and chemical properties and their possible application in optoelectronics, recording media, sensing devices, catalysis and medicine [2]. Nanoparticles exhibit completely new or improved properties based on specific characteristics such as size distribution and morphology.

A number of approaches are available for the synthesis of nanoparticles for example, reduction in solution, chemical and photochemical reactions in reverse micelles, thermal decomposition of metal compound, radiation assisted, electrochemical, sonochemical, microwave assisted process and recently via green chemistry route [3].

Biological methods of synthesis have paved way for the "Green Synthesis" of nanoparticles and these have proven to be better methods due to slower kinetics, better manipulation and control over crystal growth and their stabilization.

The use of environmentally benign materials like plant leaf extract, bacteria and fungi for the synthesis of nanoparticles offers copious benefits of eco-friendliness and compatibility for pharmaceutical and biomedical applications as they do not use toxic chemicals in the synthesis protocols. Chemical synthesis methods lead to the presence of some toxic chemicals absorbed on the surface that may have adverse effect in the medical applications [4]. Green synthesis provides advancement over chemical and physical methods as it is cost

effective, environment friendly [5], easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals. In the present communication, we present the syntheses, characterization, antimicrobial, antioxidant and corrosion inhibition potentials of silver nanoparticles obtained from the reaction between silver nitrate and the leaves extract of *Artemisia annua* and *Sida acuta* plants.

2. Materials and Methods

2.1. Materials

All chemicals used were analytical grade obtained from commercial source and used as received. The chemicals were silver nitrate ($AgNO_3$), Hydrochloric acid (HCl), Ascorbic acid, 1,1-diphenyl–2–picrylhydrazyl (DPPH) and Methanol (CH_3OH).

2.2. Instruments

UV-Vis spectra were recorded using Unicam Helios (λ) spectrophotometer.

All electrochemical measurements were carried out at 25 ^oC using Princeton Applied Research Versa STAT 400 advanced electrochemical system. Antioxidant activities were measured at 517 nm using Spectrophotometer.

2.3. Collection and Identification of Plant Materials

Fresh leaves of both plants [*Sida acuta* and *Artemisia annua* (Figures 1a and 2a)] were collected without infection from farms in Ibong Ikot Akan, Ikot Ekpene and Ikpa Road, Uyo, respectively and were identified and authenticated at the Department of Botany and Ecological Studies University of Uyo, Uyo, Akwa Ibom State, Nigeria.

2.4. Preparation of the Leaves / Extraction

The collected leaves were thoroughly washed with distilled water, chopped into pieces, sun dried for days, ground into powder using an electric blender and stored in well labeled air tight containers.

For the extraction, 20g of ground leaves of *Sida acuta* and *Artemisia annua* were respectively added to 200mL of distilled water in 250mL beakers. The mixtures were boiled for 15 minutes, allowed to cool and filtered through Whatman No.1 filter paper. The freshly prepared aqueous extracts were used immediately after filtration. Fresh extracts were used for all the analysis.

2.5. Synthesis of Silver Nanoparticles

Aqueous solution of $AgNO_3$ (0.1 M) was prepared and used for the synthesis of silver nanoparticles. 5mL of aqueous extract of *Artemisia annua* was added to 95mL of 0.1M aqueous solution of silver nitrate in 250mL Erlenmeyer flask, mixed thoroughly by manual shaking and placed under sunlight for reduction into silver nanoparticles (AgNPs). The same was done for *Sida acuta*. After 10 minutes both solutions turned from yellow to yellowish red and to dark brown, indicating the formation of silver nanoparticles.

2.6. UV-visible Analysis/Characterization

The characterization of the synthesized *Artemisia annua* and *Sida acuta* silver nanoparticles were carried out using UV-visible spectroscopy. The reduction of silver ions was monitored from 200 nm-800 nm by Unicam Helios (λ) spectrophotometer after 10-fold dilution of the samples with distilled water. The spectral data recorded were then plotted.

2.7. Corrosion Inhibition Studies

Electrochemical experiments were carried out for both *Artemisia annua* and *Sida acuta* silver nanoparticles in the conventional three-electrode cell with a platinum counter electrode (CE) and a saturated calomel electrode (SCE) coupled to a fine Luggin capillary as the reference electrode. In order to minimize ohmic contribution, the Luggin capillary was placed close to the working electrode (WE) which was in the form of square mild steel embedded in polyvinyl chloride (PVC) holder using epoxy resin so that the flat surface was the only surface in the electrode. The working surface area was 1.0 cm × 1.0 cm. Before measurement, the electrode was immersed in test solution (0.5 M HCl) at open circuit potential (OCP) for 30 minutes to be sufficient to attain a stable state. All electrochemical measurements were carried out at 25 ^oC using Versa STAT 400 advanced electrochemical system. The potential of potentiodynamic polarization curves system was increased at $0.5mVS^{-1}$ and started from a potential of -250 to + 250mV versus OCP. Inhibition efficiency (Ep%) was calculated using.

$$Ep(\%) = \left(\frac{i_{corr} - i^{`*}_{corr}}{i_{corr}} \right) \times 100 \quad (1)$$

where i_{corr} and i^*_{corr} are the uninhibited and the inhibited corrosion current densities, respectively.

2.8. Antioxidant Activity Studies

The antioxidant activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free – radical scavenging method [6]. 1.0g of DPPH, a stable radical was dissolved in 100mL of methanol. 3.0mL of different concentrations of the test samples were added to 3.0ml of a 0.004% methanol solution of DPPH and incubated for 30 minutes at room temperature. The decrease in absorbance of the solution brought about by the test samples were measured at 517nm using spectrophotometer. Ascorbic acid, which is a known antioxidant, was used as a reference standard. The radical scavenging activity was calculated as the percentage inhibition of DPPH discoloration using the equation below:

$$\%inhibition = \left(\frac{A_{blank} - A_{sample}}{A_{blank}} \right) \times 100 \qquad (2)$$

where A_{blank} is the absorbance of the control reaction solution (containing all reagents except the test compound) and A_{sample} is the absorbance of the test compound.

2.9. Antimicrobial Analysis

The assessment of antimicrobial activity was carried out with the following strains (microorganisms): *Staphylococcus aureus, Escherichia coli, Streptococcus faecalis* and *Candida albican* with Gentamycin as control using agar well diffusion method. The culture media was prepared by the method of Fawole and Oso [7]. 7.0g of Muller Hinton agar was dissolved in 200mls of sterile water and allowed to soak, thereafter, it was boiled to jell. 9 mL dilution blanks were prepared and sterilized alongside with the medium at 121^{o}C for 10 minutes. On cooling, about 25mL of the molten medium was aseptically poured into sterile Petri dishes and allowed to set (solidified). 0.1 mL of the different standardized working isolates were used to seed the sterile plates respectively. A lawn was made with hockery stock to have a uniform distribution of the inoculum on the plates. Wells of 4 mm x 4 mm were bored in the medium using sterile cork borer and 0.1mL of different antimicrobial agents were dropped into the holes aseptically. The assay plates were held at 4 ^{o}C for 1 hour after which they were incubated at 37 ^{o}C for 24 hours for sensitivities. After the incubation, inhibition zones for the respective antimicrobial agents were measured in diameter.

3. Results and Discussion

3.1. Synthesis and UV-Vis Characterization

The synthesis of silver nanoparticles by reduction of aqueous silver nitrate into silver nanoparticles (AgNPs) during exposure to leaves extract can be easily monitored by using UV-visible spectrophotometer. Gradual changes in colour from yellow to reddish yellow and from reddish yellow to dark brown were observed for mixtures containing aqueous solution of 0.1 M silver nitrate, *Sida acuta* and *Artemisia annua* leaves extract respectively, indicating the formation of silver nanoparticles. These characteristic colour variations are due to the metal nanoparticles. Silver nanoparticles (AgNPs) have free electrons, which give rise to a surface plasmon resonance (SPR) absorption band [8], due to the combined vibration of electrons of silver nanoparticles in resonance with the light wave [9]. Figures 1a and 2a show *Sida acuta* and *Artemisia annua* plants while Figures 1b and 2b show the gradual colour changes of the *Sida acuta* and *Artemisia annua* leaves extract and their AgNP's respectively.

(a) (b)

Fig. 1. (a) *Sida acuta* plant (b) Left: *Sida acuta* AgNPs; Right : Aqueous extract

The reduction of Ag^{+} ions into AgNPs was further confirmed using UV-Vis spectroscopy. Figures 3 and 4 depict the UV-Vis spectra of the synthesized nanoparticles from *Sida acuta* and *Artemisia annua* . A strong, broad absorption band with maxima located at 450 nm confirms the formation of *Sida acuta* and *Artemisia annua* AgNPs, respectively.

(a) (b)

Fig. 2. (a) *Artemisia annua* plant (b) Left: Aqueous extract; Right: *Artemisia annua* AgNPs.

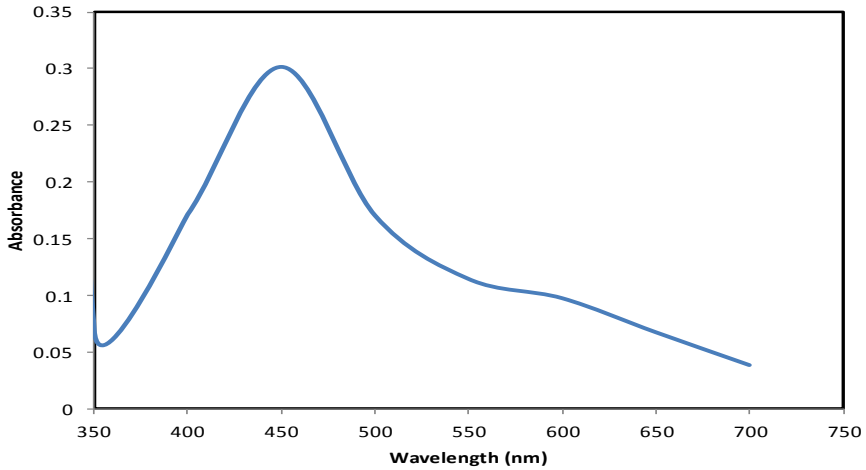

Fig. 3. UV-Vis absorption spectrum of silver nanoparticles synthesized from *Sida acuta* leaves in 0.1M silver nitrate.

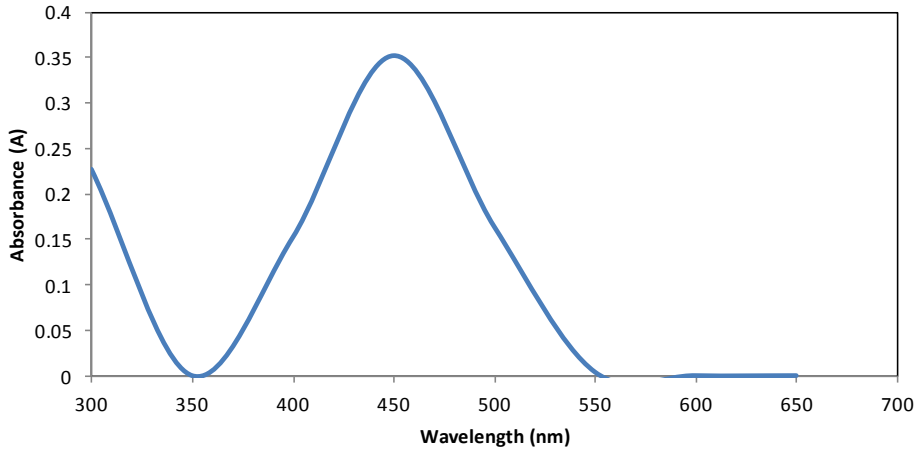

Fig. 4. UV-Vis absorption spectrum of silver nanoparticles synthesized from *Artemisia annua* leaves in 0.1 M $AgNO_3$.

3.2. Anti-microbial Activity

The result of antimicrobial activity with zones of inhibition measured in millimeters is as shown in Table 1.

Table 1. The results of antimicrobial activity with zones of inhibition

Micro-organisms	Zones of inhibition (mm)					
	Artemisia extract	*Artemisia* AgNP	*Sida acuta* extract	*Sida acuta* AgNP	Silver nitrate	Gentamycin
Staphylococcus aurus	NA	14	15	10	NA	35
Escherichia coli	NA	15	NA	13	10	38
Streptococcus faecalis	NA	NA	NA	NA	12	40
Candida Albicans	13	18	NA	21	10	-

Where NA = No Activity and (-) = undetermined

The aqueous extract of *Artemisia annua* showed good inhibitory effect against the fungus, *Candia albican* with inhibition zone of 13 mm but no activity against the bacterial strains (*S. Aureus, E. coli and S. faecalis*). The *Artemisia* AgNP exhibited good inhibitory effect against *S. aureus, E.coli* and *Candida albican* with inhibition zones of 14, 15 and 18 mm, respectively but no inhibitory effect was found against *S. faecalis*. *Sida acuta* AgNP showed good inhibitory effect against *S.aureus* (10mm), *E.coli* (13 mm) and *Candida albican* (21 mm) but no activity against *S. faecalis*. Silver nitrate ($AgNO_3$) exhibited inhibitory effect against all the tested strains [*E.coli* (10 mm), *S. faecalis* (12mm) and *C albican* (10mm)] except *S. aureus*. More so, *Sida acuta* extract showed inhibitory effect against *S. aureus* only with inhibition zone of 15 mm but no antimicrobial activity against other tested strains. Finally, the standard (Gentamycin) showed the highest inhibitory effect against all the micro-organisms with inhibition zone of 35 mm for *S. aureus*, 38 mm for *E. coli* and 40 mm for *streptococcus faecalis*.

Figure 5 shows antimicrobial activity of *Sida acuta* and *Artemisia annua* aqueous extracts, AgNP's, $AgNO_3$ and control against pathogenic bacterial and fungal strains.

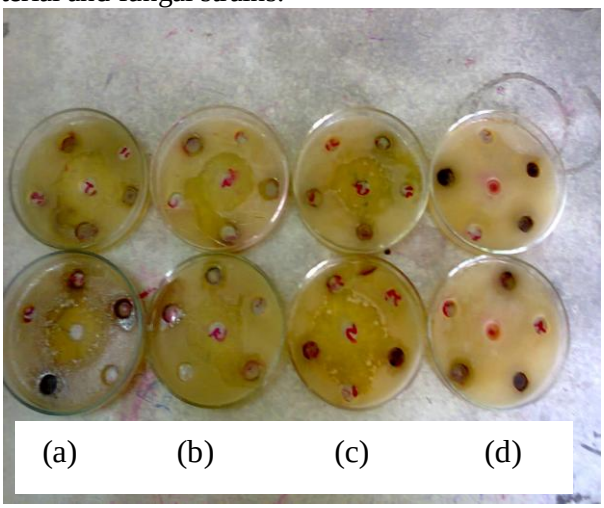

Fig. 4. UV-Vis absorption spectrum of silver nanoparticles synthesized from *Artemisia annua* leaves in 0.1 M $AgNO_3$.

In recent times, nanoparticles have gained importance in the field of biomedicine. The
The most important and distinct property of nanoparticles is that they exhibit larger surface area to volume ratio. Specific surface area is relevant for catalytic reactivity and other related properties such as antimicrobial activity in AgNPs. As specific area of nanoparticles increased, their biological effectiveness can increase due to the increase in surface energy [10].

Thus, a decrease in the AgNP's size can lead to an increase in the specific surface of a bacterial specimen, inducing an increase in their ability to penetrate cell membrane and thus improving antibacterial activity. Several studies proposed that AgNPs may attach to the surface of the cell membrane disturbing permeability and respiratory functions of the cell [11, 12]. It is also possible that AgNPs not only interact with the surface of membrane but also penetrate inside the microorganism [13].

The bacterial activity is presumably due to certain changes in the membrane structure of bacteria cell as a result of the interaction with the embedded AgNPs which leads to the increased membrane permeability of the bacteria and consequently, leading to their death.

3.3. Antioxidant Activity

The effect of antioxidants on DPPH is thought to be due to their hydrogen donating activity [14]. As DPPH is considered as the lipophilic radical, it readily accept electron from the anti-oxidant compound and converts its colour from violet to yellow which was detected at 517nm. Thus, DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. At 517nm, the absorbance of the DPPH solution (i.e. the blank) was 0.502nm. The reduction in absorbance of DPPH at 517nm caused by the samples was measured in triplicate after 10min. the tested samples showed very good activity when compared with the standard used (Table 2).

There was a decrease in absorption at 517nm indicating that the leaves extract and AgNPs have hydrogen donating ability or can scavenge free radicals. From the analysis, the samples showed a concentration dependent radical scavenging ability. This observation was further corroborated by calculating percentage inhibition for all the test samples.

It was observed that *Artemisia annua* extract exhibited good activity as free radical scavenger compared to the control. The extract gave percentage inhibition of 80% while that of the control was 78% at 0.002 mg/mL. *Artemisia annua* AgNPs and *Sida acuta* AgNPs were 37% and 21% respectively at 0.002 mg/mL. The antioxidant activity may be due to the flavonoids present in the plant extract.

Table 2. Absorbance values from scavenging effect of test samples on DPPH at 517nm.

Concentration (mg/mL)	*Artemisia* extract	*Artemisia* AgNP	*Sida acuta* AgNP	Ascorbic acid
0.001	0.099	0.315	0.397	0.109
0.002	0.104	0.339	0.320	0.095
0.003	0.139	0.374	0.346	0.115
0.005	0.161	0.406	0.392	0.107

3.4. Corrosion Inhibition Studies

Potentiodynamic polarization curves of mild steel in 0.5M HCl with and without (%v/v): 2, 4 and 6 *Artemisia annua* AgNP and 2, and 4 *Sida acuta* AgNP at 25◦C are shown in Figures 6 and 7 respectively. In all cases, increase in the concentrations of the *Artemisia annua* AgNP and *Sida acuta* AgNP caused a remarkable decrease in the corrosion rate i.e. shifts both anodic and cathodic curves to lower current densities, in other words, both cathodic and anodic reactions of mild steel electrode are drastically inhibited by the AgNPs. The electrochemical corrosion parameters including corrosion current densities (I_{ccor}), corrosion potential (E_{corr}), cathodic Tafel slope (b_c), anodic Tafel slope (b_a) and corresponding inhibition efficiency (Ep) for *Artemisia annua* AgNP and *Sida acuta* AgNP are given in Tables 3 and 4, respectively.

Table 3. Potentiodynamic polarization parameters for corrosion of mild steel in 0.5M HCl solution containing different concentrations of inhibitor at 25° C (Immersion time is 30 minutes).

Inhibitor	E_{corr}	I_{corr}	b_c	b_a	
	(mVvsSCE)	(μAcm^{-2})	($mVdec^{-1}$)	($mVdec^{-1}$)	Ep(%)
Blank	-529.57	557.61	161.72	99.25	-
2% *Artemisia annua*	-495.40	359.36	100.02	62.89	35.55
4% *Artemisia annua*	-495.91	91.67	73.09	46.78	86.56
6% *Artemisia annua*	-486.94	67.72	90.91	57.99	87.85

It is apparent that i_{corr} decreases considerably with increasing AgNP concentration. Correspondingly, inhibition efficiency (Ep) increases with increasing concentration of inhibitor due to the increase in the blocked fraction of the electrode surface by adsorption of the AgNPs onto the steel surface [15, 16-24]. Inhibition efficiency (Ep) of 35.55% for 2%, 83.56% for 4%, 87.85% for 6% *Artemisia annua* AgNP and 59.31% for 2%, 78.80% for 6% *Sida acuta* AgNP were obtained, respectively, which confirmed that that AgNP from *Artemisia*

annua and *Sida acuta* are good corrosion inhibitors for mild steel in 0.5M HCl. This work adds to recent research on bio-synthesis and application of silver nanoparticles using plant extracts [25-28].

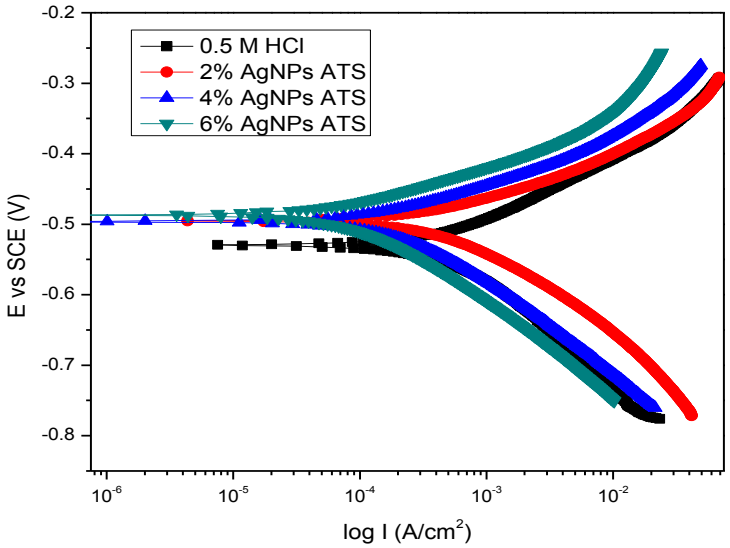

Fig 6. Potentiodynamic polarization curve for mild steel in 0.5M HCl at different concentrations AgNP from *Artemisia annua* at 25C.

Table 4. Potentiodynamic polarization parameters for the corrosion of mild steel in 0.5M HCl solution containing different concentrations of inhibitor at 25°C (immersion time is 30 minutes).

Inhibitor	E_{corr} (mV vs SCE)	I_{corr} (μAcm^{-2})	b_c ($mVdec^{-1}$)	b_a ($mVdec^{-1}$)	Ep(%)
Blank	-529.57	557.61	161.72	99.25	
2% *Sida acta* AgNPs	-498.00	226.90	69.37	47.98	59.31
6% *Sida acta* AgNPs	-492.13	118.22	53.75	39.25	78.31

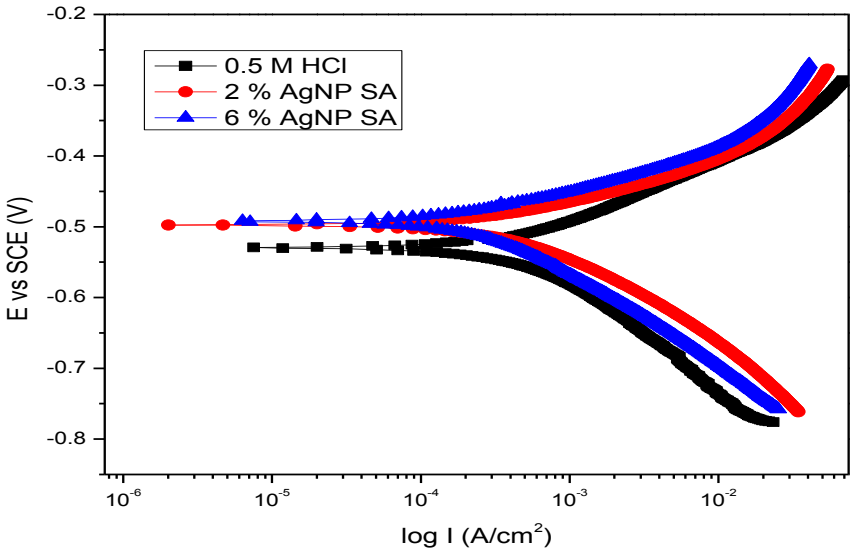

Fig 7. Potentiodynamic polarization curve for mild steel in 0.5M HCl at different concentrations of AgNP from *Sida acuta* at 25°C (immersion time 30 minutes).

Conclusions

The extracts of *Artemisia annua* and *Sida acuta* were found capable of producing silver nanoparticles and were quite stable in solution. The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction.
The plant extracts and AgNPs showed good potential as antimicrobial and antioxidant agents. Also the plant-mediated AgNPs acted as good inhibitors for the corrosion of mild steel in 0.5M HCl solutions. Inhibition efficiency increases with inhibitor concentration.

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