



GREEN SYNTHESISED SILVER NANOPARTICLE OF COLOCASIA ESCULENTA: INVITRO ANTIOXIDANT AND ANTIULCER ACTIVITY

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Abstract: In the present research, a methanolic extract of *Colocasia esculenta* leaves is used to produce silver nanoparticles in an environmentally friendly manner. The chemical families of flavonoids and phenols were highly present in this therapeutic plant. Once silver nitrate was transformed into silver nanoparticles (Ag NPs), Zeta potential, particle size analysis, and FT-IR were used to characterize the particles. Numerous functional groups surrounding Ag NPs were verified by the FT-IR investigation. It was discovered through particle size analysis that the synthesised silver nanoparticles were nano in size. Ag NPs' calculated zeta potential of -46.5 mV confirms particle repulsion and hence raises the stability of the formulation. Investigating Ag NPs' antioxidant and anti-ulcer qualities, the findings showed a strong capacity to scavenge free radicals and a notable boost in the extract's capacity to neutralize acids. It was discovered that the greenly synthesized silver nanoparticles were a potentially effective phytomedicine for ulcer treatment.

Keywords: Antiulcer activity; Antioxidant activity; Green synthesis; phytochemical studies; silver nanoparticle.

INTRODUCTION

Nanomedicine is a prominent area of research these days. The main goal of science is to create drugs that are less costly, less dangerous, and safe for treating conditions like ulcer, diabetes, cancer, epilepsy, etc. Owing to these nanoparticles' site-specific activities, drug molecules can only be administered at a safe and advised dosage, which helps to minimise unintentional toxicity. The targeted activity of these nanoparticles increases the drug's efficacy. They have an advantage because of their small size, which enables them to evade immune reactions and pass through frequently impermeable membranes. The green synthesis of nanoparticles is preferred since chemical synthesis necessitates the reduction of metals using many hazardous agents. In order to carry out green synthesis, plants naturally contain both primary and secondary metabolites. In industry, green synthesis techniques for producing silver nanoparticles are widely used for a range of purposes, such as antiulcer, anti-diabetic, antimicrobial, antioxidant, and anticancer applications¹⁻⁵.

One of the most common causes of being hospitalised is ulcers, and their prevalence is rising over the world. Peptic ulcers are a non-fatal medical disorder characterized by severe discomfort, disruption of daily routines, disorientation, and mental distress in addition to recurrent episodes of epigastric pain that are often relieved by food or alkali. People may develop ulcers due to a variety of factors, including stress and prolonged use of anti-inflammatory drugs. While the exact aetiology of ulcers is still mostly unknown, it is generally accepted that the imbalance between hostile factors and the mucosa's ability to be maintained by the endogenous defense mechanism is what leads to ulcers. A

medication for peptic ulcers must either raise the mucosal resilience to aggravating stimuli or diminish their effects on the gastroduodenal mucosa. An erosion of the lining of the stomach or duodenum is called a peptic ulcer. Ulcers can range in depth from deeper abrasions to superficial epithelial irritation, causing organ bleeding and perforation⁶⁻¹⁰.

Colocasia esculenta (L.) Schott commonly called taro or cocoyam, is a perennial herbaceous plant, with large spherical corm from which a few heart-shaped leaves emerge at the tip on long petiole reaching 1-2m height grown in many parts of India and is a member of the Araceae-Arums family anti-inflammatory, antioxidant, hepatoprotective, hypolipidemic, anticancer, anti-melanogenic, anti-inflammatory, antimicrobial, anti-helminthic, estrogenic, and neuropharmacological properties of *Colocasia esculenta*, are employed. In traditional medicine, the leaves of *Colocasia esculenta* are used as good stimulant, expectorant, astringent, appetizer and otalgia. These plants have yielded a number of isolated substances, including phenols, terpenoids, saponins, flavonoids, and tannins¹¹⁻¹².

The current study synthesises, characterises, and examines the antioxidant and antiulcer characteristics of Ag NPs generated from a methanolic leaf extract of *Colocasia esculenta* using FTIR, UV-vis spectroscopy, particle size analysis, and zeta potential. The acid neutralising ability of a *Colocasia esculenta* methanol extract was used in this investigation to assess its antiulcer efficaciousness. Thus, an effort was made to support its conventional claim as an anti-ulcer agent using these chosen techniques.

METHODS

COLLECTION OF PLANT

The leaves of *Colocasia esculenta* were harvested in November from Hyderabad district in Telangana, and the authenticity of the leaves was confirmed by Osmania University botanist Balakrishna. After drying in the shade for roughly 20 days at room temperature, the leaves were ground into a coarse powder using a mixer grinder. The powdered material was stored or taken up for extraction process.

Preparation of plant extract

20 grams of *Colocasia esculenta* dry leaf powder was dissolved in 200 ml of methanol in a beaker and covered properly with aluminium foil and leave it for 24 hours, in between shake it from time to time so the compounds which are soluble in methanol get dissolved or soluble in methanol and after that filter the product. Now evaporate the filtrate product in room temperature for 4 to 5 days. After complete evaporation of filtrate scrap out the extract which is obtained and measure it¹³.

Preliminary phytochemical screening

Tannins and phenols

(Ferric Chloride Test)

FeCl₃ (0.1%) was added to 1 ml of the extract and observed for brownish green or a blue-black coloration shows the presence of tannins and phenol.

Flavonoids

(Alkaline reagent test)

Two to three drops of sodium hydroxide were added to 2 mL of extract. Initially, a deep yellow colour appeared but it gradually became colourless by adding few drops of dilute HCL, indicating that flavonoids were present.

Terpenoids

(Salkowski Test)

5 ml of aqueous solution of extract sample was mixed with 2 ml of CHCl₃ in a test tube. 3 ml of concentrated H₂SO₄ was carefully added to the mixture to form a layer. An interface with a reddishbrown coloration was formed which showed the presence of terpenoids

Saponins

(Foam Test)

20 ml of the extract sample was measured into test tube and boiled in a water bath, then filtered. Half of above sample after filtration was shaken vigorously for consistent froth formation. To this froth olive oil (few ml) was added. Presence of saponins is ascertained if emulsion forms¹⁴.

Green synthesis of Ag NPs

Plant extract was prepared from the leaves of *Colocasia esculenta*. Before the preparation of extract, leaves were washed with distilled water to remove dust particles and other impurities. Shade dried leaves were powdered by using mixture grinder. 8 gm of leaf powder was added into 200 ml methanol and heated at 60 °C for 15 min then cooled and supernatant was filtered using Whatman filter paper No. 1. 2 mM solution of silver nitrate was prepared by dissolving 0.0225 gm of AgNO₃ in 75 ml of distilled water. Next, 100 ml of leaf extract was combined with 50 ml of silver nitrate

solution. A colour Change from light green to colloidal yellowish green black colour indicated the formation of silver nanoparticles. After 30 min, 100 ml of reaction mixture was centrifuged for 5 min. at 5000 rpm. The solid particles as sediment were collected, washed with distilled water followed by methanol and further dried at room temperature. Formation of silver nanoparticle was further checked by UV-Visible spectroscopy¹⁵.

Quantitative estimation of phenol by FC method

Procedure:

To 1ml plant extract add 4ml of distilled water. To this mixture add 1.5ml of the FC reagent. For a quick spin the contents are placed on the cyclometer and leave it for 5minutes. After that add 5ml of Na₂CO₃ to the contents and incubate for 1 hr a bluish colour is formed. Observe the absorbance at 650nm under UV spectrometer¹⁶.

Quantitative estimation of flavonoids

Procedure:

In this method, 100μL of plant extract, NPCE, 400 μL of methanol was added. Quercetin was used as standard to make the calibration curve at different concentration (20, 40, 60, 80, 100 μL) dissolved in methanol. Give a quick spin and then add 100 μL of 10% AlCl₃ and 1M sodium acetate was added again give a quick spin on addition of each. Incubate MECE, NPCE, standard in room temperature away from light for 40-45 min. check absorbance at 415nm¹⁷.

In-vitro antioxidant activity:

DPPH Method

Procedure:

The capacity of sample extracts to reduce DPPH as been assessed by this method. 0.004% of DPPH solution was prepared by dissolving 4mg of DPPH in 100 ml of methanol. Plant extract and NPCE of different concentrations (100, 200, 300, 400, 500 μg/ml) was utilized followed by makeup the volume up to 3 ml with methanol. Ascorbic acid was used as standard. Add 1ml methanolic DPPH to each test tube. Incubate at room temperature for 30 min and measure the absorbance at 517nm and calculate IC₅₀ value¹⁸.

Nitric oxide scavenging assay

Procedure:

Extract, nanoparticles prepared in methanol, was added to different test-tubes in varying concentrations (100, 200, 300, 400, and 500 μg/ml). Each test tube received 5 mM sodium nitroprusside in phosphate buffer to fill it to the full 1.5 ml capacity. For thirty minutes, solutions were incubated at 25°C. Thereafter, 1.5ml of Griess reagent (1% sulphanilamide, 0.1% Naphthyl ethylene diamine dichloride and 3% phosphoric acid) was added to each test tube. at 546 nm, the absorbance was measured right away, and the percentage of scavenging activity was calculated using ascorbic acid as a standard¹⁹.

Invitro antiulcer activity:

Acid neutralizing assay

Procedure:

Colocasia esculenta methanolic extract's acid neutralizing capacity (ANC) values at various doses (100, 200, 500, and 1000 mg/ml)

were compared to the standard antacid, MCCC (magnesium carbonate + calcium carbonate -500 mg/ml). To make the total volume up to 70 ml, water was added to the 5 ml quantity of each extract separately and thoroughly mixed. Following 15 minutes of stirring the standard and test preparations with 30 milli litres of 1N HCl, two to three drops of phenolphthalein solution were added and combined. As soon as possible, the excess HCl was titrated using a 0.5N sodium hydroxide solution drop by drop until a pink hue emerged. Acid neutralizing capacity (ANC) per gram of antacid = moles of HCl neutralized / Grams of Antacid/Extract. This formula yields the moles of acid neutralized: moles of acid neutralized = (vol. of HCl × Normality of HCl) - (vol. of NaOH × Normality of NaOH)²⁰.

Characterization of Ag NPs

To ascertain the biochemical characteristics, a silver nanoparticle of *Colocasia esculenta* leaves was synthesised and characterised. The Nano Particles of *Colocasia esculenta* were characterised using FTIR, particle size analysis, and zeta potential.

RESULTS

The green synthesised silver nanoparticle of *Colocasia esculenta* was investigated for its antiulcer and antioxidant properties in vitro. The outcomes of the study were all included below.

Preliminary Phytochemical analysis

Preliminary phytochemical investigation of leaves extract of *Colocasia esculenta* showed the presence of phenols, saponins, flavonoids, terpenoids, tannins etc.

Table 1: Preliminary Phytochemical analysis

Phytochemical Constituents	Results
Tannins	++
Flavonoids	++
Terpenoids	++
Phenols	++
saponins	++

UV-Spectral Analysis

Formation of silver nanoparticle was further checked by UV–Visible spectroscopy. A broad absorption peak was observed at 415 nm as shown in the figure 1. this band corresponds to the absorption by colloidal silver nanoparticles in the region (400–450 nm) due to the excitation of surface plasmon vibration.

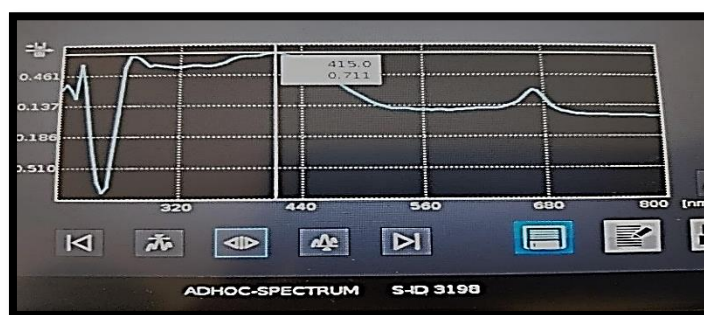


Figure 1: UV–Visible spectra of synthesized Ag NPs

Estimation of Phenolics by FC method

The MECE and NPCE was subjected to Estimation of Phenolics by FC method. It was performed using gallic acid as standard determined by spectrophotometric analysis with Folin-Ciocalteu reagent. $Y = 0.2353x - 0.0289$ ($R^2 = 0.9983$) was the linear equation derived from the gallic acid calibration curve. *Colocasia esculenta* leaves had a total phenolic content of 81 mg GAE/g and 76 mg GAE/g of NPCE and MECE in the methanolic extract.

Table 2: Estimation of Phenolics by ascorbic acid, MECE and NPCE

Concentration	Abs of sample	m= Wt of plant extract	c	C	V	c*V	a=(c*V)/m
µg/ml			µg/ml	mg/ml	mL	mg	mg/gm
100	0.981	0.0001	8.1	0.0081	1	0.0081	81mg GAE/gram
100	0.914	0.0001	7.6	0.0076	1	0.0076	76 mg GAE/ gram

Estimation of Flavonoids.

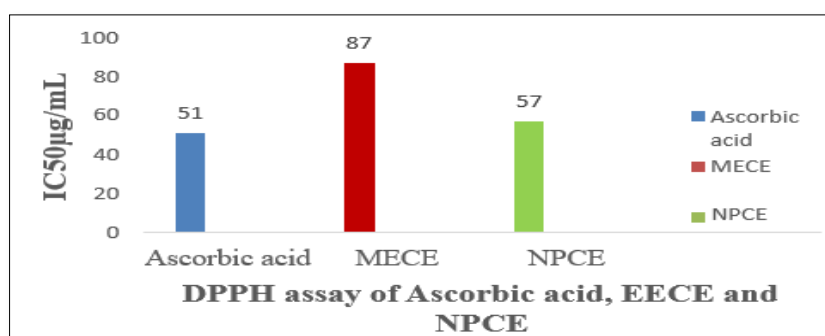
The MECE and NPCE was subjected to estimation of flavonoids. It was performed using Quercetin as standard. The linear equation $Y = 0.183x + 0.0828$ ($R^2 = 0.9997$) was derived from the Quercetin calibration curve. Using the aforesaid equation, the total flavonoid contents of the methanolic extract of the leaves of *Colocasia esculenta* were found to be 57 mg QE/g and 56 mg QE/g of NPCE and MECE, respectively.

Table 3: Estimation of Flavonoids by Quercetin, EECE and NPCE.

Concentration	Abs of sample	m= wt of plant extract	c	C	V	c*V	a=(c*V)/m
µg/ml			µg/ml	mg/ml	mL	mg	mg/gm
100	0.978	0.0001	5.7	0.0057	1	0.0057	57 QE/gram
100	0.955	0.0001	5.6	0.0056	1	0.0056	56 QE/gram

Invitro antioxidant assay**DPPH assay****Table 4: DPPH Method of Ascorbic acid, EECE and NPCE**

S. No.	Compounds	Concentration (µg/ml)	%Inhibition	IC ₅₀ values
1.	Ascorbic acid	100	58.4	51
		200	63.0	
		300	72.3	
		400	91.2	
		500	98.0	
2.	Methanolic extract of Colocasia Esculenta (MECE)	100	55.3	87
		200	60.5	
		300	67.1	
		400	90.7	
		500	97.4	
3.	Nanoparticle of Colocasia Esculenta (NPCE)	100		57
		200	51.1	
		300	67.8	
		400	82.7	
		500	84.8	

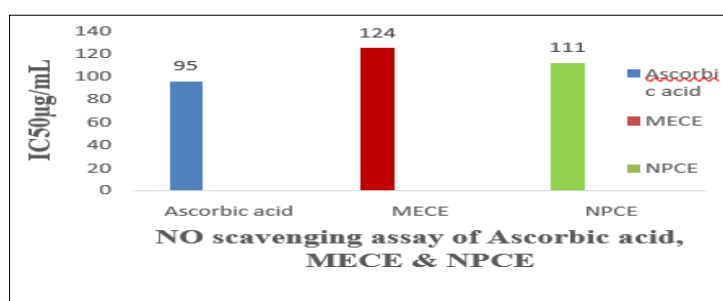
**Figure 2: DPPH Method of Ascorbic acid, EECE and NPCE**

DPPH Method, IC₅₀ values of standard drug, MECE and NPCE was found to be 51, 87 and 57 µg/ml respectively. From the above result it is clear that NPCE showed more antioxidant activity compared to MECE. The results were expressed in the table 4 and the profile was expressed in the figure 2.

Nitric oxide scavenging assay:

Table 5: NO scavenging assay of Ascorbic acid, EECE and NPCE

S. No	Compounds	Concentration (µg/ml)	%inhibition	IC ₅₀ values
1.	Ascorbic acid	100	57.2	95
		200	55.7	
		300	67.3	
		400	88.3	
		500	98.6	
2.	Methanolic extract of Colocasia Esculenta (MECE)	100	37.0	124
		200	66.9	
		300	84.7	
		400	96.8	
		500	98.3	
3.	Nanoparticle of Colocasia Esculenta (NPCE)	100	39.1	111
		200	67.3	
		300	87.2	
		400	97.4	
		500	98.5	

**Figure 3: NO scavenging assay of Ascorbic acid, MECE & NPCE**

Nitric oxide radical scavenging assay, IC₅₀ values of standard drug, MECE and NPCE was found to be 95, 124 and 111 µg/ml respectively. From the above result it is clear that NPCE showed more antioxidant activity compared to MECE. The results were expressed in the table 5 and the profile was expressed in the figure 3.

In vitro antiulcer activity

The MECE and NPCE was subjected to invitro antiulcer activity. Invitro antiulcer activity was performed using Calcium carbonate and Magnesium Carbonate in combination as a standard. NPCE (100, 200, 500, and 1000 mg) and a normal dose of 500 mg of calcium carbonate + magnesium carbonate [CaCO₃ + MgCO₃], the neutralising impact of the NPCE was investigated. Using standard and four concentrations (100, 200, 500, and 1000 mg), the neutralising impact of the NPCE was investigated. The results demonstrated that the extract's capacity to neutralise acid dropped considerably at doses of 100, 200, 500, and 1000 mg, or by 80, 62.5, 14 and 6.5 mg/mg, respectively, when compared to the standard of 10 mg/mg. It has been discovered that when diluted to a concentration of 1000 mg, the extract significantly increases the ability of acid to be neutralised. The outcomes are listed in table 6 & 7.

Table 6: Effect of MECE on acid neutralizing capacity:

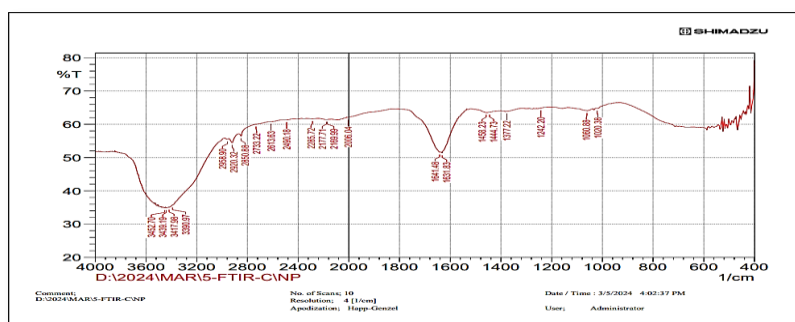
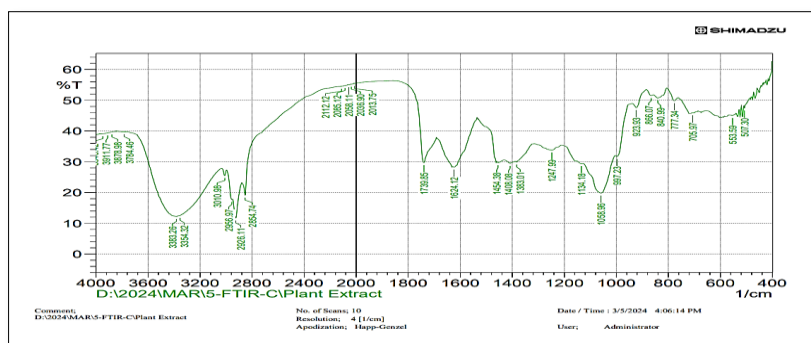
S. No	Compounds	Concentration mg	Volume of <u>NaoH</u> consumed (mL)	meq of acid consumed	ANC per gram of acid
1.	MECE	100	38.8	10.6	106
2.	MECE	200	31.2	14.4	72
3.	MECE	500	47.1	6.45	12.9
4.	MECE	1000	45	7.5	7.5
5.	Standard	500	50	5	10

Table No 7: Effect of NPCE on acid neutralizing capacity

S. No	Compounds	Concentration mg	Volume of NaoH consumed (mL)	meq of acid consumed	ANC per gram of acid
1.	NPCE	100	44	8	80
2.	NPCE	200	35	12.5	62.5
3.	NPCE	500	46	7	14
4.	NPCE	1000	47	6.5	6.5
5.	Standard	500	50	5	10

FT-IR spectroscopic analysis

FT-IR measurements were performed to identify the potential biomolecules in the leaf of *Colocasia esculenta* responsible for the formation of silver nanoparticles. The FT-IR spectrum of methanolic leaf extract of *Colocasia esculenta* showed several absorption peaks at 3383, 2956, 2854, 1624, 1454, 1247, 1058, 923 cm^{-1} . The FT-IR spectrum of silver nanoparticles showed absorbance peak at 3390, 2958, 2850, 1631, 1458, 1242, 1060, 920 cm^{-1} represent O-H stretching (alcohol, amides), C-H stretching (alkane, ether), O-H stretching (carboxylic acid), C-H bending (aromatic compounds), C-C-C (allene), C-C (phenol). C-O stretching (ester), C-N stretch (amine), analysis of the plant extract using FTIR measurements confirmed the presence of active biomolecules that acted as capping and reducing agents. functional groups present in sample might be responsible for bio reduction C-C (conjugated alkene), O-H bending C-Cl stretch (halo compounds) action of Ag^+ to Ag nanoparticle.

Figure 4: FT-IR Spectrum of synthesized silver nanoparticle using leaves extract of *Colocasia esculenta*Figure 5: FT-IR Spectrum of *Colocasia esculenta* leaves extract

Particle size and zetapotential:

NPCE:

Particle size and zetapotential for nanoparticle of *Colocasia esculenta* was found to be 205 nm and -46.5 mV respectively. Zeta potential shows the charge on the particle surface which indicates the physical stability of the dispersed systems. A high zeta potential will confer stability of the preparation that will resist aggregation.

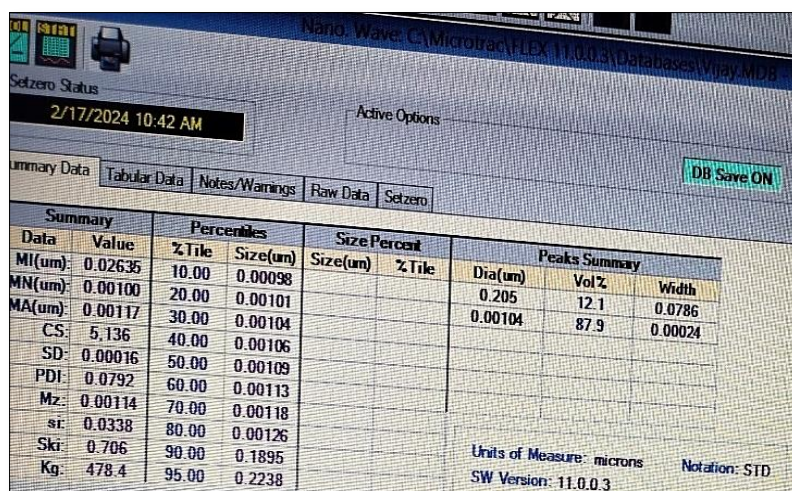


Figure 6: Particle size of NPCE

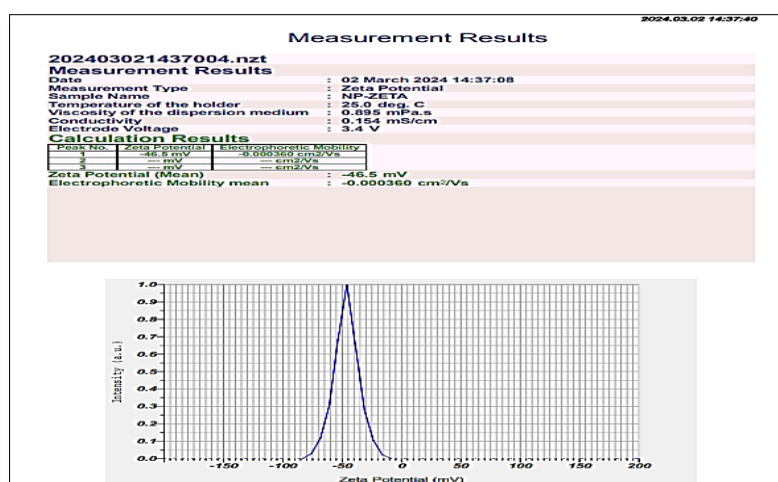


Figure 7: Zeta potential of NPCE

CONCLUSION:

Methanol was used in the maceration procedure to extract the plant after it was collected. The resulting plant extract was put through the following tests using conventional techniques. A preliminary phytochemical analysis was conducted on the methanolic extract derived from *Colocasia esculenta*. It revealed the presence of flavonoids, phenols, terpenoids, tannins, and saponin. Estimation of phenol and flavonoid of MECE and NPCE showed the presence of phenol & flavonoids in sample extracts. Using the DPPH assay and the nitric oxide scavenging assay, MECE and NPCE have demonstrated significant antioxidant activity. The outcome demonstrated that NPCE had stronger antioxidant properties than MECE. Both MECE and NPCE have demonstrated a strong antiulcer effect using the acid neutralising capacity approach. In comparison to MECE, the results indicated a significant antiulcer property for NPCE. *Colocasia esculenta* silver nanoparticles were prepared, and their effects on particle size, zeta potential, and Fourier transform infrared spectroscopy were assessed. We may conclude that *Colocasia esculenta* nanoparticles exhibit strong antioxidant and antiulcer properties. Although the results are noteworthy, more research is needed to determine the exact mechanism of action of this product using in vivo models before it

can be recommended as a therapeutic medication for the treatment of ulcers.

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