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CARALLUMA TUBERCULATA- THERAPEUTIC POTENTIAL OF BIODEGRADABLE NANOPARTICLES

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ABSTRACT: *Caralluma tuberculata* have great importance due to it's various medicinal properties. The purpose of this research was the structural and biochemical analysis of *C. tuberculata* extracts and nanoparticles. Extraction was done in methanol, *n*-hexane and aqueous solvents by microwave assisted method and ion gelation assay was used to synthesize chitosan-based nanoparticles. Antioxidant, antidiabetic, antibacterial, anti-inflammatory and cytotoxicity analysis was done for the assessment of bioactivities and for structural characterization FTIR (Fourier transformed infrared spectroscopy) and HPLC (high performance liquid chromatography) were used. The results showed that methanolic extract had highest antioxidant (48.65%) and antidiabetic potential (14.71%). *n*-hexane extract (91.41%) showed highest anti-inflammatory potential. Cytotoxicity analysis proved the non-toxicity of *n*-hexane (4.40%) and aqueous extract (0.87%) while the methanolic extract showed mild toxicity (5.33%). The aqueous sample had 8 mm zone of inhibition against *Staphylococcus aureus* and *n*-hexane extract showed inhibition zone of 16mm against *Escherichia coli*. The nanoparticles were toxic and showed less antioxidant, anti-inflammatory and anti-diabetic potential than extracts. It may be due to the dilution of nanoparticles or any environmental factor. Nanoparticles were 332.2nm in size with +20.7mV potential. Phytochemicals like aldehyde, chlorides, sulfonamides, nitro group, aromatic, primary and secondary amines, amides, carboxylic acid, ether and ester were identified by FTIR while HPLC indicated that p-coumaric acid, benzoic acid, caffeic acid and chlorogenic acid are present. More research is required in the field of nanoparticles to enhance the efficacy of this plant.

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INTRODUCTION

The most essential natural products for human health are found in the plant kingdom. Through experience, migration, careful observation and divine power, humans have learned how to use plants for medicinal purposes. Herbal medicines have regained popularity efficacy, safety, and cost. Furthermore, more than 25% of prescribed pharmaceuticals come from wild plant species, while up to 80% of individuals in developing countries only use herbal remedies for their primary medical care (1).

A member of the *Asclepiadaceae* family is *Caralluma tuberculata*. It contains vitamins, proteins, carbohydrates, cumarine, flavonoids, phenol, steroids, tannins, saponins, alkaloids and flavone glycosides. In traditional medicine, *C. tuberculata* is used to cure fever, paralysis, rheumatism, leprosy, diabetes and joint discomfort.

It is also used as an antipyretic, carminative and rheumatism remedy. *Caralluma* extract contains anti-inflammatory and anti-cancer properties (2, 3). The entire plant is utilized as a vegetable and as a diabetic treatment in the District Buner, Khyber Pakhtunkhwa, Pakistan. People in central Kurrum used the aerial part to treat high blood pressure, diabetes and stomach issues (4).

To maximize the therapeutic benefit and reduce the negative side effects linked to other medications, encapsulated therapeutic agents are synthesized from biodegradable polymers like

chitosan. Chitosan has drawn attention as a nanocarrier because to its non-toxicity, biocompatibility and biodegradability (5). The purpose of the researcwas to prepared *Caralluma tuberculata* extract and their nanoparticles to evaluated biochemical and structural characterization.

MATERIALS AND METHODS

Extract And Nanoparticles Sample Preparation

Plant samples (fleshy leafless stem) were collected from Soon Valley, Khushab District. Plants were identified at the Department of Botany at the University of Agriculture, Faisalabad, Pakistan and voucher specimen (209-1-2023) was saved. Aqueous, methanol and *n*-hexane extracts were prepared as described earlier (6). Crab shell was the source of chitosan. Nanoparticles were synthesized using the ionic gelation process (7).

Biochemical Analysis

Antioxidant Profile

Na_2CO_3 solution, test samples and FC reagent were incubated for two hours. At 765 nm, the absorbance was measured. The total phenolic contents (TPC) were presented as mg GAE/mL. Using the AlCl3 colorimetric technique, total flavonoids contents (TFC) were measured and the findings were presented as µg CE/mL. The anti-radical efficacy was measured by DPPH scavenging assay and expressed as percent DPPH inhibition (8).

Microbicidal Activity

The microbicidal ability was assessed by agar diffusion assay with bacterial strains *Staphylococcus aureus* and *E. coli*. Ciprofloxacin was positive control. The diameter of the growth inhibitory zone was recorded as millimeter (9).

Antidiabetic And Anti-Inflammatory Activity

Alpha amylase inhibition assay was done to measure antidiabetic potential and source of the enzyme was *Bacillus subtilis* (8). Percentage inhibition of alpha amylase was calculated (8). The *in vitro* BSA denaturing protocol was applied to evaluate the anti-inflammatory property. The positive control was diclofenac sodium (11).

Cytotoxicity – Red Cell Lysis Test

The human blood was used to separate red blood cells. Test sample and cell suspensions were incubated to measure cell lysis along with non-ionic detergent Triton X-100 (control). The absorbance was noted at 576 nm (10).

Fourier transform infrared spectroscopy (FTIR) was used for functional groups identification (8). Model was Bruker Tensor 27 FTIR spectrometer. High performance liquid chromatography (HPLC) was done to detect bioactive compounds (12).

Zeta Sizer Nano Sizer

Morphological Characterization and Particle Size Analysis

The average size and distribution (polydispersity index) of nanoparticles were measured using photon correlation spectroscopy (Zetasizer, Beckman Coulter Inc., Delsa Nano 4C) at HI-Tech Laboratory, University of Agriculture, Faisalabad as described earlier (13).

STATISTICAL DATA ANALYSIS

Data is described as average ± Standard Deviation. Analysis was performed by using Statistix 9 software by one of the co-authors.

RESULTS

The percentage yield of methanolic extract (10.98%) was the highest as compare to aqueous extract (6.22%) and *n*-hexane extract (2.22%).

Antioxidant Activity

TPC of methanolic extract (124.48±0.73 mg GAE /mL) of *Caralluma tuberculata* was the highest as compare to aqueous extract (103.57±0.45 mg GAE /mL), *n*-hexane extract (23.63±0.27 mg GAE /mL) and nanoparticles (21.02±0.10 mg GAE /mL). TFC of *n*-hexane extract (128.46±0.15 µg CE/mL) was the highest than that of methanolic extract (99.78±0.15 µg CE/mL), aqueous extract (48.81±0.52 µg CE/mL) and nanoparticles (16.53±0.60 µg CE/mL). Methanolic extract had highest antioxidant potential (48.65±0.13% inhibition) than *n*-hexane extract which showed 40.22±0.39% inhibition, aqueous extract that showed 40.22±0.22% inhibition and nanoparticle that showed 1.83±0.22% inhibition.

not restricted by other extracts and control gave the inhibition zone of 33mm (fig 1). Similarly, in case of *Staphylococcus aureus,* 8mm zone of inhibition by the aqueous extract was observed. Other extracts had no microbicidal potential and control gave the inhibition zone of 50mm (fig 2).

Alpha Amylase Inhibition

Anti-diabetic activity was determined by alpha-amylase inhibition assay. Methanolic extract showed 14.71±1.45% inhibition, *n*-hexane extract showed 5.41±1.22 % inhibition and aqueous extract showed 11.44±1.38% inhibition. Moreover, chitosan-based nanoparticles of methanolic extract showed 9.10±1.33% inhibition.

Cytotoxicity

Hemolytic activity

Hemolytic activity was used to measure the sample's cytotoxicity. Hemolytic activity of methanolic extract was 5.33±0.18% and that of *n*-hexane extract it was 4.40±0.08%. Hemolytic activity observed in aqueous extract was 0.87±0.14%. Hemolytic activity of chitosan nanoparticles of methanolic extract was 25.92±3.70%. It showed that all the extracts were non-toxic and safer to use but nanoparticles were toxic.

Anti-Inflammatory Activity

Maximum anti-inflammatory activity was exhibited by *n*-hexane extract (91.41±42.16%) as compared to other samples; aqueous (57.93±1.37%), methanol (21.77±5.38%) and nanoparticles (18.66±1.33).

Fourier-Transform Infrared Spectroscopy

FTIR results were shown in figure 3 and table 1. Table 1 list the expected absorption values that indicated various functional groups. A peak at 3278.2 cm^{-1} showed the presence of stretching NH bending while peak at 2918.5 cm^{-1} indicated carboxyl group and stretch alkane. The peak at 2849.5cm^{-1} was the indication of carboxyl group and aldehyde. Bend primary and secondary amines and amides were indicated at 1578.5cm^{-1} band. Peak at 1457.4 cm^{-1} showed the presence of nitro(R-NO2) group, while the peak at 1541.3 cm^{-1} showed the presence of aromatic and nitro(R-NO2) group. The peaks at 1418.3cm^{-1} showed the presence of CH3 and nitro(R-NO2) group. A peak at 1395.9cm^{-1} showed the presence of amino substituted alkyl group. The presence of sulfones, sulfonyl chlorides, sulfates and sulfonamides were indicated by band 1319.5 cm^{-1} and 1375.4 cm^{-1}, while a peak at 1258.0 cm^{-1} showed the presence of alcohol, ethers, esters, carboxylic acid and anhydride. Alcohols, ethers, esters, carboxylic acid, anhydrides and amines were indicated at 1012.0cm^{-1} and 1032.5 cm^{-1}band. Alkenes and aromatic bend were indicated at 833.1cm^{-1} band.

High Performance Liquid Chromatography

The result of HPLC indicated the presence of various compounds that were shown in figure 4. In our study five compounds were identified by HPLC. Chlorogenic acid was observed at retention time 2.831min. The retention time for p-coumeric acid was 3.274min with 422,715.3 peak area. HB acid was observed at retention time 6.755min with peak area 8,648.7. Similarly, retention time for caffeic acid and benzoic acid were 7.478min and 18.315min respectively.

Characterization Of Nanoparticles

Nanoparticles were characterized through zeta sizer and zeta potential by using litesizer. According to zeta sizer and potential, the nanoparticles were 332.2nm in size with +20.7mV potential respectively. The results of zeta sizer and zeta potential are shown in figure 5 and figure 6 respectively.

DISCUSSION

Percentage yield of different extracts of *C. tuberculata* were determined by many researchers. They suggested that the percentage yield of *n*-hexane extract of *Caralluma tuberculata* was 39% (14) and methanolic extract showed 9.6% yield. While in current research, methanolic extract showed 10.98% yield which was near to the previous studies (15), with 10.78% yield of aqueous extract. In current research, *n*-hexane and aqueous extract showed 2.22% and 6.22% yields. *C. tuberculata* loaded CS-TPP (chitosan

tripolyphosphate) nanoparticles were synthesized to access their anticancer activity whose size was 140nm with +59.5± 0.61mV potential (5), while our nanoparticles were 332.2nm in size with 20.7mV potential.

The TPC of *n*-hexane and aqueous extract was 0.02±0.01 GAE mg/g extract and 0.09±0.02 GAE mg/g respectively (4), while in our research TPC of *n*-hexane and aqueous extract was 23.63±0.27 mg GAE /mL and 103.57±0.45 mg GAE /mL respectively and in previous study the TPC of methanolic extract was 13.57 ± 1.714 mg GAE/g (4), while TPC of our methanolic extract was 124.48±0.73 mg GAE /mL. In case of selenium nanoparticles, they showed 3.91 mg GAE/g total phenolic content (17), while TPC of our chitosan nanoparticles was 21.02±0.10 mg GAE /mL. In a previous research, the TFC of methanolic extract was 30 ± 0.48 mg QE/g (4), while in our study TFC of *n*-hexane, aqueous and methanolic extract was 128.46±0.15 µg CE/mL, 48.81±0.52 µg CE/mL and 99.78±0.15 µg CE/mL respectively. It was reported that that selenium nanoparticles had 1.8 mg QAE/g flavonoid content (17), while the TFC of our chitosan nanoparticles was 16.53 ±0.60 µg CE/mL. Previously the *n*-hexane and aqueous extracts showed 53.60±2.26% and 55.70±1.90% DPPH percentage inhibition respectively (16) and methanolic extract showed 86.25% inhibition (5), while in our research, percentage inhibition of *n*-hexane, aqueous and methanolic extract was 40.22±0.39%, 48.81±0.52% and 48.65±0.13% respectively. It was suggested that selenium nanoparticles showed 82% DPPH inhibition (17).

In case of antimicrobial activity, *n*-hexane extract showed 11.66mm inhibition zone against *E. coli* (19) and no inhibition against *S. aureus* (16), methanolic extract showed 0.53mm inhibition zone against *S. aureus* (19) and 34.33mm inhibition zone against *E. coli* (20), while the aqueous extract showed 8mm inhibition zone against *E. coli* (16) and 16mm inhibition zone against *S. aureus* (18). In our research, *n*-hexane sample inhibited growth (16mm) against *E. coli* and in case of *staphylococcus aureus,* the aqueous extract showed inhibition (8mm).

In case of alpha amylase inhibition assay, the methanolic and aqueous extract of *C. tuberculata* showed 99 % inhibition and 29% inhibition respectively (4), while our methanolic and aqueous extract showed 48.65±0.13% and 11.44±1.38% inhibition. It was reported that the selenium nanoparticles showed 78.24 % inhibition (17), while our chitosan nanoparticles showed 9.10±1.33% inhibition.

In case of hemolytic activity, aqueous extract of Caralluma species had antihemolytic property (21). *C. tuberculata* loaded chitosan nanoparticles showed almost 5% hemolysis (5), while in our *in-vitro* research it is concluded that *n*-hexane (4.40±0.08% hemolysis) and aqueous extract (0.87±0.14% hemolysis) was non-toxic, methanolic extract was slightly toxic (5.33±0.18% hemolysis) and nanoparticles (25.92±3.70 % hemolysis) was toxic to use.The *n*-hexane extract of the Caralluma specie showed the least anti-inflammatory activity as compare to the methanol-aqueous extract and ethyl acetate extract (22), the aqueous and the methanolic extract showed 83.41 ± 0.49% and 77.52 ± 0.38% inhibition respectively (15), while in our research *n*-hexane extract showed highest anti-inflammatory activity while the aqueous and methanolic extract showed 57.93±1.37% and 21.77±5.38% inhibition respectively. The down regulation of AKT, mTOR and pI3K signaling pathways was carried out by gold nanoparticles of Caralluma specie that produce anti-inflammatory effect (23), while our chitosan nanoparticles showed less anti-inflammatory effect (18.66±1.33%).

Wavelength ranges between 3250cm^{-1} - 3650cm^{-1}, showed the presence of hydrogen bond in H2O/-OH/NH3/-NH2 and methylene (>CH2) group was observed at wavelength ranges from 2845 cm^{-1} –2865 cm^{-1}. methoxy, methyl ether O-CH3 and C-H stretch containing compounds were reported at wavelength ranges from 2915 cm^{-1} - 2935 cm^{-1} /2815 cm^{-1} –2850 cm^{-1} while at wavelength ranges from 1550 cm^{-1}-1650 cm^{-1}, secondary amine, N-H bend,

carboxylate (carboxylic acid salt) and open-chain azo (-N=N-) were observed. Vinyl C-H in-plane bend and carbonate ion was observed at wavelength ranges from 1410 cm^{-1}-1490 cm^{-1}. Aromatic ring, methylene C-H bend compounds were present at wavelength ranges from 1485 cm^{-1} - 1560 cm^{-1}. Organic sulfates, phenol or tertiary alcohol, OH bend, methyl (−CH3), aliphatic nitro compounds and nitrate ion were reported at peak ranges from 1310 cm^{-1}-1420 cm^{-1} and at wavelength ranges from 1000 cm^{-1}-1600 cm^{-1}, hydrogen bond in H2O/-OH/NH3/-NH2, phosphate ion, triple bond of C≡C, cyclohexane ring vibrations, aliphatic fluoro compounds, primary amine,aromatic tertiary amine, carboxylate (carboxylic acid salt), phenol or tertiary alcohol and OH bend compounds were identified. Organic phosphates (P=O stretch), dialkyl/aryl sulfones, aromatic primary amine and secondary amine, CN stretch, aliphatic compound and aromatic ring were observed at wavelength ranges from 720 cm^{-1}-1470 cm^{-1} (21). All these results were almost similar to our results. The presence of caffeic acid at retention 7.694min (15) and the presence of chlorogenic acid, p-coumaric acid and benzoic acid (22) were confirmed in Caralluma species. All such compound were also identified in our research.

CONCLUSION

The most essential natural products for human health are found in the plant kingdom. *Caralluma tuberculata* have great importance due to it's various properties. In the current research, structural characterization confirmed the presence of various functional groups and compounds.

Through biochemical characterization, it was proved that the extracts showed notable antioxidant, antidiabetic, antimicrobial and anti-inflammatory potential. Moreover, they were non-toxic to use. On the other hand, nanoparticles showed least biochemical potential and proved to be toxic for use. Dilution of nanoparticles, environmental factor and human error may be the reason behind this. Further research is required in this field to enhance the efficacy of this plant through nanoparticles synthesis to make it an effective drug in future.

Informed Consent

Not Applicable.

Conflicts Of Interest

We, the authors have "no conflict of interest"

Authors' Contributions

All authors contributed equally

Ethics Approval

Not Applicable

Consent For Publication

All authors gave consent for this publication

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Table 1 : FTIR spectrum chart indicating the identical functional groups

Sr. No.	Characteristic Peak	Compounds	Functional group	Intensity	Sr. No.	Characteristic peak	Compounds	Functional group	Intensity
1	3278.2	Stretching NH bending	OH NH	S	9	1418.3	-CH3 Nitro (R-NO2)	C-H N=O	**MS**
2	2918.5	Carboxyl group, stretch Alkane	C-H	S	10	1395.9	Amino substituted Alkyl group	N=O Nitro(R-NO2)	**S**
3	2849.5	Carboxyl group, Aldehyde	C-H	W	11	1375.4	Sulfones, Sulfonyl chlorides, Sulfates, Sulfonamides	C-H bend CH3 S=O	**MS-S**
4	2372.5				12	1319.5	Sulfones, Sulfonyl chlorides, Sulfates, Sulfonamides	S=O	**S**
5	2322.1				13	1258.0	Alcohol, Ethers, Esters, Carboxylic acid, Anhydride	C-O C-N	**S** **M-S**
6	1578.5	Primary and secondary amines and amides	N-H bend	M-S	14	1032.5	Alcohol, Ethers Esters, Carboxylic acid, Anhydride Amines	C-O C-N	**S** **M-S**
7	1541.3	Aromatic Nitro (R-NO2)	C=C N=O	M-W S	15	1012.0	Alcohol, Ethers, Esters, Carboxylic acid, Anhydride Amines	C-O C-N	**S** **M-S**
8	1457.4	Nitro (R-NO2)	N=O Nitro(R-NO2)	S	16	833.1	Alkenes, Aromatic bend	C-H	S

Figure 1: Inhibition zone against *E.coli*

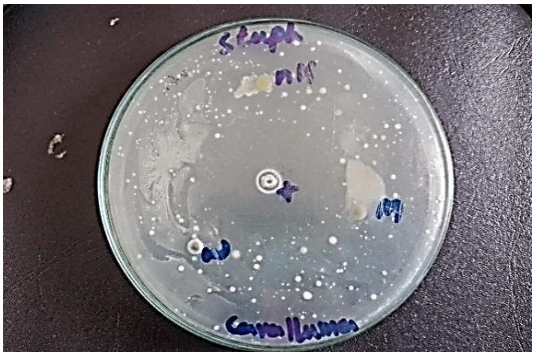

Figure 2: Inhibition zone against *S. aureus*

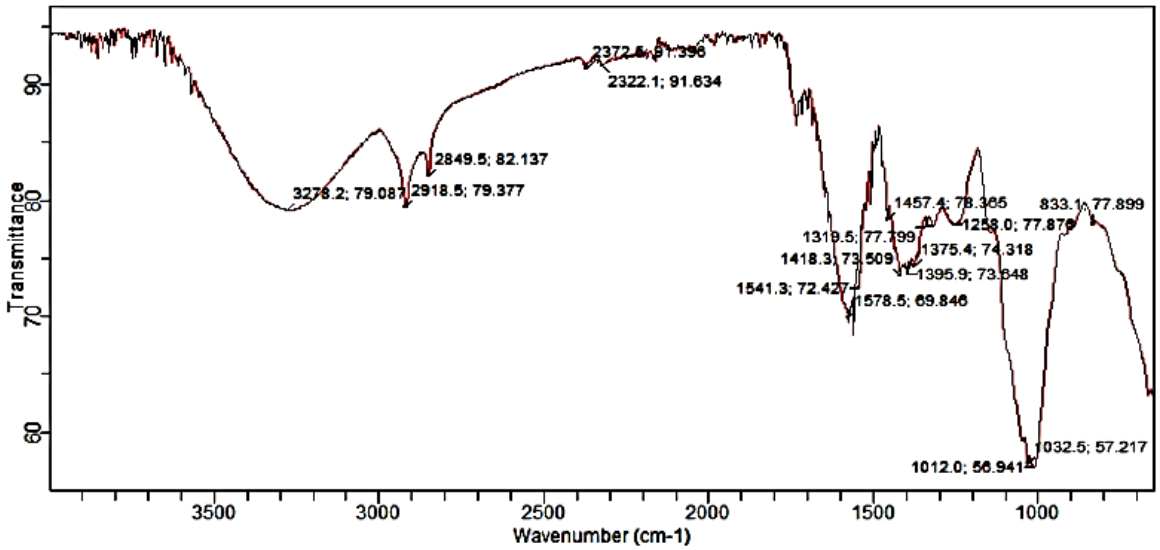

Figure 3: FTIR spectra of *C. tuberculate*

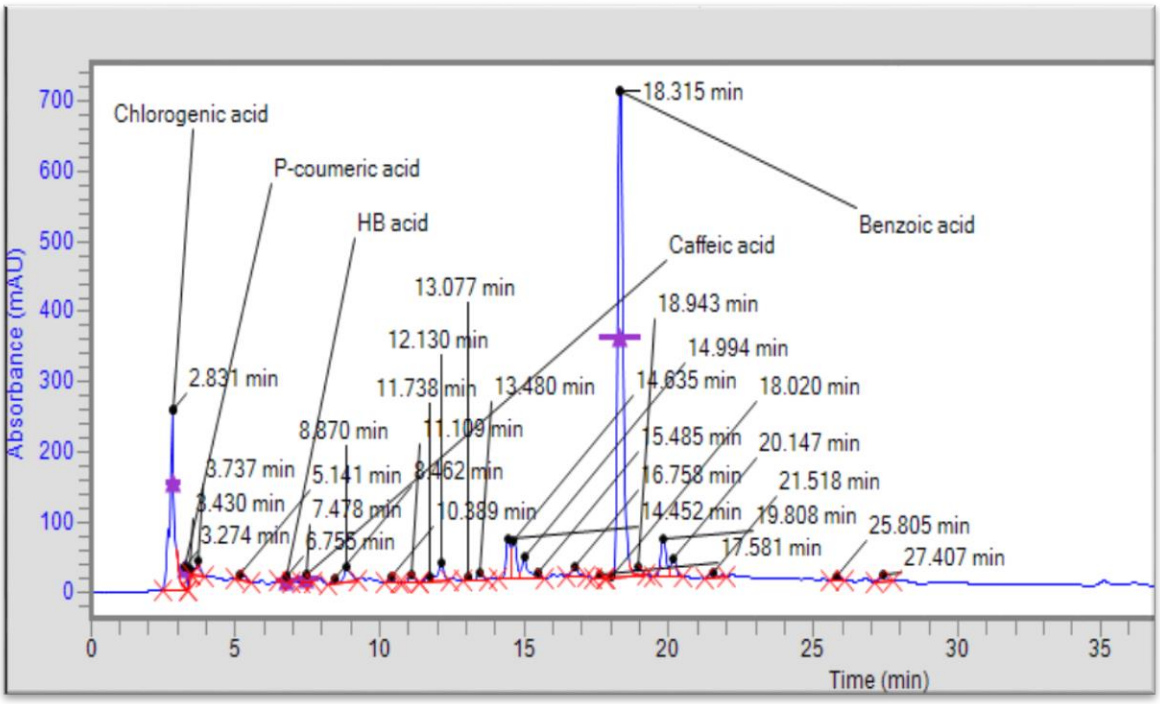

Figure 4: HPLC spectra of *C. tuberculate*

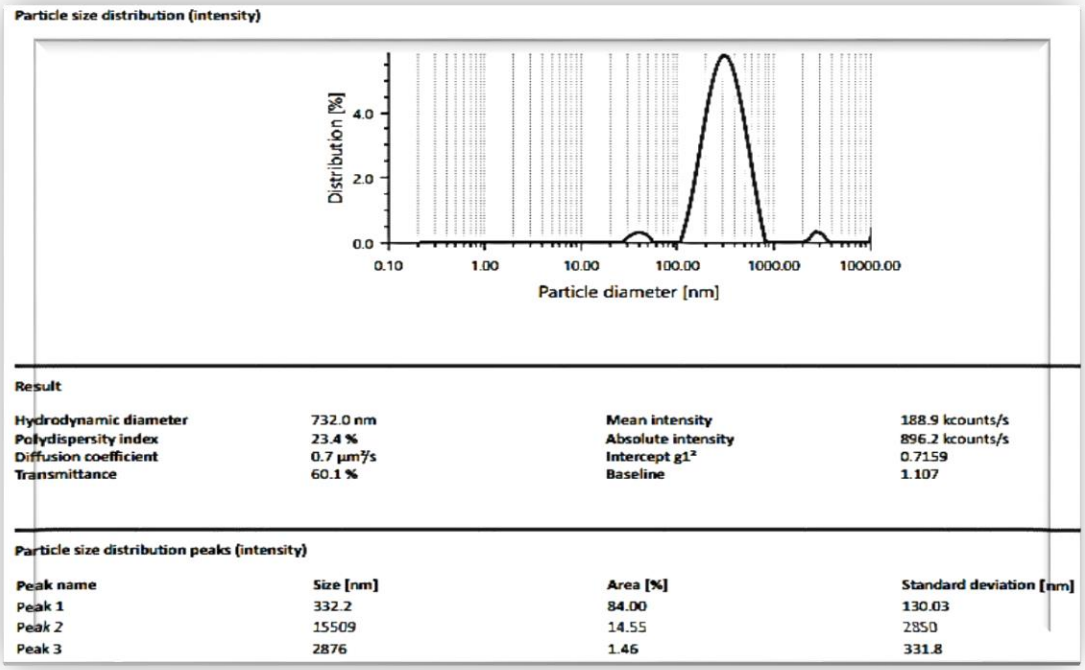

Figure 5: Result of zeta sizer

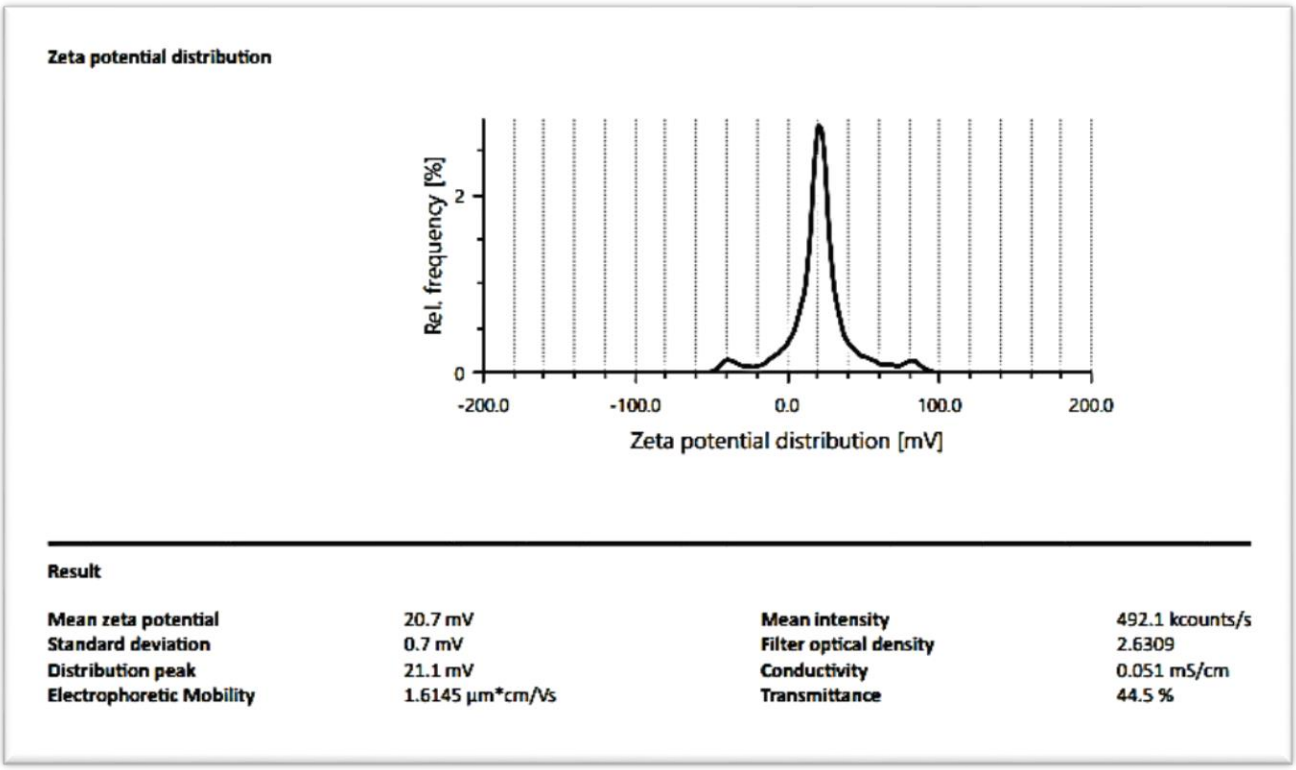

Figure 6: Result of zeta potential