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EXTRACTION, CHARACTERIZATION AND CYTOTOXICITY STUDIES OF SILICA PARTICLES FROM WILD CYMBOPOGON FLEXUOSUS (NEED EX STEUD.) LEAF

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ABSTRACT

Silica particles were extracted and characterized efficiently and effectively from wild *Cymbopogon flexuosus* leaf. The highest percentage (81.03 ± 0.03 %) yield of silica particles was extracted from the plant when burnt at 600 °C for 2 h. SEM analysis data revealed that silica particles were uniformly distributed in the agglomerated spherical shape with nanosized particles ranging from 50-60nm. FT-IR data revealed the presence of hydrogen bonded silanol and siloxane groups in silica nanoparticles. EDAX report gave the elemental details compossing carbon, oxygen, potassium, calcium along with silica. XRD analysis showed the crystallinity of the silica particles with amorphous nature. BET analysis gave the physical parameters such as size, surface area and internal pore size. MTT assay to check for the cytotoxicity studies using the extracted silica particles showed IC_{50} value of 26.837µg/mL compared to standard Cisplatin IC_{50} value of 15.00µg/mL.

Keywords: Cymbopogon flexuosus, Extraction, Characterization, Silica particles, Cytotoxicity

1. INTRODUCTION

Cymbopogon flexuosus (Need ex Steud.) Wats is a perennial grass belonging to the family Graminaceae and is grouped under the genus Cymbopogon. It is of indigenous origin and is a medicinal and aromatic plant. *C. flexuosus* is grown in East Indian States which is famous for its oil and has a good market. The origin is India and grown in states like Kerala, Karnataka, Tamil Nadu, Sikkim, Bengal, Madhya Pradesh, Arunachal Pradesh and Maharashtra. It is also grown in Brazil, Guatemala, Argentina, West Indies, Vietnam, Thailand, Sri Lanka and China. Wild species of lemon grass are found in tropical region of Asia, Africa and Latin America. Lemon grass has many contributions in the medical field. This plant is used as a folk remedy for coughs, gingivitis, malaria, pneumonia and vascular disorder.

Silica (SiO_2) is one of the valuable inorganic multipurpose chemical compounds. It can exist in gel, crystalline and amorphous forms [1] and is the most abandon material on the earth's crust. However, manufacturing pure silica is energy intensive. The soluble silicates produced from silica are widely used in the glass, ceramics, cement and as major components in pharmaceuticals, cosmetics and detergents industries as bonding and adhesive agents [2, 3].

Silica is deposited in plants as hydrated amorphous silica (SiO_2 nH_2O) through the polymerization of monosilicic acid ($Si(OH)_4$) absorbed by roots from soil solutions [4].

Silica nanoparticles (SNPs) have gained a great attention due to its highly reactive surface area to volume ratio, chemical and physical stability, low toxicity and straight forward surface chemistry [5]. It shows applications in industrial manufacturing, packaging, ceramic and synthesis of high molecule composites material, drug delivery, cancer therapy, disease labeling, biosensor, food and agriculture [6].

Lemon grass is composed of crude protein (15.68%), ash (23.40% to 25.00%), crude fiber (27.72%), fat (1.25%) and carbohydrates (38.44%) [7]. Besides proximate analysis the plant consists of many crucial minerals such as Carbon, silica, phosphorus, potassium and calcium. However, the amount of minerals that can be found in lemon grass can vary according to the methods of sample preparation and experimentation [8]. According to Ogie-odia *et al.,* [9]. The silica bodies are present on the adaxial surface. The silica can be extracted and used as a source of amorphous or porous silica. Besides, silica which is abundant in soil and rock can also be transmitted to the tissue of root plants such as rice husk, wheat husk, sugar cane and lemon grass [10]. Hence this silica can be extracted from plants and used as an alternative source

for commercial silica that is presently being utilized in industry [11]. Silica from natural fiber is usually extracted or precipitated from ash content by implementing the acid washing and gasification methods. These methods are popularly utilized for recovering silica from rice husk and bagasse [12, 13]. According to Pekarovic *et al.*, [10] silica is soluble in highly alkaline solutions.

The reduction of tetrazolium salts is widely accepted as a reliable way to examine cell proliferation. The yellow tetrazolium MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) is reduced by metabolically active cells, in part by the action of dehydrogenase enzymes, to generate reducing equivalents such as NADH and NADPH. The resulting intracellular purple formazan can be solubilized and quantified by spectrophotometric means [14]. The assay measures the cell proliferation rate and conversely, when metabolic events lead to apoptosis or necrosis, the reduction in cell viability [15].

The present investigation was carried out with an aim to explore the extraction and characterization of silica particles from wild *C. flexuosus* leaf and to determine its cytotoxicity effect by MTT assay on HUVEC cell lines.

2. MATERIALS AND METHODS

2.1. Collection and identification of wild *Cymbopogon flexuosus*

Wild *C. flexuosus* (Need ex Steud.) (RRCBI-mus231) was collected at Sri champakadhama Swamy temple located in sub-locality of Bangalore district, Karnataka. The Latitude and Longitude of Bannerghatta champakadama swamy temple is 12.8138 and 77.5762 respectively. The species survived in clay-heavy soil containing high silica content.

2.2. Extraction of Silica (Si) particles from leaf

Leaves were separated and washed under tap water to remove impurities and dried at 105°C for 24 h. The dried leaves were powdered and sieved for further analysis. The sample was soaked in 5M HCl for 3 h at 50°C. The treated sample was rinsed using warm distilled water and oven dried overnight at 105°C. The dried sample was calcined at 600°C until it became white ash. The ash were further dispersed in water and sonicated for 2 hrs to obtain spherical nanosized particle.

2.3. Characterization of Si particles

The ash obtained from the leaf sample was characterized using Scanning Electron Microscope (SEM) and was carried out using a computer-controlled field emission used to produce images of the sample (model JSM 6460 LA, JEOL, USA). To identify the types of chemical bonds (functional groups), the diffuse reflectance Fourier Transform Infrared Spectroscopy (FTIR) technique was performed for all the samples using Spectrometer. The spectra were scanned in the range of 4000 to 400cm^{-1} with a resolution of 4 cm^{-1} (model L1280044, Perkin Elmer, USA). EDAX is used to identify the elemental composition of materials. Phase identification of extracted silica was determined by X-ray powder diffraction (XRD) using Bruker D2 phase X-ray diffractometer. Brunauer–Emmett–Teller (BET) was carried out using Quantachrome Surface analyzer instrument version 3.0 is measure the specific surface area of material.

2.4. Cytotoxicity studies using Si particles

The ash obtained from the leaf sample was subjected to cytotoxic studies by MTT assay. For the cytotoxicity studies, Human Umbilical Vein Endothelial Cells (HUVEC) cells cultured in T-75 flasks were trypsinized and aspirated into a 5mL centrifuge tube. Cell pellet was obtained by centrifugation at 300 x g. The cell count was adjusted, using DMEM HG medium, such that 200µl of suspension contained ~15,000 cells. To each well of the 96 well microtitre plate, 200µl of the cell suspension was added and the plate was incubated at 37°C and 5% CO_2 atmosphere for 24 hr. After 24 hr, the spent medium was aspirated. 200µl of different test concentrations of standard drug Cisplatin (0.5, 2.5, 5, 10 & 20µg/mL diluted from stock) and Si particles (0.01, 0.1, 1.0, 10 & 100µg/mL serially diluted from stock) were added to the respective wells. The plate was incubated at 37°C and 5% CO_2 atmosphere for 24 hr. The plate was removed from the incubator and Si particles containing media was aspirated. 200µl of medium containing 10% MTT reagent was then added to each well to get a final concentration of 0.5mg/mL and the plate was incubated at 37°C and 5% CO_2 atmosphere for 3 hr. The culture medium was removed completely without disturbing the crystals formed. Then 100µl of solubilization solution (DMSO) was added and the plate was gently shaken in a gyratory shaker to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 570 nm and 630 nm. The percentage growth inhibition was calculated, after subtracting the background and the blank, and concentration of Si particles needed to inhibit cell growth by 50% (IC_{50}) was generated from the dose-response curve for the cell line.

3. RESULTS AND DISCUSSION

3.1. Yield of Si particles from leaf of *C. flexuosus*

The EDAX report showed 81.03% of Si content in the leaf sample.

3.2. Structural and Morphological characteristics of Si particles

3.2.1. Scanning Electron Microscopy (SEM)

The morphology of ash obtained from leaf sample of *C. flexuosus* treated at 50°C temperature is shown in Fig. 1. SEM analysis revealed spherical aggregates of Si nanoparticles with nano sizes of 52.30nm, 50.93nm, 58.30nm, 60.48nm and 62.14nm.

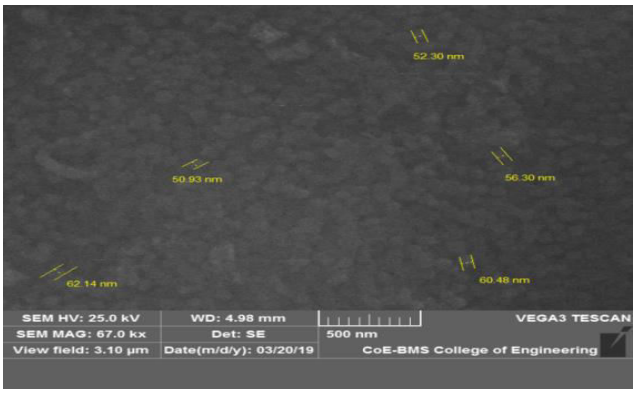

Fig. 1: SEM analysis of silica particles from leaf ash of *C. flexuosus*

3.2.2. Fourier Transform Infrared Analysis (FTIR)

FTIR spectroscopic studies of leaf ash showed the major functional groups which represent silica between the wave numbers 3300 to 3200 cm^{-1} present in the acid leached leaf sample (Fig. 2). The peaks obtained corresponded to the stretching vibrations of Si-OH or H-OH. The fibers of *C. flexuosus* are hydrophilic in nature. The hydroxyl group present in the cell wall forms hydrogen bond with water molecules [16]. In this study, the strongest broad IR band recorded was at 1200 cm^{-1}. The peaks in this range revealed asymmetry between Si-O and Si-O-Si bonds. The peaks ranging from 800 to 700 cm^{-1} are due to the symmetric stretching mode of the Si-O-Si bond and the band from 450 to 600 cm^{-1} corresponds to the Si-O-Si bending vibration, which is in agreement with the previous report [17].

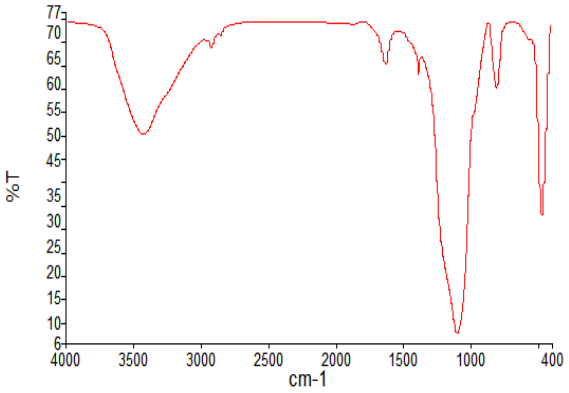

Fig. 2: FTIR spectrum of silica particles from Leaf ash of *C. flexuosus*

3.2.3. Energy Dispersive X-ray Spectrometer (EDAX)

The elemental analysis of leaf ash of *C. flexuosus* was studied using EDAX detector (Fig 3). EDAX spectra were measured with a Si (Li) EDS detector having an active area of 10 mm. The leaf sample contained mostly silica (Si) in addition to Carbon (C), Oxygen (O), potassium (K) and Calcium (Ca) (Table1) which is consistent with previous reports involving Cymbopogon sp. [18].

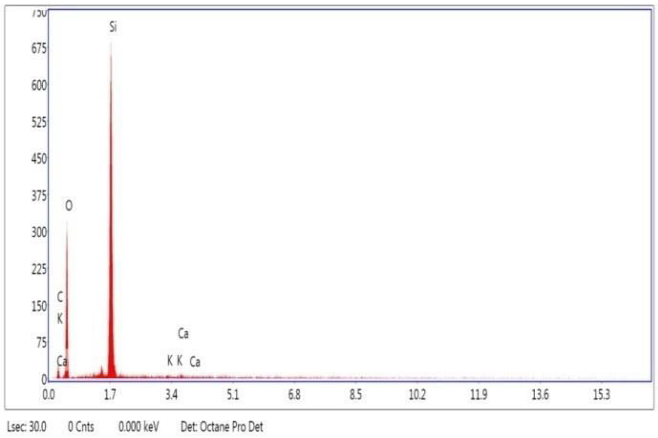

Fig. 3: EDAX analysis of silica particles from Leaf ash of *C. flexuosus*

Table 1: EDAX analysis showing elements in leaf ash *C. flexuosus*

Element	Weight %	Atomic %
C K	18.32	25.95
O K	**54.02**	**57.42**
SiK	**27.01**	**16.35**
K K	0.28	0.12
CaK	0.38	0.16

3.2.4. X-Ray Powder Diffraction (XRD)

X-ray diffractograms were recorded from the leaf ash of *C. flexuosus* treated at 50°C leaching temperature and calcined at 600°C (Fig. 4). They exhibited sharp crystallinity peaks at 2θ values of 28.6°, 29.7°, 33.4°, 41.1° and 45.2°. These peaks indicated d-values of 3.11, 3.00, 2.67, 2.19 and 1.81 respectively. They showed less impurity as the acid leaching temperature was 600°C [19].

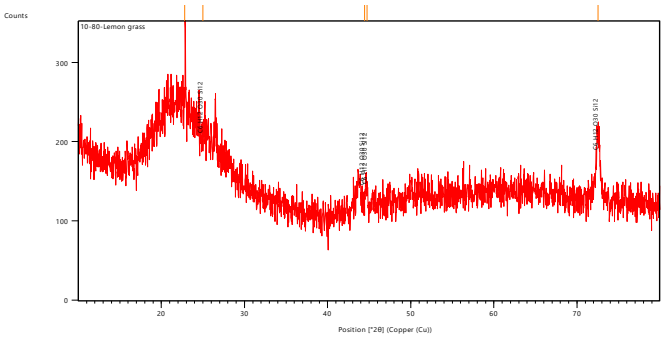

Fig. 4: XRD pattern of silica particles from leaf ash of *C. flexuosus*

Table 2: BET analysis showing surface area

Diameter	Pore Volume	Surf Pore Area	dV(d)	dS(d)	dV(logd)	dS(logd)
nm	cc/g	m²/g	cc/nm/g	m²/nm/g	cc/g	cc/g
1.2830	2.3995e-03	7.4807e+00	7.3367e-03	2.2873e+01	2.1557e-02	6.7206e+01
1.5741	3.9566e-03	1.1437e+01	6.1064e-03	1.5518e+01	2.2084e-02	5.6119e+01
1.7705	4.7777e-03	1.3293e+01	5.9559e-03	1.3456e+01	2.4268e-02	5.4828e+01
1.9639	6.3056e-03	1.6405e+01	6.1379e-03	1.2502e+01	2.7719e-02	5.6457e+01
2.1591	7.2236e-03	1.8105e+01	6.4856e-03	1.2015e+01	3.2232e-02	5.9713e+01
2.3638	8.7503e-03	2.0689e+01	5.7020e-03	9.6489e+00	3.1001e-02	5.2461e+01
2.5791	9.5676e-03	2.1956e+01	5.0180e-03	7.7826e+00	2.9790e-02	4.6202e+01
2.8153	1.1077e-02	2.4101e+01	4.8772e-03	6.9296e+00	3.1584e-02	4.4875e+01
3.0592	1.2210e-02	2.5583e+01	6.3512e-03	8.3043e+00	4.4726e-02	5.8480e+01
3.3181	1.7705e-02	3.2207e+01	1.6195e-02	1.9523e+01	1.2362e-01	1.4903e+02
3.6965	2.4400e-02	3.9452e+01	1.6038e-02	1.7355e+01	1.3636e-01	1.4756e+02
4.0838	2.6104e-02	4.1120e+01	4.7678e-03	4.6699e+00	4.4804e-02	4.3885e+01
4.4563	2.8084e-02	4.2898e+01	5.1091e-03	4.5860e+00	5.2392e-02	4.7027e+01
4.9431	3.2348e-02	4.6348e+01	7.2781e-03	5.8895e+00	8.2741e-02	6.6955e+01
5.5915	3.9394e-02	5.1389e+01	9.9102e-03	7.0895e+00	1.2742e-01	9.1153e+01
6.2918	4.8598e-02	5.7240e+01	1.3348e-02	8.4858e+00	1.9318e-01	1.2281e+02
7.1415	6.6213e-02	6.7107e+01	1.7442e-02	9.7696e+00	2.8634e-01	1.6038e+02
8.2693	8.6205e-02	7.6777e+01	1.6047e-02	7.7621e+00	3.0497e-01	1.4752e+02
9.6913	1.0323e-01	8.3805e+01	1.0654e-02	4.3973e+00	2.3720e-01	9.7903e+01
11.5884	1.1132e-01	8.6595e+01	3.6814e-03	1.2707e+00	9.7938e-02	3.3805e+01
14.4947	1.1541e-01	8.7726e+01	1.1330e-03	3.1265e-01	3.7616e-02	1.0381e+01
19.8888	1.1935e-01	8.8517e+01	5.4854e-04	1.1032e-01	2.4846e-02	4.9970e+00
32.2036	1.2336e-01	8.9015e+01	2.2990e-04	2.8556e-02	1.6621e-02	2.0645e+00

3.2.5. Brunauer–Emmett–Teller (BET) Surface Analysis

BET Surface Area Analysis provides specific nanoparticle surface area evaluation *via* nitrogen multilayer adsorption, measured as a function of relative pressure. Here, it was determined that silica nanoparticle surface area was 73.212 m²/g (Table 2).

BJH analysis was employed to determine the pore area and specific pore volume using adsorption and desorption techniques. This technique characterizes pore size distribution independent of the external area due to the particle size of the sample. Pore size analysis using BJH showed Pore Volume of 0.123 cc/g and Pore Diameter Dv(d) of 7.141 nm.

3.2.6. Cytotoxicity studies of Si particles

The cytotoxicity of wild *C. flexuosus* leaf was predicted with MTT assays (Figs. 5-9).

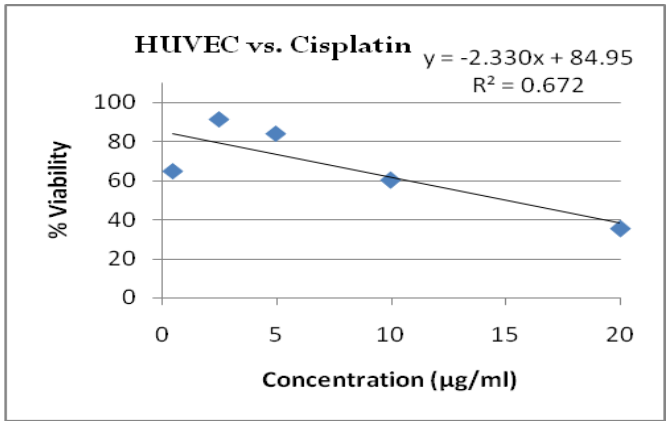

Fig. 5: Cytotoxic potential of Cisplatin against HUVEC cell lines

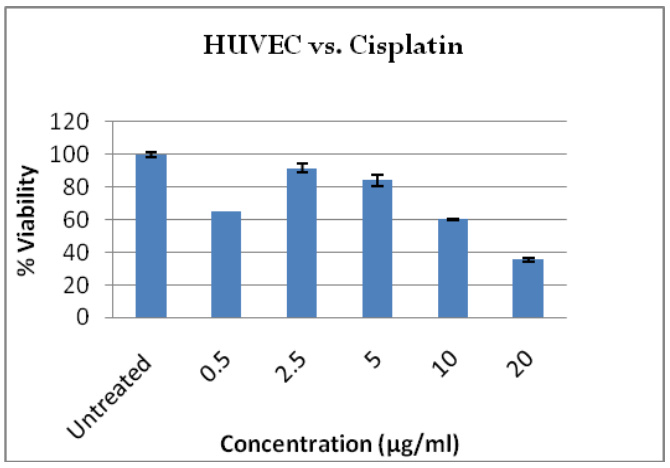

Fig. 6: Cell viability (%) of HUVEC cells with Cisplatin treatment (µg/ml)

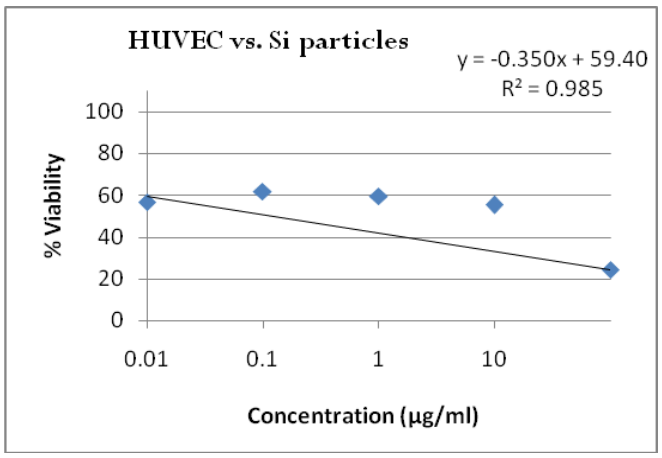

Fig. 7: Cytotoxic potential of Si particles against HUVEC cell lines

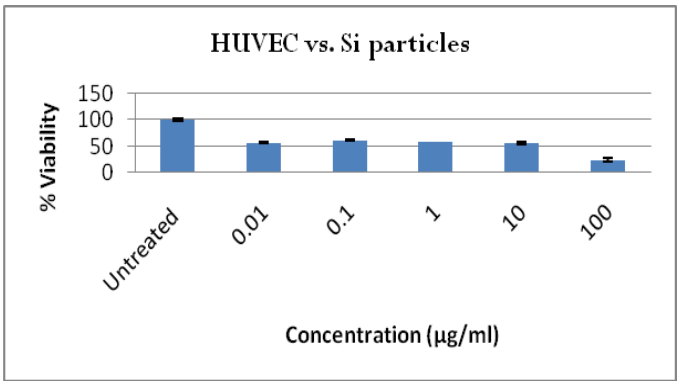

Fig. 8: Cell viability (%) of HUVEC cells with Si particles treatment (µg/ml)

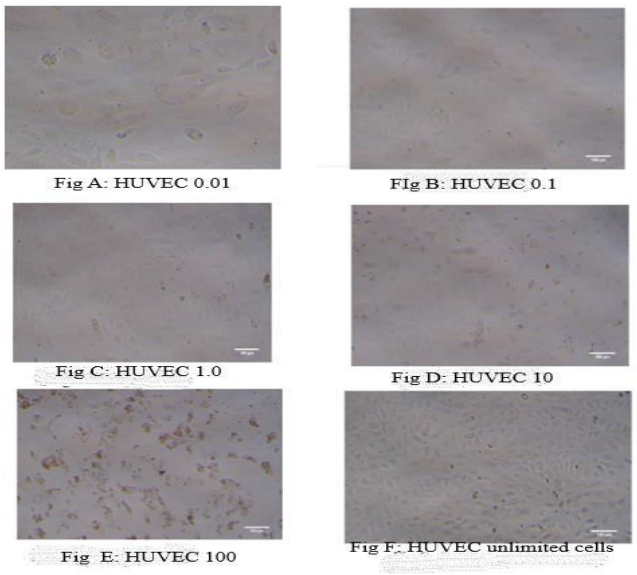

Fig. 9 (A-F): Microscopic images of HUVEC cells treated with MTT salt

The cytotoxic activity of wild *C. flexuosus* leaf (IC_{50} = 26.83 µg/mL) on HUVEC cells was comparable to that of the standard Cisplatin drug used in this study which had IC_{50} of 15.00 µg/mL (Table 3-5). The cytotoxic effect is mediated by permeabilization of the cytoplasmatic membrane and reduction of the redox potential of the cells, suggesting a decrease of the mitochondria activity.

Table 3: The IC50 values of the test samples for 24 hour treatment

Sample	HUVEC cell line IC_{50} 24hr
Cisplatin (Std)	15.00µg/mL
Si Particles	26.837µg/mL

Table 4: MTT data analysis of HUVEC cells vs. Std Cisplatin

HUVEC vs. Cisplatin	Test concentrations (µg/mL)						
	Blank	Untreated	0.5	2.5	5	10	20
Reading 1	0.0016	0.43675	0.279	0.383	0.376	0.261	0.157
Reading 2	0.0016	0.42275	0.28	0.405	0.348	0.258	0.148
Mean OD	0.0016	0.42975	0.2795	0.394	0.362	0.2595	0.1525
Mean OD-Mean Blank		0.42815	0.2779	0.3924	0.3604	0.2579	0.1509
Standard deviation		0.0098995	0.000707	0.015556	0.019799	0.002121	0.006364
Standard error		0.007	0.0005	0.011	0.014	0.0015	0.0045
% Standard error		1.634941	0.116782	2.569193	3.269882	0.350345	1.051034
% Viability		100	64.90716	91.65012	84.17611	60.2359	35.24466
IC_{50}= 15.00 µg/ml							

Table 5: MTT data analysis of HUVEC cells vs. Silica particles

HUVEC vs. Cisplatin	Test concentrations (µg/mL)						
	Blank	Untreated	0.5	2.5	5	10	20
Reading 1	0.0016	0.43675	0.239	0.269	0.258	0.245	0.118
Reading 2	0.0016	0.42275	0.25	0.264	0.256	0.233	0.094
Mean OD	0.0016	0.42975	0.2445	0.2665	0.257	0.239	0.106
Mean OD-Mean Blank		0.42815	0.2429	0.2649	0.2554	0.2374	0.1044
Standard deviation		0.0098995	0.007778	0.003536	0.001414	0.008485	0.016971
Standard error		0.007	0.0055	0.0025	0.001	0.006	0.012
% Standard error		1.634941	1.284597	0.583908	0.233563	1.401378	2.802756
% Viability		100	56.73245	61.87084	59.65199	55.44786	24.38398
IC_{50}= 26.837 µg/ml							

4. DISCUSSION

The Silica precipitates out from various types of bio-waste such as rice husk, rice hull, bagasse and lemon grass. The grasses belonging to family Poaceae, contain high silica content and are present predominantly in leaf, leaf sheath and inflorescence compared to its stem counterparts. The Silica from grasses is obtained from heating to high temperatures by eliminating carbon and other volatile compounds. As reported earlier, Silica is extracted by acid leaching and gasification methods [20]. The epidermal and phytochemical analysis of *C. citratus* revealed the presence of silica on adaxial surface of the leaf [9]. Silica can be obtained in amorphous (lechatelierite) and crystalline (crystobalite) forms through combustion process [21].

During the present investigation, Silica particles were extracted using leaf sample leaf sample of *C. flexuosus* to obtain high silica content when subjected to 50°C and calcined at 600°C.

Enhanced agglomeration with uniformed spherical Silica nanoparticles were observed with a size ranging between 50-60nm.The results obtained revealed the presence of amorphous nature of the silica particles. The leaf sample of *C. flexuosus* showed 81.03% of silica. It is reported that the amorphous silica is pure having small particle size with high surface area. The cytotoxicity studies of extracted silica particles showed IC_{50} value of 26.837µg/mL compared to standard Cisplatin IC_{50} value of 15.00µg/mL.

The present study showed that, the treated ash obtained from the leaf of *C. flexuosus* contained spherical silica particles predominating in high range with no original compounds.

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