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Therapeutic Approach and Characterisation of Watermelon (Citrullus lanatus) Rind against Acrylamide Toxicity

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ABSTRACT

Watermelon (Citrullus lanatus) is a cheap and easily available fruit in the local markets of India. The rind, which is the outer layer of watermelon, is completely edible. It is the only fruit with 90 per cent of water, and is fully edible including its rind and seeds as they contains different types of nutrients which are needed by our body in day to day life. The benefits in our body, includes reduced blood pressure, presence of different types of vitamins, such as vitamin A, B & C, as well as different types of minerals required by our body. The present study aims in evaluating the presence of different secondary metabolites in the watermelon rind. The therapeutic efficacy of watermelon rind against acrylamide toxicity in the lymphocyte cell line is studied. As selenium is an important micronutrient, an attempt has been made to prepare the selenium nanoparticles followed by its characterisation.

Keywords: Selenium Nanoparticles; UV visible; Fourier Transform Infrared; Differential Scanning Calorimetry; Particle Size Analyser

1. INTRODUCTION

Efficient green chemistry methods for synthesis of nanoparticles from plant sources are an emerging field of research. Plants seem to be the best candidates for nanoparticle production as they are more stable and easily available. The advantages in the usage of plant-materials for the biosynthesis of nanoparticles are their varied shapes and sizes when compared with those that were formed by the other organisms. Globally, an effort has been made to extract out something beneficial from the plant extract using different experimental methods[1]. The antioxidants that were found in vegetables or in medicinal plants are vitamin C, and E, carotenoids, phenolic compounds and flavonoids and usually in combination with other elements. In the present era, there is increased attention towards the diet of humans in details and their studies have been shown that a high intake of plant products is associated with the reduced risk of harmful diseases such as cancer. The antioxidant properties which were found in chocolates with cocoa content is also beneficial for disease prevention[2]. Bio-nanotechnology is an ecofriendly and nontoxic perspective for the value of biomaterials along with the nanoparticles[3] Fig.1

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1.1. Watermelon

Watermelon *(Citrullus lanatus)* is a widely acceptable, edible fruit. Red part is sweet, edible but the outer part is usually discarded and considered as waste[4]. Watermelons are reported to be rich in carotenoids like include lycopene, phytofluene, phytoene, beta-carotene, and lutein. It consists of different types of protein, functional group such as hydroxyl, carboxylic, pectin, citrulline, cellulose[4,5]. On an average, the watermelon consists of 68 per cent pulp. 30 per cent rind and rest seeds which are discarded can be used in different ways like feeds of the cattle's, and as vegetables in different parts of the country. The studies revealed the presence of different phytochemicals constituents in the rind; that has immense biological significance. Watermelon rind has also been used as the bio sorbent for the removal of dyes and heavy earth metals from the sample solution[6].

1.2 Acrylamide

There is high production of chemicals that were having adverse effect in the different products that were made in the industries[7]. According to World Health Organisation (WHO), more than 100,000 compounds are discharged in the society every year from different industries. The exposures of chemicals are also found in foods, and different food products; such as polycyclic aromatic hydrocarbons, aromatic amines, amino dyes,

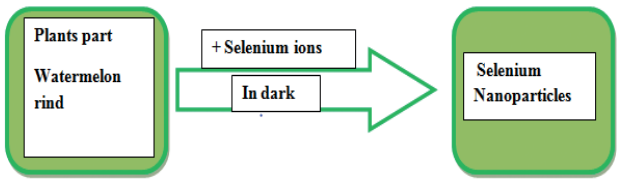


Figure 1. Flow Chart representation of formation of selenium nanoparticles.

alkenes; that may also cause cancer[8].

Acrylamide has low molecular weight and composed of carbon, hydrogen, nitrogen and oxygen atoms and can be soluble in water[9]. It is used in different industries in the form of polyacrylamide that were utilised as flocculent for the waste water treatment, utilised as adhesives, soil stabilisers, in laboratory gels, by product of temperature processed foods[10]. Acrylamide is considered as environmental and occupational pollutants. It can be formed during the Millard reaction, especially in the products containing asparagine and glucose that were processed and cooked, which leads to the formation of acrylamide in the foods. Its residues were mostly found in different types of processed foods such as bread, potato, different types of cereals and breakfast[11].

1.3 Selenium Nanoparticles

The plants carries sources for many biological products, and are mostly required for nanoparticles, and many plants extracts has been tested for this purpose in these years. Plants are less sensitive to metal toxicity, when compared with other alternatives like algae and bacteria. Plants synthesised nanoparticles are much better in terms of synthesis, safety, simplicity and ecological considerations[12].

The interest in nanoparticles has evolved due to their novel approach towards the drug discovery and other related fields such phytochemistry, electronics, energy production area, computer productsetc[13]. A nanoparticle increases the therapeutic efficiency of ionised drugs to penetrate inside the cells and in other biological components like proteins, peptides, DNA, RNA and other components[14].

The biosynthesis of nanoparticles is more preferred over green synthesis of nanoparticles and its preparation has been observed to have a wide range of activities which include anti hydroxyl radical property protective against the oxidation of DNA, and also used as an important element that acts as semiconductor to enhance the activity of seleno–enzyme, i.e. glutathione peroxidase which helps in the prevention of free radical damage to the cells and tissues invivo[14,15]

Selenium is among those trace elements, which is daily nutritional supplement for an adult at a dose of approximately 40 mg – 300 mg/day[14,15]. It is necessary to contain at least 40 µg of selenium to increase the maximal expression of Se-enzymes and maximal intake of 300 µg/day reduces the risk of cancers. Low selenium intake can contribute to morbidity and mortality, which is caused due to infectious as well as chronic diseases[16,17]. The Recommended Dietary Allowance (RDA) for selenium as per Food and Nutrition Board—USA, for adults is 55 µg/day (0.7 µmol/day)[18]. SeNPs shows free radicals scavenging effects as well as ant carcinogenic properties in both in-vitro and in in-vivo condition. A study reveals that the selenium nanoparticles show the anti-carcinogenic activity against some types of cancers. Unique antimicrobial activities have been exhibited against Candida albicans, Proteus mirabilis and Pseudomonas aeruginosa[18]. Hence the present study aims towards the therapeutic approach of selenium nanoparticles in watermelon rind study the secondary metabolites, toxicity of acrylamide, in lymphocyte cell lines, analysis of different functional groups, formation of selenium nanoparticles and the characterisation of selenium nanoparticles.

2. METHODOLOGY

2.1 Extract Preparation

The fresh fruits of Watermelon were purchased from the local vendor. The watermelon was separated out and watermelon rinds were washed thoroughly and chopped into pieces and dried for days under shadow (to remove moisture), then weighed and kept in oven at 85°C for 48 hour. A fine powder was obtained with the help of mortar pestle which is than sieved and stored in desiccator for further use[17].

2.2 Aqueous Extract Preparation

2.2.1 Decoction

The fine powder of watermelon rind (1 g) was extracted by boiling with distilled water (1:20) for around 4-6 hours, then filtered and the extraction is repeated until the extract is colorless. The filtrate was concentrated followed by evaporation in water bath for further use[18]. 1 g was dissolved in 20 ml of water and the aqueous extract was used for further analysis.

2.3 Biosynthesis of Selenium Nanoparticles

Two ml of aqueous extract was added to 10 ml of 10 mM of sodium selenite solution which is then kept in magnetic stirrer condition. Then the solution was allowed for reduction in dark at 27 °C ± 2 °Cin orbital shaker for 24 hours till the color change is observed[17].

2.3.1 Chemical Profile of Watermelon rind extract

Plants and its materials contain a varied range of secondary metabolites, which shows their potential to reduce substances for biosynthesis of nanoparticles. The constituents of different phytochemicals constituents of watermelon rind were determined and evaluated for suitability of the extract in the synthesis of nanoparticles[17].

2.4 Phytochemical Screening of Watermelon Rind Extract[19,20]

2.4.1 Test for Alkaloids (Mayer's test)

One mg of rind extract was dissolved in few drops of acetic acid followed by Mayer's reagent. No white precipitate indicates the absence of alkaloids.

2.4.2 Test for Carbohydrate (Fehling's test)

One mg of rind extract was added to 1 ml of alcoholic solution followed by 1 ml of Fehling solution A:B (1:1). Formation of red precipitate indicates the presence of carbohydrate.

2.4.3 Test for Steroids (Liebermann test)

Twenty mg of rind extract was dissolved in 1 ml of chloroform, 1 ml of acetic acid, and 1 ml of anhydride acetate. The solution is heated for 2-3 minutes which results in the conversion of pink color to green color solution thereby indicating the presence of steroids.

2.4.4 Test for Saponins (Foam test)

One mg of rind extract was diluted in 7 ml-8 ml of distilled water. This results in the development of stable foam, thereby indicates the presence of saponins.

2.4.5 Test for Tannins (Ferric chloride test)

One mg of rind extract was diluted in 1 ml of distilled water followed by addition of 1 ml of 5 per cent ferric chloride solution. Dark green color in watermelon rind extract indicates the presence of tannins.

2.4.6 Test for Phenols (Ferric chloride test)

One mg of rind extract was diluted in 1 ml of distilled water followed by addition of 1 ml of 5 per cent ferric chloride solution, the blue or bluish black color is obtained, Watermelon rind extract, shows no color change, which indicates the absence of phenols in the solution.

2.4.7 Test for Coumarins (Sodium hydroxide test)

Two mg–4 mg of rind extract was taken in a test tube and 1 ml of ethanol followed by 1 ml of 2N sodium hydroxide solution was added which results in the formation of dark fluorescence.

2.4.8 Test for Carboxylic acid (Effervescence test)

Twenty mg of rind extract was diluted in 1 ml of distilled water in a test tube followed by addition of 1 ml of sodium bicarbonate solution and dark bubble was obtained thereby indicating the presence of carboxylic acid.

2.4.9 Test for Resin (Acetone test)

Twenty mg of rind extract was diluted in 1 ml distilled water and added 1 ml of acetone solution and the solution becomes turbid, this results in the presence of resin. Watermelon rind extract, shows the turbidity in the obtained solution, which indicates the presence of resin.

2.4.10 Test for quinone (Sulphuric acid test)

Twenty mg of rind extract was taken in a test tube and 1 ml of ethanol, 1 ml of 2N sulphuric acid was added, which results in the formation of pink/purple/red colour of the solution, thereby confirming the presence of quinone.

2.5 Characterisation

2.5.1 UV Vis Spectroscopy

The selenium nanoparticles prepared were characterised in a PerkinElmer UV Vis spectrophotometer, The scanning range of the sample ranges from 200 nm–1000 nm at a speed of 480 nm/min. The data taken by the UV Vis spectrophotometer is recorded and analysed by UV Winlab software to determine the analyte concentration or the chemical changes of a component in a solution.

2.5.2 FTIR (Fourier Transform Infrared)

Fourier Transform Infrared Spectrophotometer is used to determine different functional group present in the solution of the watermelon rind extract, in the solution of selenium nanoparticles. The rind extract was dried and grounded with mortar pestle and spectrum was observed at a wavelength of 4000–400 cm^{-1}.

2.5.3 PSA (Particle Size Analyser)

The particle size were analysed with the instrument Particle size analysis, Shimadzu SALD–2300, the measurement was taken and refractive index of the medium was taken at 1.07 (Water)[21].

2.5.4 DSC (Differential Scanning Calorimetry)

The thermal analysis was conducted with the differential scanning calorimetry, TGA– 50, SHIMADZU Thermogravimetric Analyzer and the instrument was calibrated with the sample from 15 °C to 300 °C[22].

2.6 In vitro study

2.6.1 Chemical and Reagent

Acrylamide, Roswell park memorial institute medium (RPMI 1640) were procured from Sigma Aldrich, ficoll plaques, EDTA, fetal bovine serum (FBS), $NaHCO_3$, streptomycin, gentamycin, penicillin G, KCl, NaCl, KH_2PO_4, triple distilled water, trypan blue dye, MTT assay, acetic acid, TCA, tris base. All the chemicals were of analytical grade.

2.6.2 Treatment and Dose Preparation

A suspension of (5 mM) acrylamide was prepared in triple distilled water.

2.6.3 In vitro Therapeutic Efficacy

2.6.3.1 Watermelon Rind Aqueous Extract

Watermelon rind aqueous extract (15 mg) was dissolved in RPMI medium and volume made up to 3 ml and a different dose of WR aqueous extract was administered to the 96 well plates to select the optimum dose of aqueous extract.

2.6.4 In vitro Experimental Design

Preparation of Roswell park memorial Institute medium (RPMI 0.82 g RPMI, 100 mg NaHCO3, 20 mg streptomycin, 37.5 ul gentamycin, 6 mg penicillin G was dissolved in 20 ml autoclaved triple distilled water and 10 ml Fetal Bovine Serum (FBS) was added to it.

2.6.5 Isolation of Lymphocyte

A blood sample was derived from a healthy female rat and collected with the help of capillary in the test tube with one pinch of EDTA, 2 ml of phosphate buffer (PBS) pH 7.4 was used for the dilution. Air was layered on 4 ml Ficoll plaque, centrifuged for 10 mim at 2000 rpm. The white buffy layer containing lymphocytes was separated and transferred to a new tube. Collected lymphocyte layer was diluted with PBS pH 7.5 in the ratio of 1:1 and centrifuged at 2000 rpm for 10 min and the pellet was collected. After washing cell with RPMI 1640 (containing 10% fetal bovine serum) twice, the cell were cultured using RPMI 1640 (containing 10% FBS) and 1 per cent antibiotic in the flask and incubated in CO_2 incubator containing 5 per cent CO_2 at 37 °C[23].

2.6.6 Maintenance

Lymphocytes cell were inoculated and grown in tissue culture flask with complete growth at 37 °C in the atmosphere of 5 per cent CO_2 and 90 per cent humidity in CO_2 incubator. The medium was changed as the colour changes. The fresh medium was placed in culture flask 5-7 under sterile condition. Passaging was done at the sub confluent stage of cells which depends on the mass doubling time of cell.

2.6.7 Subculturing

The exhausted medium was changed by the fresh medium as per requirement. The medium of the flask having sub confluent growth was changed followed by centrifugation at 2000 rpm for 10 min. After centrifugation pellet was collected, washed with phosphate buffer saline. The tube was centrifuged at 2000 rpm at 10 min and the supernatant was discarded. The cells were resuspended in the complete growth medium and were counted and checked for viability with trypan blue. After achieving 70 per cent–80 per cent confluence, the next sub-culturing was performed[24].

2.6.8 Cell Viability Assay

Cell viability is the number of healthy cells in the sample, based on a total cell sample.

2.6.9 Calculation

$$\% \text{ cell growth} = \frac{\text{cell growth in the presence of test material}}{\text{cell growth in the absence of test material}}$$

$$\% \text{ growth inhibition} = 100\% \text{ cell growth}$$

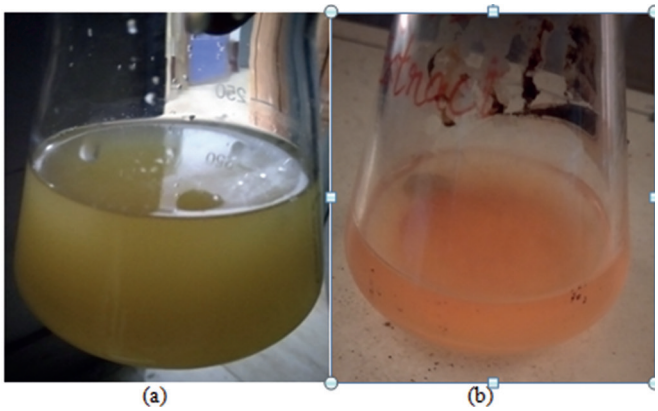


Figure 2. (a) The starting of the incubation of extract and (b) The changes in the color after the completion of incubation period.

Table 1. Phytochemical screening of aqueous extract of watermelon rind

S. No.	Absorption (cm^{-1})	Group	Compound	Appearance
1	3278.45 cm	O-H stretching	Alcohol	Strong
2	2921 cm	C-H stretching	Methylene asymmetric	Strong
3	2849 cm	O-CH_3stretching	Methoxy	Medium
4	1638 cm	C=C stretching	Alkene	Strong
5.	1535 cm	>N-H stretching	Secondary amine	Medium
6.	1395 cm	O-H stretching	Tertiary alcohol	Medium
7.	1238 cm	C-O stretching	Alkyl aryl ether	Weak
8.	1030 cm	C-C stretching		Strong
9.	539 cm	OH stretching	Phenolic	Strong

3. RESULTS & DISCUSSION

3.1 Biogenic Synthesis and Characterisation of SeNPs

It was observed that the extraction efficiency achieved by using boiling water was much effective and greater than that achieved with other methods using 80 per cent methanol and other alcoholic method[25-26].

Initially, the sodium selenite solution was colorless which turned into brick-red after the addition of Watermelon rind extract, and incubation for 24 hour. The formation of brick-red solution was due to the excitation of the surface plasmon resonance and indicated reduction of sodium selenite into elemental selenium. (Fig. 2) The reduction of sodium selenite into SeNPs can occur by the action of phenolics, flavonoids, and tannins in watermelon rind. This is then further confirmed by the UV Visible Spectrophotometer[17].

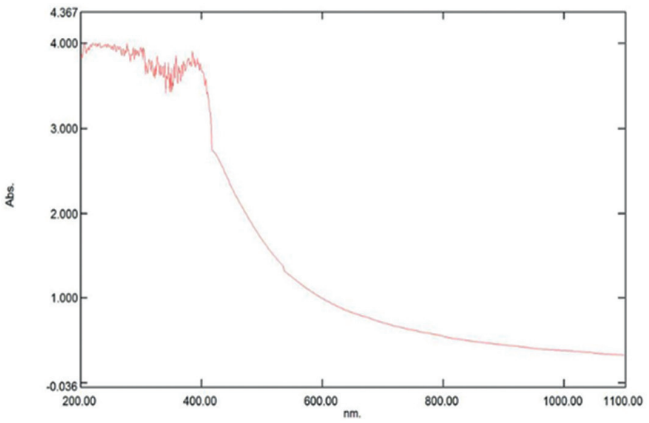
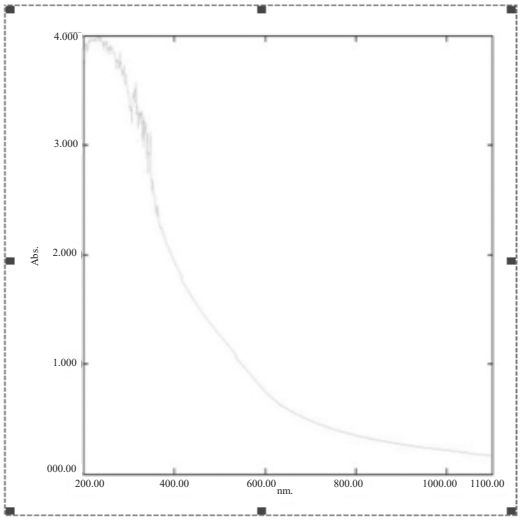
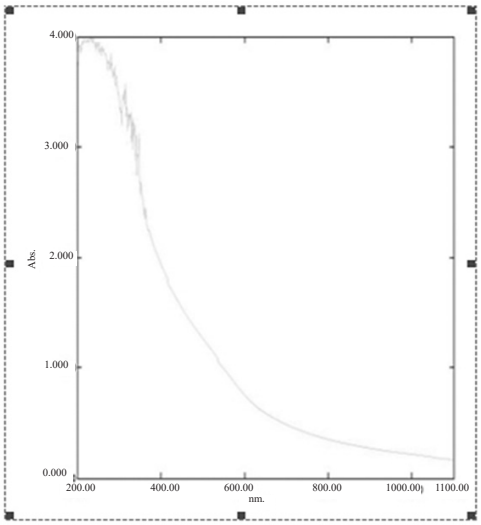


Figure 3. UV visible spectrum of the aqueous extract of watermelon rind



(a)

Figure 4. (a) UV Vis spectra which for the presence of SeNPs after the incubation of the solution prepared.



(b)

Figure 4. (b) UV Vis Spectra before the solution kept in incubation.

Table 2. Functional groups of aqueous extract watermelon rind

S. No.	Absorbance (cm^{-1})	Group	Compound	Appearance
1.	3340.03	O - H stretch	Alcohol	Strong
2.	1634.72	C = C stretch	Alkene	Medium

3.2 Phytochemical Screening of Aqueous Extract of Watermelon Rind

The result of the phytochemicals analysis of aqueous extract of watermelon rind shows the presence of different Phytochemicals in varied amount. In the extract carbohydrate, steroids, saponins, tannins, coumarins, carboxylic acid, quinine, and rennin were present in higher amount whereas phenol was relatively less with no alkaloid detected. The phytochemicals analysis provides an insight about the different bioactive elements that were present in the extract. (Table 1)

3.3 UV- Vis Spectroscopy

Synthesis of selenium nanoparticles using sodium selenite has been reported by the UV–Vis spectroscopy. To see the presence of the selenium nanoparticles present in the solution UV–Vis spectra of SeNPs were recorded, the formation of selenium nanoparticles were visualised with the change in the colour of the solution. The spectra show that there is increase in the spectra as moving forward from 200 nm. The absorption maximum is shown after 203 nm which determines that the extract is reduced and SeNPs has been stabilised at this wavelength. The graph of selenium nanoparticles has increased in the spectra from the range of 200 nm–300 nm (Fig. 3) which indicates the presence of selenium at the range from 220 nm to 385 nm in the aqueous extract of the watermelon rind. There was a high peak at 200 nm to 358 nm in (Fig. 4(a)) before the incubation period and after the incubation period the high peak at 203 nm to 288 nm in the (Fig. 4(b)). The UV data analysis supports the formation of selenium nanoparticles from the watermelon rind extract at 200–300 peak[17].

3.4 FTIR (Fourier Transform Infrared)

The Fourier transform infrared analysis of aqueous extract of watermelon rind shows the major absorption bands which appears at 3340.03 and another at 1634 cm^{-1} which is due to

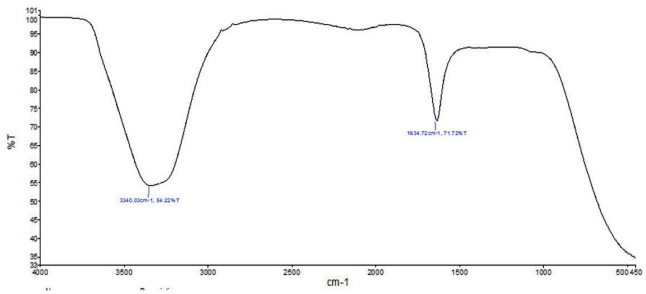


Figure 5. Fourier transform infrared spectrum of aqueous extract of watermelon rind.

Table 3. Functional groups of selenium nanoparticles extracted from watermelon

S. No.	Absorption (cm^{-1})	Group	Compound	Appearance
1	3278.45cm	O-H stretching	Alcohol	Strong
2	2921 cm	C-H stretching	Methylene asymmetric	Strong
3	2849 cm	O-CH_3stretching	Methoxy	Medium
4	1638 cm	C=C stretching	Alkene	Strong
5.	1535 cm	>N-H stretching	Secondary amine	Medium
6.	1395 cm	O-H stretching	Tertiary alcohol	Medium
7.	1238 cm	C-O stretching	Alkyl aryl ether	Weak
8.	1030 cm	C-C stretching		Strong
9.	539 cm	OH stretching	Phenolic	Strong

of O–CH_3 bond of methoxy group. The strong band at 1638 cm^{-1} shows the presence of C=C which is of alkene. Another band is at 1535 cm^{-1} shows the presence of > N–H which is of secondary amine. The short band at 1395 cm^{-1} shows the presence of O–H which is of tertiary alcohol. The short band at 1238 cm^{-1} is due to C–O stretching which is alkyl aryl ether. The strong band at 1030 cm^{-1} is due to C–C stretching vibration and at 539 cm^{-1} OH bending of the phenolic group. (Table 3)

Fourier transform infrared spectrum indicates that the variation in the graph indicates the presence of secondary metabolites which are responsible for the reduction of the selenium ions and the formation of SeNPs due to their reduction and capping process. This implies that Fourier transform infrared results analysed indicates that the SeNPs were successfully synthesised using the watermelon rind extract[21,29] (Fig. 6).

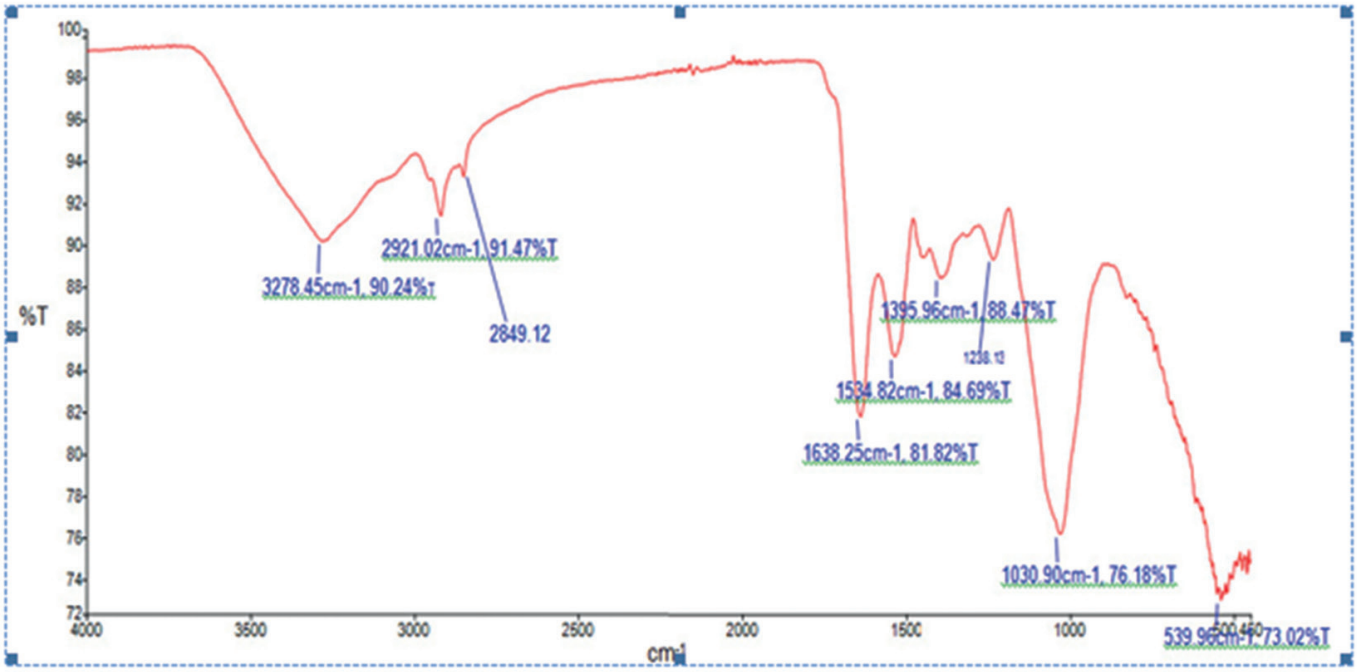


Figure 6. Fourier transform infrared spectrum of selenium nanoparticles synthesized from watermelon rind extract.

the presence of O–H stretching, which is alcohol and another is due to the C=C stretching, which is alkene; respectively. (Fig. 5 and Table 3)

The Fourier transform infrared analysis of SeNPs of watermelon rind extract shows some major absorption bands which appeared at 3278.45, 2921, 2849, 1638, 1535, 1395, 1238, 1030, 539 cm^{-1}. The bands which appeared at different wavelength have different stretches at 3278.45 cm^{-1} which is due to the presence of O–H stretching, of carboxylic acid. Another absorption peak at 2921 $cm^{-1}$is due to the presence of C–H bond of methylene. The band at 2849 cm^{-1} is due to the presence

3.5 PSA & DSC

The particle size analysis was determined by Particle size analyser. The laser diffraction studies reveals that the particle size obtained from highly dispersed mixture was in two areas, on the scale of normalised particle amount. In 0-10 the particle diameter was 0.05-0.4 and in 0-5 the particle diameter found to be 48-100 µm in range[27,28]. The image (Fig. 7) shows that the size of the nanoparticles in nanoscale.

The differential scanning calorimetry, gives us the thermograph of the nanoparticles with multiple peaks which determines the different crystalline features of the

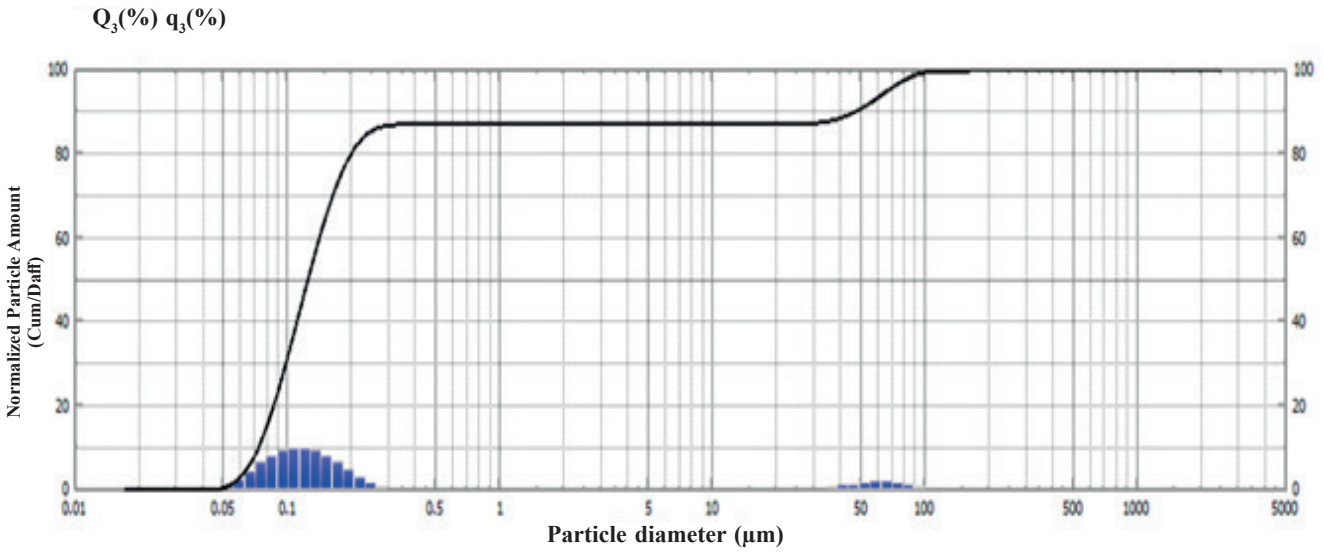


Figure 7. Particle size analysis of selenium nanoparticles.

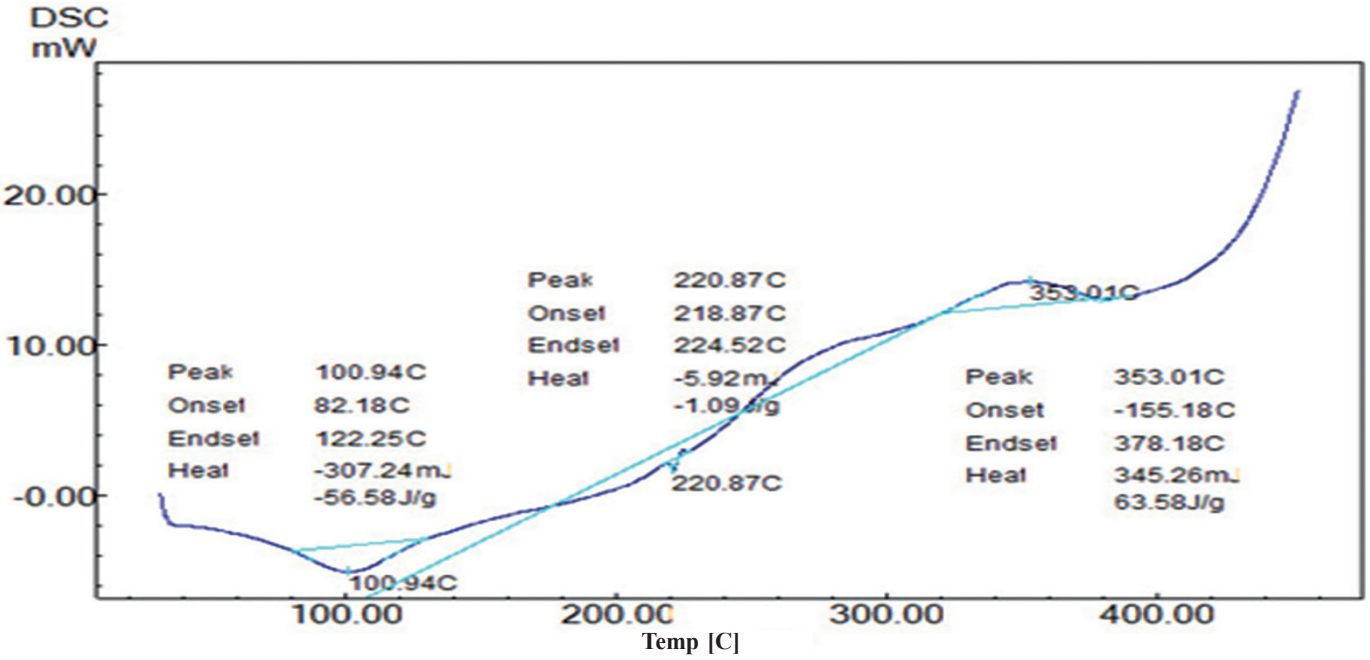


Figure 8. Differential scanning calorimetry analysis of selenium nanoparticles.

particles provided. The particles were recorded at 50°C, the exothermic transition of the particles and at 300°C, the endothermic melting peaks were observed[29-30]. The graph (Fig. 8) shows the nanocrystalline nature of the particles losing their capability at 353°C.

3.6 In Vitro Studies

3.6.1 Evaluation of therapeutic effectiveness of queous extract of watermelon rind against acrylamide induced cytoxicity on isolated lymphocyte

Cytotoxicity of acrylamide was measured on isolated lymphocytes. The therapeutic effectiveness of aqueous extract of watermelon rind was evaluated after acrylamide exposure. Control group depicted 98 per cent cell viability while acrylamide exposed group at 5 mM concentration revealed that the cell viability was decreased potentially upto 24 per cent treatment with aqueous extract of watermelon rind on acrylamide exposed group showed the significant lymphocyte protective activity in concentration dependent manner. Lymphocytes were treated with aqueous extract of watermelon rind at 6 different concentration ranges from 25 ug/ml-500 ug/ml after acrylamide exposure on lymphocytes. Treatment with aqueous extract of watermelon rind at a dose of 500 ug/ml concentration showed maximum cell viability.

Rind aqueous extracts exhibited iron and copper ions chelating activity. The proliferation was inhibited by 20 per cent-85 per cent of extracts at 0.1 mg/ml-1.0 mg/ml in renal carcinoma, cervical adenocarcinoma and carcinoma. Thus, watermelon rinds

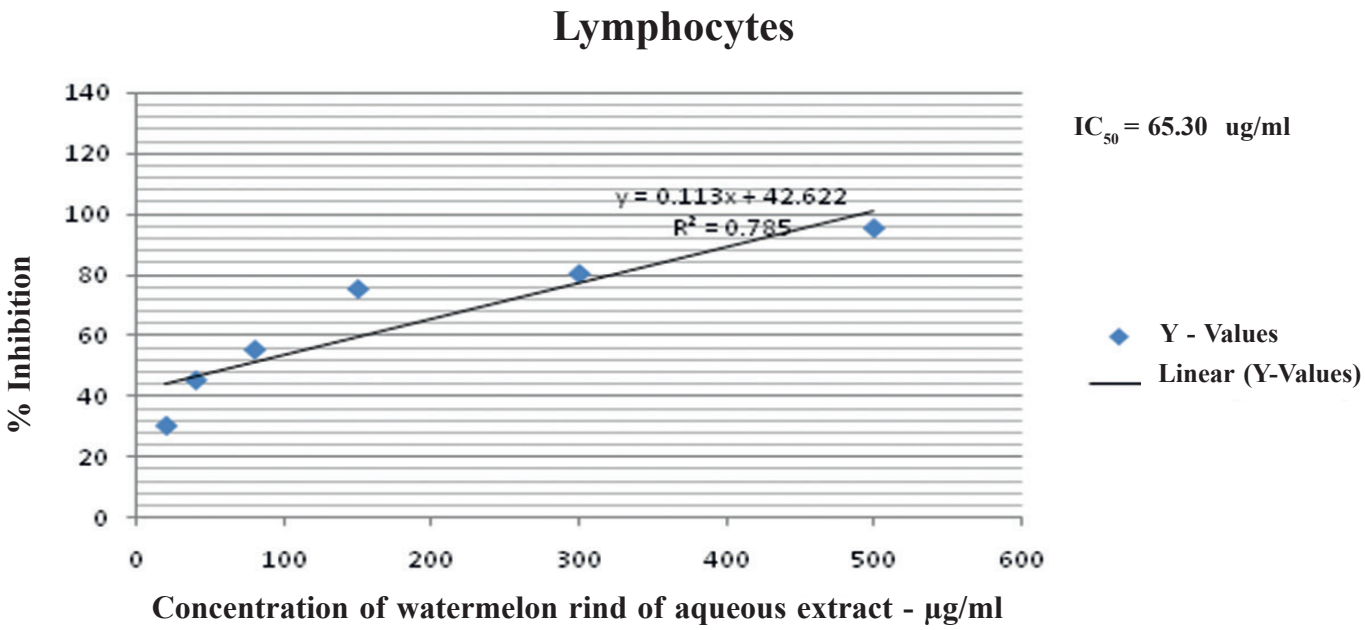


Figure 9. IC50 of aqueous extract of watermelon rind.

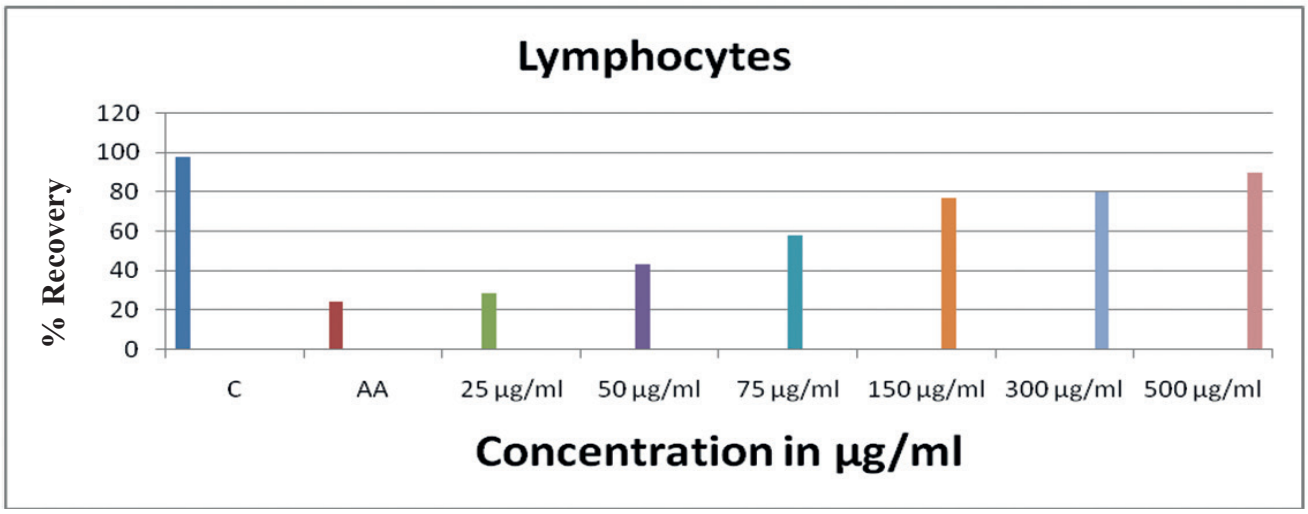


Figure 10. Effect of aqueous extract of watermelon rind on acrylamide exposed lymphocytes.

extracts display high antioxidant activity in *in vitro* assays and have effective biological activity against the growth of human tumor cells[31].

Based on *in vitro* studies, aqueous extract was more effective on lymphocyte. Extremely significant activity was observed in acrylamide exposure on lymphocyte i.e., IC_{50} = 65.30 µg/ml. Aqueous extract of watermelon rind showed significant activity with lowered IC_{50}. IC values of aqueous extract of watermelon rind were calculated to compare the therapeutic potential of plant extract against AA induced cytotoxicity. The lower (IC_{50}) value corresponds to the maximum activity of treatment against particular cell[24,31].

4. CONCLUSION

The present studies show that the biosynthesis of SeNPs by using watermelon *(Citrullus lanatus)* rind extracts which is economically environmental friendly and non toxic process. The characterisation of the nanoparticles through UV Visible spectroscopy reveals the presence of selenium nanoparticles that were responsible for the absorption of the light. FTIR analysis confirms the presence of different functional groups which belongs to biomolecules on the surface of the SeNPs. The DSA and PSC reveals size of the particle and the nature of the particles present in the solution of the nanoparticles, their nature at different temperatures that determines the nanosize of the particles. Determination of secondary metabolites, *in vitro* approach against acrylamidetoxicity, Characterisation by UV Visible spectroscopy, FTIR, followed by the biosynthesis of SeNPs, promotes further research in field of medicine, nano drugs.

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