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Research Article

PREPARATION AND CHARACTERIZATION OF INTEGRATED BOSWELLIC ACID AND PHYCOCYANIN NANOPARTICLE FOR MEDICAL APPLICATIONS

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ABSTRACT

The integrated Boswellic acid and Phycocyanin nanoparticle has important medical applications and major advantage than using individually. This study investigates various properties of integrated nanoparticle in comparison with individual particles. The Boswellic acid was extracted from *Boswellia serrata* and Phycocyanin from *Oscilllatoria cortiana*. The compounds were purified using precipitation, dialysis and chromatography methods. The characterizations of the purified compounds were done using SDS-PAGE, HPLC and FTIR. The purified compounds were then integrated and characterized using SEM, Zeta potential measurements, EDAX and cytotoxicity studies. The Boswellic acid extracted from Boswellia serrata had a total organic content of 55%. The Oscillatoria cortiana gave high yield of 63% of Phycocyanin. The integrated Boswellic acid and C-PC coated selenium nanoparticle had a size of 210 nm determined using SEM and the stability was high with -50.0mV by Zeta potential measurements compared to individual compounds. The elemental composition using EDAX studies showed selenium 3.07% by weight and carbon 65.70% by weight along with oxygen, nitrogen and negligible quantities of sodium and chlorine. The best IC_{50} was observed for integrated Boswellic acid and C-PC coated selenium nanoparticle at 16.87µg/ml. The herbal extracts find several medical applications and act as potential alternatives for various drugs. The integration of poly herbal drug finds more advantage and efficacy when compared to that of the individual compound.

KEYWORDS: Boswellic acid, Phycocyanin, nanoparticle

INTRODUCTION

Cancer is probably the biggest medical concern of people living in affluent societies. Cancer refers to a group of disease in which a cell or a group of cells divide and replicate uncontrollably, leading to intrusion into adjacent cells and tissues (invasion) and ultimately spreading to other parts of the body other than the location at which they originated metastasis[1]. Cervical cancer, next to breast cancer, is the second common and fifth most deadly cancer in women. The urgency to manage the disease is reflected from the fact that more than 500 000 new cases of cervical cancer are diagnosed yearly of which nearly 80% account from developing countries resulting in more than 250 000 deaths[2]. There are many factors involved in the development of cervical cancer but the main risk factor seems to be human papilloma virus (HPV) infection. Even though there are several strains of HPV, two strains (HPV 16 and 18) account for more than 70% of all cervical cancer cases[3]. Additional factors that increase the risk of developing cervical cancer includes smoking, oral contraceptive use, high parity (3 births or more), weak immune system and multiple sexual partners[4]. Most cervical cancer patients receive combined therapy of radiation as well as chemotherapy that decreases the risk by 30–50% where the prognosis remains very poor in advanced cervical cancer[5]. With the chemotherapeutics, only 5-yr survival rate is increased in patients with advanced cervical cancer due to poor chemosensitivity of cervical cancer cells to chemotherapeutics[6]. Therefore, novel therapeutics need to be developed that is useful to manage the progression of cervical cancer and to have an effective quality of life. Traditional medicine uses sources from nature particularly plants and an impressive number of modern drugs have been isolated. Novel natural product leads will be optimized on the basis of their biological activities to yield effective chemotherapeutic and other bioactive agents using medicinal chemistry and combinatorial chemical and biosynthetic technology[6]. Currently more than 50% of drugs are derived from medicinal plants, which still continue to provide a novel source of anticancer therapeutics[3]. *Boswellia serrata* is a kind of deciduous tree native to arid parts of India and China, containing Boswellic acids that chemically resemble steroids[7]. In addition, the boswellic acids and their analogs such as 11-keto-b-boswellic acid, 3-O-acetyl-b-boswellic acid and 3-O-acetyl-11-keto- boswellic acid also exhibit strong anti-cancer activities against brain tumors and leukemia cells[8]. The pentacyclic triterpenic acid named Boswellic acid present in the gum resin is responsible for its anti-inflammatory property[7]. Besides anti-inflammatory effects, recent research studies have shown that Boswellic acid also has anticancer effects[8]. Boswellic acid causes anticancer effect by triggering apoptosis in cancer cells via a pathway dependent on caspase 8 activation[9] and it shuts down the master inflammation regulatory complex NF-κβ in tumor cells, bringing about early tumor death and regression[10]. Boswellic acid also acts as leukotriene inhibitor and provides protection against gastric ulcer[11]. In addition to the plant derived anticancer property another promising lead is the Phycobiliproteins. These are responsible for about 50% of light capitation from cyanobacteria and red algae and are accessory photosynthetic pigments that participate in an extremely efficient energy transfer chain in photosynthesis. Phycocyanins, Allophycocyanins and Phycoerythrins forms three main groups of phycobiliprotein[12]. Phycocyanin (PC) is a blue protein pigment located in the thylakoid system in the cytoplasmic membrane of Cyanobacteria. The phycocyanins have an apparent molecular mass of 140–210 kDa and two subunits, α and ß. Studies of crystal structure in phycocyanin have shown that all of these have a very

similar three-dimensional structure. The subunits are associated in an (αß) protomers, which in turn can be associated in trimmers (αß)3 and hexamers (αß)6[13]. The pigment has a single visible absorption maximum between 615 and 620 nm and a fluorescence emission maximum at ~650 nm. C-phycocyanin (C-PC) extracted from cyanobacteria is widely used as a natural blue dye in the food and cosmetic industry. Recent studies have demonstrated the hepatoprotective[14], anti-inflammatory[2,14] and antioxidant properties of C-PC. Phycocyanin has been reported to exert a strong photodynamic action on tumor cells and no side effects have been observed. Hence it can be potentially used as a new type of phytodynamic therapeutic agent[15]. Animal model studies have reported that phycocyanin administration enhances bone marrow reproduction, thymus growth and spleen cell proliferation and in some cases, the stimulation of hematopoiesis (especially erythropoiesis). This property of phycocyanin is a promising agent in the treatment of leukemia[16]. Phycocyanin induces apoptosis in cancer cells by reducing the levels of anti-apoptotic proteins like Bcl-2 and promoting the expression of death receptor genes like Fas/FasL and ICAM. Phycocyanin promotes higher level expression of caspase cascades which play a central role in all known apoptotic signaling pathways[17].

MATERIALS AND METHODS

Materials and Equipments

Boswellia serrata resin was purchased from a local herbal sale market, Coimbatore. All the chemicals used in the present study were of highest purity and of analytical grade. The analytical instruments used in the present study include UV Spectrophotometer (Make: Perkin Elmer; Model: Lambda 35 & Country: USA), Electronic Balance (Make: Mettler Toledo; Model: AB 265-S & Country: Switzerland), pH meter (Make: Sartorius; Model: PB-11 & Country: India), Orbital shaker (Make: Scigenics & Model: Orbitek-L), Hot Air Oven (Make: Heraeus 7000 series; Model: UT12 & Country: Germany), Lyophilizer (Make: Christ & Model: Alpha 1-2 LD Plus), Gel Documentation System (Make: Bio-Rad), High Performance liquid Chromatography (HPLC) (Make: Shimadzu & Model: LC20AD), Magnetic stirrer (Make: Remi & Model), SEM equipped with EDAX (Philips, Netherlands), Zeta Potential (Zeises), FTIR (PerkinElmer, Spectrum GX, USA).

Extraction of Boswellic acid and Nanoparticle Synthesis

12.5 g of *Boswellia serrata* resin was added to 50 ml of methanol with continuous stirring using a magnetic stirrer to dissolve it which was then concentrated under high vacuum. This concentrated extract was subjected to repeated solvent extraction using hexane (pH 12). The obtained extract was further concentrated under high vacuum which was precipitated with concentrated HCl. The precipitate was then dried using vacuum oven at 45˚C. The powder thus obtained was subjected to solvent-antisolvent precipitation (nano-precipitation). The addition of water to ethanol dissolved powder will show turbid nature which indicates the presence of Boswellic acid nano-particles[18].

Estimation of Total Organic Acids in the extract

The estimation of total organic acid content in the extract prepared was done by volumetric analysis. The Boswellic acid powder was mixed with ethanol and neutralized with 0.1M NaOH under stirring condition. The organic content is evaluated using the formula,

Organic acid content = (V*N*75*100)/(1000*volume of sample)[18]

HPLC Analysis

Boswellic acid extract was characterized according to a HPLC characteristic spectrum compared with the commercial sample, which was used as a control. Chromatography conditions for detecting Boswellic acid included a C_{18} reverse phase chromatographic column (46 mm × 250 mm, 5 µm); the mobile phase, which was a mixture of Acetonitrile and water (at a ratio of 90:10); a flow velocity of 2.0 mL/min; a column temperature of 27°C; an injection volume of 20 µL; and a detection wavelength of 260 nm[19].

FTIR Characterization

IR spectroscopy is one of the most powerful analytical techniques, which offers the possibility of chemical identification. The IR spectra of Boswellic acid were obtained by KBr pellet method. Spectral scan analysis was carried out at wave number ranging from 400 to 4000 cm^{-1} by using a FT-IR spectrometer (Perkin Elmer, Spectrum GX) with resolution of 0.15 cm^{-1} to evaluate functional groups that might be involved in particle formation process[18].

Culture conditions and growth of Cyanobacteria for Phycocyanin preparation

The Cyanobacterial cultures procured from Bharathidasan University, Trichy, India were grown in standard ASN III medium at room temperature for 30 days with intermittent shaking. The light intensity is provided for a period of 16hrs and maintained at dark for 8hrs. The growth was monitored to select best Cyanobacterial species to obtain maximum phycocyanin from the cells.

Freezing-Thaw cycle

Fully grown cell culture was harvested by centrifugation at 10000 g for about 20 min at 4ºC to settle the biomass. One volume of washed cell mass was re-suspended in five volumes of the phosphate buffer and subjected to repeated freeze–thaw cycles of -20 °C and 4 °C temperature shocks for the release of Phycocyanin[20]. The cell debris was removed by centrifugation at 10,000 g for 20 min at 4 °C. The supernatant was pooled and citric acid was added to stabilize the pigment coloration[21]. The amount of phycocyanin was calculated as mg phycocyanin per ml using the equation

(O.D at 615 nm – 0.474 (O.D at 652 nm))/5.34

The blue supernatant crude extract was stored at 4^0C for further purification process.

Purification of C-PC

The precipitation of C-PC from crude extract was carried out by slow addition of ammonium sulphate [$(NH_4)_2SO_4$] at 4°C under constant stirring. 50% saturation of $(NH_4)_2SO_4$ was used to get maximum yield of C-PC. This C-PC containing higher salt concentration is dialyzed with the membrane having a molecular cut-off 12,000 Da. This ensures the removal of unwanted salt from C-PC which will hinder further purification process[22].

Size Exclusion Chromatography

The powdered Sephadex G-100 matrix was regenerated at 90 °C for 5 h and packed in the column (150 mm×10 mm, bed height 75 mm). A 0.1 M Phosphate buffer (pH 7.0) was used as the mobile phase. Elute was collected in 1 ml fractions. Each fraction was analyzed by UV-VIS spectroscopy and same fractions were

pooled. The purified sample was analyzed by polyacrylamide gel electrophoresis[23].

Spectrophotometric estimation of Phycocyanin

The UV-Vis absorbance spectra (250–650 nm) were recorded during each stage of purification by UV Spectrophotometer. The amount of C-PC in the sample was calculated from the following equations[12].

Formula: C-PC (mg/ml) = (OD_{620}- 0.474×OD_{652})/5.34

HPLC Analysis of Phycocyanin

The purified fraction was characterized according to a HPLC characteristic spectrum compared with the commercial sample (C-PC, from Sigma), which was used as a control. Chromatogram conditions for detecting PC included a C_{18} reversed phase chromatographic column (46 mm × 250 mm, 5 µm); the mobile phase, which was a mixture of deionized water and Acetonitrile (at a ratio of 40:60); a flow velocity of 1.0 mL/min; a column temperature of 30 °C; an injection volume of 20 µL; and a detection wavelength of 280 nm[24].

Phycocyanin characterization by SDS-PAGE

To check the purity of the phycocyanin and to estimate molecular mass, sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) was performed[25]. Stacking (7.5%) and separating (12.5%) gels were prepared and 30 µl of samples (Crude and purified samples of each stage) were loaded in the wells. Mixed protein markers of Myosin 205 kDa, Phosphorylase B 97.4 kDa, BSA 66 kDa, Ovalbumin 43 kDa, Carbolic Anhydrase 29 kDa, Soyabin Trypsin Inhibitor 20.1 kDa, Lysozyme 15.3 kDa, Aprotinin 6.5 kDa and Insulin 305 kDa were used[26]. Sample was electrophoresed at 100 V for 90 minutes. Coomassive Brilliant Blue G250 solution was used for staining. The gel was visualized in the Gel documentation system and the molecular weight of the sample was found with the help of the molecular weight of marker.

C-PC Nano-particle synthesis

In a typical synthesis of selenium nano-particles, the protocol consists of drop-wise addition of phycocyanin to sodium selenite solution (10 mM). Addition of the C-PC was carried out under constant stirring condition. The content was later placed on to a rotatory orbital shaker operating at 200 rpm for 24 h. The incubation of the mixture was performed at 30ºC in dark condition. The reduction of selenium ions was monitored by sampling an aliquot (3 ml) of the mixture at intervals of 12 h, followed by measurement of the UV–Vis spectra using spectrophotometer (Lambda 35). In order to find the absorption maximum, a spectral scanning analysis was carried out by measuring optical density of the content from wavelength, 200-600 nm[27].

Integration of Boswellic acid and C-PC Nano-particles

The synthesis of nanoparticle from two sources is followed by their integration into a single nanoparticle. For this, a suitable solvent capable of dissolving both the extract was identified. Prior to this a simple docking study was performed to find the extent of affinity between the compounds to be integrated. The Boswellic acid nanoparticle and C-PC nanoparticle were made to dissolve in a common solvent of acetone under constant stirring condition. In order to evaluate the efficacy of different composition as nanoparticles, five different kinds of nanoparticles with different composition were prepared. Boswellic acid nanoparticle (S1), Crude C-PC (S2), C-PC coated selenium nanoparticle (S3), integrated nanoparticle of Boswellic acid and C-PC (S4), integrated nanoparticle of Boswellic acid and C-PC coated selenium (S5).

Characterization of Integrated Nanoparticle

The average particle size of the nanoparticles was analyzed with SEM while the surface charges and the colloidal stability of particles with Zeta potential measurement. To check the various chemical compositions of nanoparticles, EDAX studies were carried out.

Cytotoxicity Studies

The human cervical adenocarcinoma cell line (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37^0C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week[28].

MTT Assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.After 48 h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula[28].

% Cell Inhibition = 100- Abs (sample)/Abs (control) x 100

Nonlinear regression graph was plotted between % Cell inhibition and Concentration and IC_{50} was determined using GraphPad Prism software.

RESULTS AND DISCUSSION

Boswellic Acid Extraction

The Boswellic acid was extracted and the total organic acid content in the boswellic acid extract was determined to be 55%, which is close to that of 63% as reported by[18].

Phycocyanin Production

Six different Cyanobacterial species *Oscillatoria cortiana* (BDU 92022), *Phormedium valderianum* (BDU 10121), *Nostoc calicola* (BDU 180601), *Synechococcus elongatus* (BDU 70542), *Spirulina subsalsa* (BDU 141021), *Oscillatoria salina* (BDU 92071) procured from Bharathidasan University was subjected to species selection based on the high phycocyanin pigment yield by spectrophotometric analysis reported by[20]. The percentage yield was calculated according to the following formula,

C-PC = (OD_{620} – 0.474 OD_{652}) / 5.34

Out of six species only two showed a promising yield from which the best Cyanobacteria was identified to be *Oscillatoria cortiana.*

Table 1: Percentage yield of Phycocyanin by two different Cyanobacterial species

Species	Absorbance at 620 nm	Absorbance at 652nm	% Yield
Oscillatoria cortiana	3.4474	0.1768	63
Pharmidium valderianum	1.2514	0.1541	22

With the isolation of C-PC from *Oscillatoria cortiana*, its identity was confirmed by UV-Spectrophotometry at 620 nm by employing a general spectral scan from 200 nm to 700 nm. Smooth increase in the peak height at 620nm substantiated the presence of C-PC in the crude extract along with other related protein as obtained by repeated freeze-thaw cycles.

The most accepted mechanism behind the success of freezing–thawing extraction protocol was that it causes cells to swell and ultimately break, due to sharp ice crystals formed during the freezing process and then contract during thawing. In this study the buffer used for extraction of Phycocyanin from *O. Cortiana* by freeze-thaw cycles of -20 °C and 4 °C was optimized and it was Sodium Phosphate buffer of neutral pH. To protect Phycocyanin from photo-denaturation, the next purification steps were carried out in dark condition at 4 °C. After freeze-thaw cycle it was separated from biomass by centrifugation at 10,000 rpm for 20 min. The supernatant showed its highest absorption peak at 615 nm indicating, a higher concentration of Phycocyanin than others phycobiliproteins like Allophycocyanin and Phycoerythrin. At this stage the A_{620}/A_{280} ratio was 1.303 (Stage I).

Ammonium Sulphate Precipitation

Two-steps of fractionation were performed during this stage of purification. First 20% saturation (initial) was done to precipitate proteins other than phycobiliproteins and then 70% saturation was done to precipitate Phycocyanin. This was clearly visible in SDS-PAGE as numbers of unwanted bands were reduced as compared to crude extract (Figure 4.12). The 70% ammonium sulfate precipitate was re-suspended in 0.1 M Phosphate buffer and dialyzed overnight at 4 °C against 50 times volume of the same buffer, with changes of buffer four times to enhance dialysis process. A purity ratio 2.425 was achieved at the end of this step (stage II). The previous study reported that purity ratio 1.26 was achieved at the end of ammonium sulphate precipitation[23].

Size Exclusion Chromatography

High molecular weight proteins other than phycocyanin were eliminated by size exclusion chromatography. Sephdex G-100, size exclusion column was loaded with 2 ml of dialyzed protein while 1 ml fractions were collected. A 0.1 M Phosphate buffer (pH 7.0) was used as mobile phase. The cobalt blue-colored Phycocyanin started eluting from the 12th fraction whereas 17th fraction was colorless, indicating an absence of Phycocyanin. 12–16 fractions were pooled after UV-VIS scan was done. The purity ratio A_{620}/A_{280} at this stage was to be 3.377 (stage III). Similar investigation reported that 20 mg of pure phycocyanin of purity ratio of 3.31 was produced from 10 g of biomass- *Oscillatoria quadripunctulata*[23].

Fraction from each purification stage were verified with SDS-PAGE of which gel filtration chromatographic fraction revealed two distinct bands at 17 and 19 KDa indicating the presence of two subunits α and ß in C-PC (Figure 1).

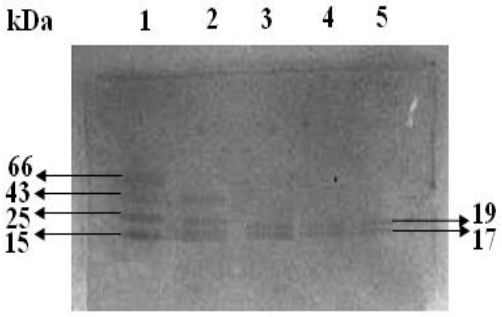

Figure 1: SDS-PAGE Profile of crude and purified C-PC fractions

High Performance Liquid Chromatography

The Figure 2. shows HPLC chromatogram of C-PC isolated from *Oscillatoria cortiana*, Standard Boswellic acid and Boswellic acid extract.

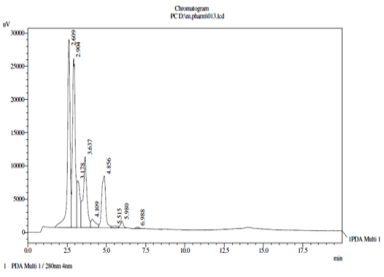

Figure 2 (a)

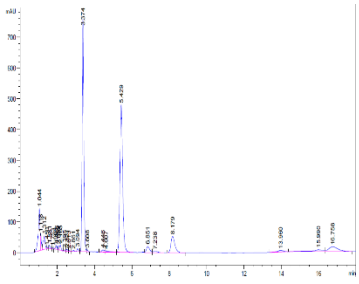

Figure 2 (b)

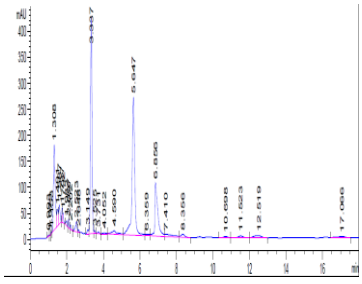

Figure 2 (c)

Figure 2: HPLC chromatogram of a) C-PC isolated from *Oscillatoria cortiana* b) Standard Boswellic acid and c) Boswellic acid extracted from *Boswellia serratta*

From the chromatogram, it was observed that the retention time for the C-PC was found to be 4.856min. In a similar manner HPLC performed for Boswellic acid isolated from *Boswellia serratta* was compared with the standard Boswellic acid available in the market of pharmacological grade. The combinations of six compounds eluted at different time interval and AKBA (3 – Acetyl -11- keto – β Boswellic Acid) fractions that is more potent among the six compounds had a retention time of 5.429min.

Fourier Transform Infrared Spectroscopy

The functional groups present in the C-PC and Boswellic acid were studied to identify the major groups present in the compounds using Fourier Transform Infrared spectroscopy and shown in Figure 3.

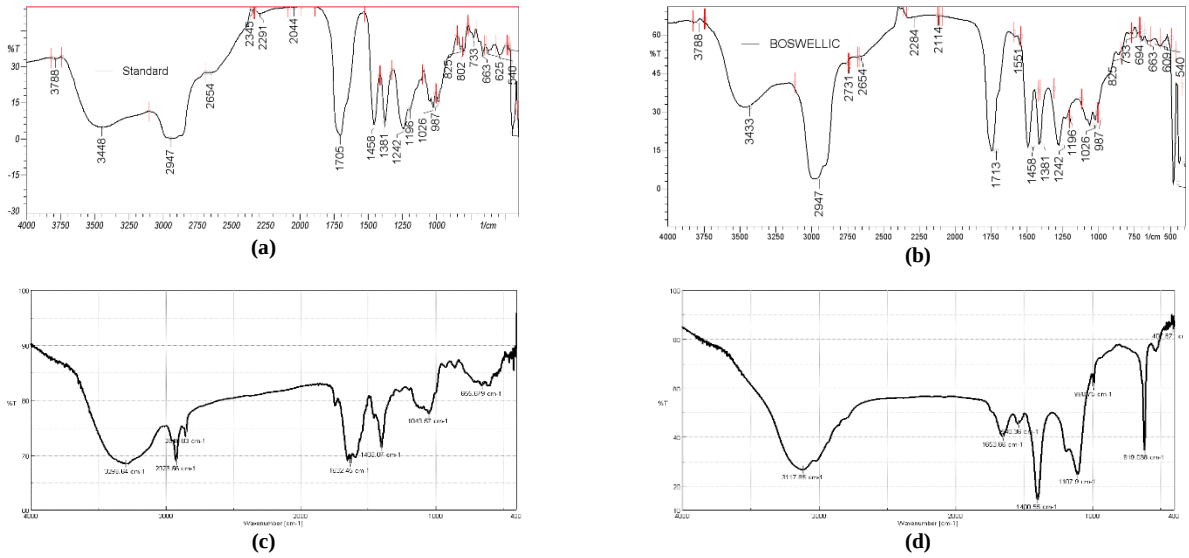

Figure 3: FTIR spectrum of (a) standard boswellic acid, (b) boswellic acid extract, (c) standard C-PC and (d) purified C-PC

By comparing the FTIR spectrum of extracted and standard Boswellic acid, the frequency range between 2950-2850 (cm^{-1}), 1700-1400(cm^{-1}) and 860-680(cm^{-1}) with wavenumber of 2947 (cm^{-1}), 1458 (cm^{-1}) and 733 (cm^{-1}) respectively indicates the exact presence of alkyl C-H stretch, aromatic C=C bending and aromatic C-H bending. With the FTIR spectra of C-PC, aromatic C-H bending, N-H amide bending, C=O amide stretching and C-N stretching were observed at 1350-1470 (cm^{-1}), 1590-1650(cm^{-1}), 1630-1695 (cm^{-1}) and 1000-1250 (cm^{-1}) respectively.

The synthesized nanoparticle C-PC coated selenium when subjected to FTIR spectra, revealed the presence of carboxylic acid derivatives, amide groups, alkenes and alkynes with a wavenumber of 1708.02(cm^{-1}) and 1242.20 (cm^{-1}), 1654.01 (cm^{-1}), 991.44 (cm^{-1}), 616.28 (cm^{-1}) respectively. FTIR spectra for integrated C-PC and Boswellic acid nanoparticles were studied. The following functional groups were present in common with Boswellic acid C-PC coated selenium nanoparticles. Carboxylic C-O bond at 1710 (cm^{-1}), C=C bending at 1450 (cm^{-1}) and aromatic C-H bending at 733-739 (cm^{-1}).

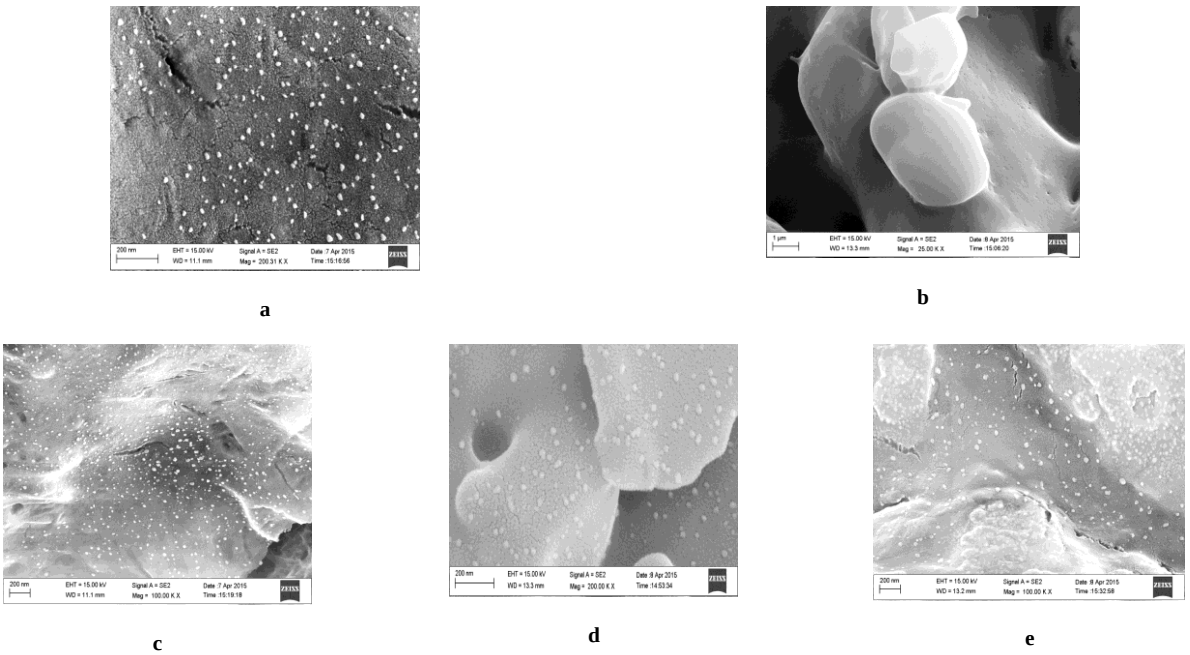

Figure 4: Scanning electron microscopic image of (a) Boswellic acid (b) Raw C-PC (c) C-PC coated selenium (d) Integrated raw C-PC and boswellic acid nanoparticle (e) Integrated C-PC coated selenium and boswellic acid nanoparticle

Scanning Electron Microscopy was employed to determine the size and morphology of the nanoparticles under study. An average particle size of 230nm, 3µm, 190nm, 200nm and 210nm were evaluated for Boswellic acid nanoparticle, raw C-PC, C-PC coated selenium nanoparticle, integrated nanoparticle of raw C-PC and Boswellic acid, integrated C-PC coated selenium and Boswellic acid respectively.

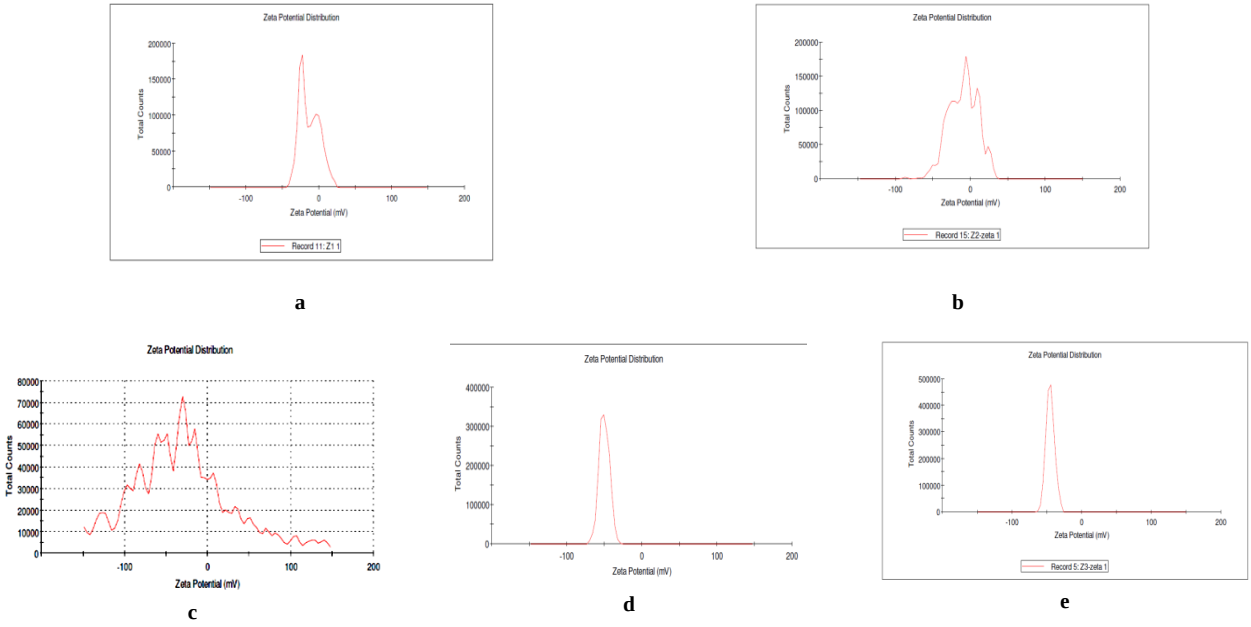

Figure 5: Zeta potential distribution of synthesized nanoparticles (a) Boswellic acid (b) Raw C-PC (c) C-PC coated selenium (d) Integrated raw C-PC and boswellic acid nanoparticle (e) Integrated C-PC coated selenium and boswellic acid nanoparticle

Stability of nanoparticles determined by zeta potential showed varying degree of stability. For Boswellic acid the stability was found to be around -39.8mV which is close to that of -42.88mV reported by[18]. For raw C-PC it was -9.66mV which indicates the pigment has lower stability. With the C-PC coated selenium nanoparticles the stability had a hike with -13.1mV. The stability increased for integrated nanoparticles of raw C-PC and Boswellic acid with -45.1mV and -50.0mV for integrated C-PC coated selenium and Boswellic acid respectively.

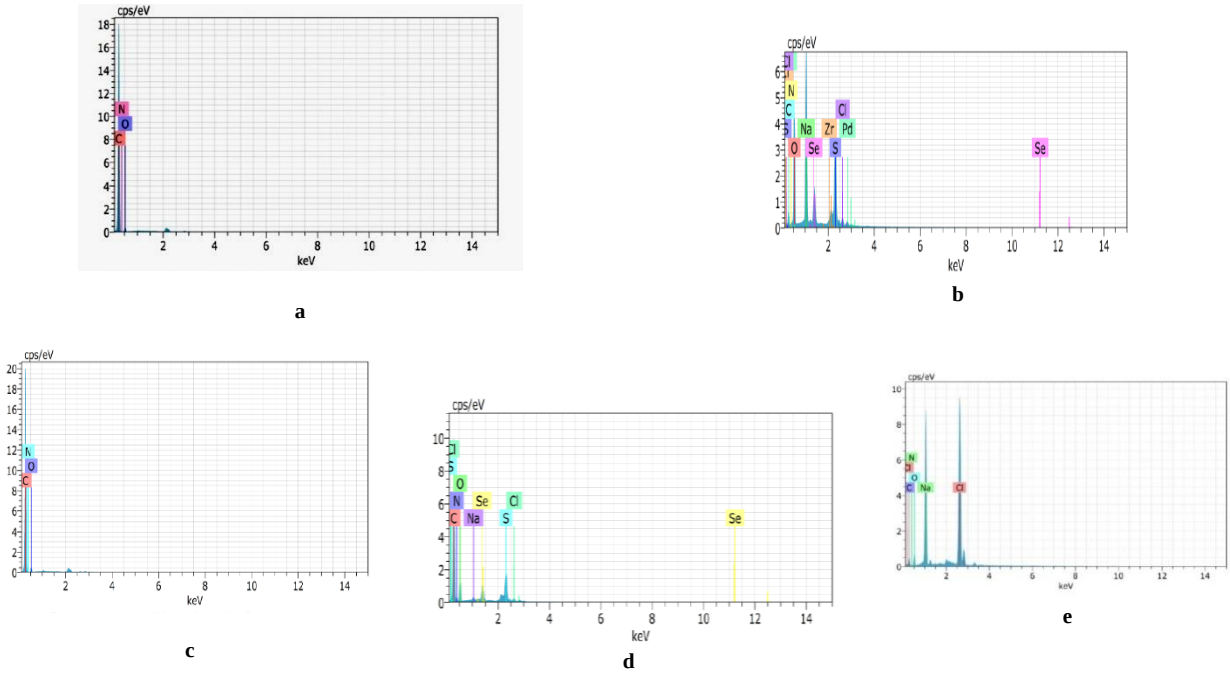

Figure 6: Elemental analysis of synthesized nanoparticles (a) Boswellic acid (b) Raw C-PC (c) C-PC coated selenium (d) Integrated raw C-PC and boswellic acid nanoparticle (e) Integrated C-PC coated selenium and boswellic acid nanoparticle

The elemental compositions of synthesized nanoparticles were confirmed with energy dispersive X-ray spectroscopic analysis. With the Boswellic acid, raw C-PC nanoparticles the major elements were Carbon, Nitrogen, and Oxygen along with Chlorine as 46.04 weight percentage in raw C-PC. EDAX analysis for C-PC coated selenium nanoparticles had Oxygen content of 35.50 weight% and selenium of 7.87 weight% with very negligible amount of Palladium and Zirconium. But with the integrated Boswellic acid and raw C-PC there were no impurities of any external elements. The carbon content higher constituted about 89.78% by weight along with oxygen and nitrogen as supporting elements. The impurities of Zr and Pd were absent in integrated nanoparticles of C-PC coated selenium and Boswellic acid with selenium comprising only 3.07% by weight and carbon 65.70% by weight along with oxygen, nitrogen and negligible quantities of sodium and chlorine.

Docking Studies

Table 2: Affinity between Boswellic acid and C-PC generated by Auto-Dock with the α-subunit

Mode	Affinity (kcal/mol)	Distance rmsd l.b.	Distance rmsd u.b
1	-7.7	0.000	0.000
2	-7.6	45.808	49.990
3	-7.5	2.727	4.104
4	-7.4	21.653	26.440
5	-7.3	17.783	21.467
6	-7.2	20.577	25.918
7	-7.2	11.490	15.146
8	-7.1	13.434	18.928
9	-7.0	12.410	17.888

Table 3: Affinity between Boswellic acid and C-PC generated by Auto-Dock with the ß-subunit

Mode	Affinity (kcal/mol)	Distance rmsd l.b.	Distance rmsd u.b
1	-7.5	0.000	0.000
2	-7.4	18.850	23.234
3	-7.2	2.050	2.757
4	-7.2	22.399	24.849
5	-7.1	62.654	65.135
6	-7.0	62.651	65.821
7	-7.0	22.633	25.206
8	-6.9	19.593	25.771
9	-6.9	62.238	65.45

A fundamental auto-dock was performed to check the affinity between Boswellic acid and two subunits (α and ß) of C-PC. From the negative free energies (-7.0 and -6.9 Kcal/mol) it was evident that the two molecules interacted positively which restrained from using couplers for integration of nanoparticles as they naturally had affinity.

Cytotoxicity Studies

Table 4: Cell inhibition percentage of samples at five different concentrations

		Cell inhibition (%)				
		S1	S2	S3	S4	S5
Concentration (µg/ml)	12.5	5.124	6.36	8.077	11.56	24.45
	25	14.28	14.98	22.521	65.60	99.30
	50	36.56	38.19	55.82	100	100
	100	56.36	71.27	100	100	100
	200	70.03	98.91	100	100	100

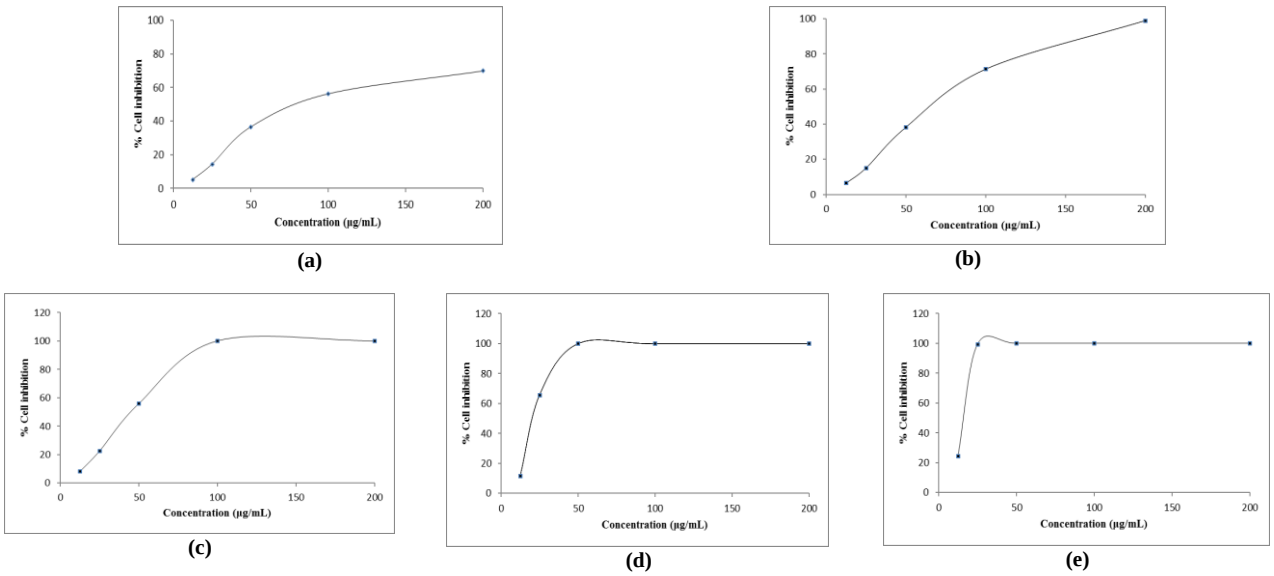

Figure 7: Cytotoxic profile of (a) Boswellic acid (b) Raw C-PC (c) C-PC coated Selenium (d) Integrated raw C-PC and boswellic acid nanoparticle (e) Integrated C-PC coated selenium and boswellic acid nanoparticle

The inherent efficiency of synthesized nanoparticles was tested for its cell cytotoxicity with HeLa cells lines meant for cervical cancer. All the five samples when tested in triplicates in five different concentrations showed varying degree of cell inhibition. Most of the integrated nanoparticles of Boswellic acid and Phycocyanin demonstrated 100% cell inhibition at a concentration range of 50 – 200 µg/mL. At a concentration of 25 µg/mL, integrated nanoparticles of Boswellic acid and raw C-PC displayed 65.60% of cell inhibition. Out of the five samples under study, integrated nanoparticles had higher inhibition rate even at lower concentration proving its efficiency.

IC_{50} values were evaluated for all the five samples. Nano-boswellic acid exerts 70% inhibition at a concentration 200 µg/ml which is relatively more efficient compared to 40% inhibition at 1000 µg/ml as reported by[29]. Raw C-PC exerts 50% inhibition at a concentration of 67.89 µg/ml this is relatively more efficient compared to 50% inhibition at 800µg/ml as reported by[30]. C-PC coated Selenium nano-particles exerted 50% inhibition at a very low concentration 47.31 µg/ml. Integrated nano-particles of boswellic acid and C-PC exert 50% inhibition at 21.48 µg/ml. Integrated nano-particles of boswellic acid and C-PC coated Selenium nano-particles exerted 50% inhibition at 16.87 µg/ml. IC_{50} value of a compound represents the maximal concentration required to inhibit biological process by half. IC_{50} value of integrated boswellic acid nano-particles and C-PC was least compared to the values of individual boswellic acid nanoparticle and C-PC. 100% cell inhibition was observed in integrated nano-particles of C-PC –Boswellic acid nano-particles and Selenium C-PC nano-particles at a concentration of 50 µg/ml, these results clearly depicts that the efficacy of integrated poly herbal drug is high compared to that of the individual compound. This suggests that various compounds having different properties can be integrated together to obtain a component with multitude properties.

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