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Synthesis of liposomes using α-phosphotidycholine and metabolites obtained from *Elephantorrhiza elephantina* and *Pentanisia prunelloides*

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Plant saponins exhibit numerous pharmacological characteristics desirable for long term hyperlipidemic therapy through their cholesterol binding capacity due to the formation of liposomes/phytosomes which ultimately decreases the gastrointestinal absorption of cholesterol. This may result in the reduction of the blood plasma cholesterol levels; hence, mitigating cardiovascular and atherosclerotic problems that are associated with elevated plasma cholesterol concentrations. In this study, we reported for the first time a potential method of synthesizing phytosomes/liposomes from two medicinal plants *Pentanisia prunelloides* (Rubiaceae) and *Elephantorrhiza elephantina* (Fabaceae) saponin extracts and fractions using α-phosphotidycholine and cholesterol. This was done to explore the possibility of cholesterol binding capacity of fractions and extracts of the two medicinal plants as a justification of their application by traditional healers in managing body weight as well as averting hyperlipidemia and atherosclerosis. Spherical nanoparticles/phytosomes/liposomes were clearly identified in the TEM images with particle sizes ranging between tens and hundreds of nanometers. The zeta potential of the nanoparticles fell between -5 and +5 mV indicating that they have a high potential for aggregation; hence, making it relatively very difficult for the complexed cholesterol molecules to permeate the microscopic pores in the alimentary tract.

Key words: Frontier transmission infra-red (FTIR), liposomes, adjuvants, zeta-potential, α-phosphotidycholine.

INTRODUCTION

Recently, much phytochemical-research attention has focused on the metabolites and pharmacokinetics of saponins due to their biological activities and absorption profile (Akihisa et al., 2007; Donya et al., 2007; Harinantenaina et al., 2006; Kimura et al., 2005). Saponins are responsible for diverse effects including anti-inflammation, anti-allergy, antitumor, augmentation of the immune responses, stimulating the apoptosis of skin cells, anti-obesity and anti-hyperlipidemia (Awad et al., 2011; Amin et al., 2010; Wang et al., 2011). Saponins are believed to form the main constituents of many drugs and folk-medicines and are considered responsible for numerous pharmacological properties (Liu and Henkel, 2002). Notably, saponins can activate the mammalian immune system, which has led to significant interest in their potential as vaccine adjuvants/liposomes (Skene and Sutton, 2006). Vaccines require optimal adjuvants including immunopotentiator and delivery systems to offer long term protection from infectious diseases in animals and man. Phospholipids like alpha- phosphodylcholine

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are thus employed in the formation of phytosomes/ adjuvants as natural digestive aids and as carriers for both fat-miscible and water miscible nutrients. Saponins on the other hand are generally used as adjuvants/ liposomes to enhance bioavailability of medication.

Adjuvants/liposomes influence the titer, duration, isotype and avidity of antibody, and affect the properties of cell-mediated immunity (Hunter et al., 1995; Kaeberle, 1986; da Silva et al.,2005). The common clinically used adjuvants are synthetic and are mostly synthesized with the inclusion of aluminium. These adjuvants have disadvantages like side effects, strong local stimulation and carcinogenesis accompanied by complicated preparations (Bowersock and Martin, 1999). This therefore calls for the preparation of physiologically friendly phytosomes; hence, this study on the use of secondary metabolites from *Elephantorrhiza elephantina* and *Pentanisia prunelloides* as potential adjuvant/phytosome precursors. The lead candidate saponin adjuvants or liposomes are Quillaja A and its derivatives Quillaja Saponin-21 (Kensil and Kammer, 1998). Quillaja saponins have been reported to have a high degree of toxicity and hence, confer undesirable haemolytic effects apart from their instability in aqueous phases which would limit their application as phytosomes/liposomes in human vaccination (Cox et al., 1999; Waite et al., 2001; Marciani et al., 2003). On the other hand, the overexploitation of the soap bark tree from which Quillaja-A saponin is obtained, *Quillaja saponaria* has caused important ecological damage and considerable shortage of available supplies (Marciani et al., 2003).

Triterpenoid glycosides were found to be predominant in both *E. elephantina* and *P. prunelloides*; hence, allaying the haemolytic effects due to the administration of the two plant metabolites in phytotherapy. Hence, the exploitation of saponins from *E. elephantina* and *P. prunelloides* could act as an alternative to Quillaja saponins. Due to the problematic nature of isolating pure saponins from plants, many reports assessing the hypocholesterolemic activity of plant saponins use 'saponin fractions containing multiple related structures rather than individually isolated saponins (Hazai et al., 1992; Southon et al., 1988; Malinow et al., 1987a,b; Oakenfull, 1986). It is for this reason that crude extracts and partially isolated saponins from *E. elephantina* and *P. prunelloides* were used in this study.

MATERIALS AND METHODS

Plant material and collection

Fresh plant rhizomes of *E. elephantina* and *P. prunelloides* were collected in June 2010 from seven different Southern African regions in Swaziland, South Africa and Zimbabwe and identified by Dr Anna Moteetee, University of Johannesburg. Voucher specimens (SJM-0, SJM-01 to SJM-08) were deposited in JRAU Herbarium, Department of Botany and Plant Biotechnology (Kingsway Campus, University of Johannesburg). Fresh plant rhizomes were washed with water, marcerated, air-dried at ambient temperature and kept in the fumehood at room temperature. The dried plant materials were then ground into fine powders, extracted as described as follows and solvents evaporated under reduced pressure while aqueous extracts were dried using the freeze drier. The dried samples were then stored in sample bottles at room temperature.

Qualitative determination of secondary metabolites in *E. elephantina* and *P. prunelloides*

The dried ground rhizome powders with a moisture content of about 10% were extracted with n-hexane three times until the solvent became clear. The extract was filtered and concentrated *in vacuo* at 40°C. The same procedure was applied consecutively with chloroform, ethyl acetate, methanol and water and percentage yields were then calculated. Standard qualitative tests were carried out (Edeoga et al., 2005; Harbone et al., 1973; Speedie and Tyler, 1996) on the crude extracts and fractions of *E. elephantina* and *P. prunelloides* as well as the Liebermann-Burchard's test for trieterpenes and sterols (Cook, 1961).

Quantitative determination of saponins

A mass of 20 g powdered sample was mixed with 100 ml of 20% aqueous ethanol. The mixture was heated in a water bath for 4 h at 55°C with constant stirring then filtered. The residue was re-extracted further with another 100 ml 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at 90°C. The concentrate was transferred to a 250 ml separatory funnel partitioned with 3 × 20 ml diethyl ether shaking vigorously. The aqueous layer was recovered while the ether layer was discarded. Partitioning of the solution was done three times with 60 ml portions of n-butanol. The combined n-butanol fractions were washed twice with 10 ml of 5% aqueous sodium chloride. The resultant solution was heated in a water bath for the vaporization of n-butanol. The final dried fraction was further dried to constant mass in an oven to constant mass. The saponin content was calculated as a percentage of the starting dried plant material.

Thin layer chromatography (TLC) of extracts

Aluminum-backed 0.3 mm silica gel 60 F_{254} pre-coated tlc plates (cut into 5 × 10 or 10 × 10 cm) were used. Each crude extract (0.1 µL) was placed on the TLC plate 0.30 mm silica gel 60 with fluorescence indicator UV_{254} Alugram Sil G UV_{254} type. Portions of both *E. elephantina* and *P. prunelloides* were spotted on separate TLC plates and eluted with butanol/acetone/water mixture (4:1:2). The developed tlc plates were sprayed with vanillin sulphuric acid and then heated in the oven at 110°C for 5 min. Portions of both plant extracts were subjected to column chromatography using the same eluent applied for prerparative PTLC. The resultant fractions of the two plant extracts were spotted on the same TLC plate against standard digitonin and saponin from Quillaja (Figure 3). This was done for comparison purposes. Preparative TLC was then used to isolate the major compounds with TLC3 ($MeOH/H_2O$/ acetone/ethyl acetate/chloroform) 10:8:30:40:12 as eluent. Standard digitonin and Quillaja-21 were also spotted against the isolates for comparison purposes.

Column chromatography analysis of extracts

The dried residue (10.0 g) was suspended in minimum amount of methanol, immobilised on silica gel (8 g), and subjected to column chromatography (CC), using a 38 × 4.5 cm glass column filled with Merck silica gel 60 F_{254} in chloroform to a level of about 8 cm from the top. The immobilized extract was added to the free volume at the head of the column. Fractionation was done by successive

Table 1. Preparation of nanoparticles/phytosomes.

Origin of saponin	Code	Saponin mass (g)	Cholesterol mass (g)	Volume of phospholipid (ml)	Volume of methanol (ml)
Zim Ee	SJ 1	0.0141	0.0138	1.5	10
KZN Ee	SJ 2	0.0154	0.0253	1.6	10
O. F. Pp	SJ 3	0.0141	0.0198	1.4	10
Sicunusa Pp	SJ 4	0.0135	0.0145	1.3	10
Saponin from Quillaja	SJ 5	0.0143	0.0143	1.2	10

Table 2. Percentage yields of moisture-free dried powders of *P. prunelloides* and *E. elephantina* for different solvents.

Extraction solvent	Sample origin and percentage yields (%)			
	Zim. (Ee)	KZN (Ee)	O. Farm (Pp)	KZN (Pp)
n-hexane	0.059	0.039	0.093	0.62
Chloroform	0.12	0,15	0.41	0.25
Ethyl acetate	0.12	0.14	0.09	0.15
Methanol	19.6	22.68	17.12	7.2

applications of 600 ml of each of the following solvents; hexane, chloroform, methanol and water. Four major fractions were collected and the solvent was evaporated in the fume hood overnight and the water extracts were dried by means of a freeze drier. The resultant fractions were weighed and percentage yields evaluated as depicted on Table 2.

FT-IR spectroscopic analysis extracts

A 2 mg of powdered plant sample was mixed with 300 mg of spectroscopic grade purity and spectroscopically dry Kbr salt in an agate mortar using a pestle, and compressed into thin pellets. Infrared spectra were recorded between 4000 to 500 cm^{-1} as Kbr pellets on the TL 8000 balanced flow FT-IR EGA (Perkin Elmer Spectrum series) instrument.

Preparation of nanoparticles using α-phosphotidycholine as precursor

Phytosomes are prepared in different ways depending on the precursor secondary metabolites and phospholipids used (Karate et al., 2013). The preparation of nanoparticles in this study was carried out as summarized on Table 1 which is similar to literature reports (Yanyu et al., 2006; Maiti et al., 2006). *E. elephantina* (Ee) samples obtained from Zimbabwe (Zim-SJ 1) and Kwazulu Natal (KZN-SJ 2) while *P. prunelloides* (Pp) samples were from Orange Farm (O.F.-SJ 3) and Sicunusa-SJ 4 and the standard saponin from Quillaja-SJ 5 were mixed with α-phosphodylcholine, cholesterol and methanol in the respective proportions summarised in Table 1. All the chemicals (Methanol, Quillaja saponin, cholesterol and L-α-phosphotidycholine) used in this study were of high purity purchased Merck.

Characterization of the synthesized nanoparticles

Nanoparticle size and zeta potential

To determine the size of the particles, 5 ml sample was sonicated after re-dispersing in methanol and placed into the analyzer chamber. Readings were collected at 25°C with a detector angle of 90° using a Malvern Zetasizer and Particle Analyzer 5000.00 (Malvern Instruments UK) at the University of Johannesburg.

Morphology study

The morphology of the nanoparticles was studied by a TEM using a JEOL Electron Microscope JEM 2100 (2000.00 kV) at CISR in Pretoria. The samples were dispersed in methanol, sonicated for 10 min and mounted on carbon coated copper grids before examination. The size, shape, membrane integrity, aggregation and fusion between vescicles were examined by means of TEM over a week during 21 days. Results for the particle sizes are shown on Table 5 (Reis et al., 2006) and for the TEM images (Figures 5 to 7).

RESULTS AND DISCUSSION

Qualitative phytochemical profiles

Phytochemical tests were carried out on the crude extracts (Table 3) as well as different fractions from the sequential fractionation (Mpofu, 2014; University of Johannesburg, PhD thesis submitted in 2013). The procedure was undertaken to confirm the partitioning of various secondary metabolites in the respective solvents applied with saponins and their derivatives expected to be predominantly found in the methanolic and aqueous fractions. The appearance of an intense reddish colour in the Liebermann-Burchard's reagent for both aqueous and methanol fractions was indicative of the presence of trieterpene saponins. UV-Vis spectra for the saponin fractions from the two plant extracts were indicative of the presence of the respective secondary metabolites which were conspicuous by their poor chromophores (Figure 1). The UV-Vis spectra for all the saponin fractions as

Table 3. The phytochemical profile of crude extracts of *E. elephantina* and *P. prunelloides* from different locations.

Major phytochemical	Sample origin and estimation of major phytochemicals			
	Zim. (Ee)	KZN (Ee)	O. Farm (Pp)	KZN (Pp)
Alkaloids	+	+	+	+
Saponins	+++	+++	++	++
Anthraquinones	+++	+++	++	++
Tannins	+++	+++	++	++
Flavonoids	+++	+++	++	++
Anthocyanidins	+++	+++	++	++

+ = Trace amount, ++ = detectable, +++ = substantially detectable, - not detectable.

Table 4. The relative saponin percentage composition of *E. elephantina* and *P. Prunelloides.*

Sample	Origin of sample	Saponin percentage (%)
E. elephantina	Zimbabwe	1.56
	KZN	1.44
P. prunelloides	Orange farm	0.24
	Sicunusa	0.71

Table 5. Zeta potential values of nanoparticles synthesized from *E. elephantina* and *P. prunelloides.*

Sample	Z-potential (mV)
SJ1 (fresh)	-4.65
SJ2 (fresh)	+0.829
SJ1 (after 3 weeks)	+2.15
SJ2 (after 3 weeks)	+0.22
SJ3	-1.38
SJ4	-0.853
SJ5	+0.807

reflected in Figure 1 exhibited a very poor chromophore with λ_{max} in the range of 208 to 210 nm which is in agreement with literature. The UV-Vis spectra for all twelve fractions of saponins of *E. eephantina* and *P. prunelloides* drawn from four different geographical locations were superimposed (Figure 1) which suggested selectivity of the extraction technique applied.

Rhizomes of both plants exhibited all the six different secondary metabolites tested although in varying degrees. Saponins and flavonoids tended to be more pronounced for both crude extracts and fractions as depicted by the intensity of colours across all samples from different geographical locations (Table 3). A further analysis showed *E. elephantina* to have a relatively greater content of the two classes of phytochemicals as exhibited by the intensity of the colour changes for the prescribed standard tests. These two classes of compounds are generally polar and this observation tentatively corroborated the findings on the quantitative determination of fractions eluted by solvents of increasing polarity (Table 2) that exhibited more polar compounds from the two species. The qualitative tests demonstrated the abundance of the major secondary metabolites that could lend support to the extensive use of the two medicinal plants in phytotherapy. Despite the fact that the samples were drawn from different geographical locations, the difference in the saponin content between these two medicinal plants was consistently conspicuous and confirmed the observations in the qualitative tests.

Quantitative composition of saponins in *E. elephantina* and *P. prunelloides*

The equantification of saponins was adopted from (Ediago, et al., 2005 and Astuti, et al., 2011). The relative percentage composition of saponins in the two plants is shown in Table 4. *E. elephantina* samples exhibited a higher quantity of saponins (1.56 and 1.44%) compared to *P. prunelloides* samples (0.24 and 0.71%) (Table 4). Despite the fact that the samples were drawn from different geographical locations, the difference in the saponin content between the two species was conspicuous. The intensity of the precipitate formed was more pronounced for both *E. elephantina* extracts compared to both aqueous and methanol *P. prunelloides* extracts. This again suggested a relatively higher saponin content of this class of phytochemicals in *E. elephantina*. This group of phytochemicals is very important in phytotherapy and may have a significant role in the treatment of ailments for which these two medicinal plants are pre-

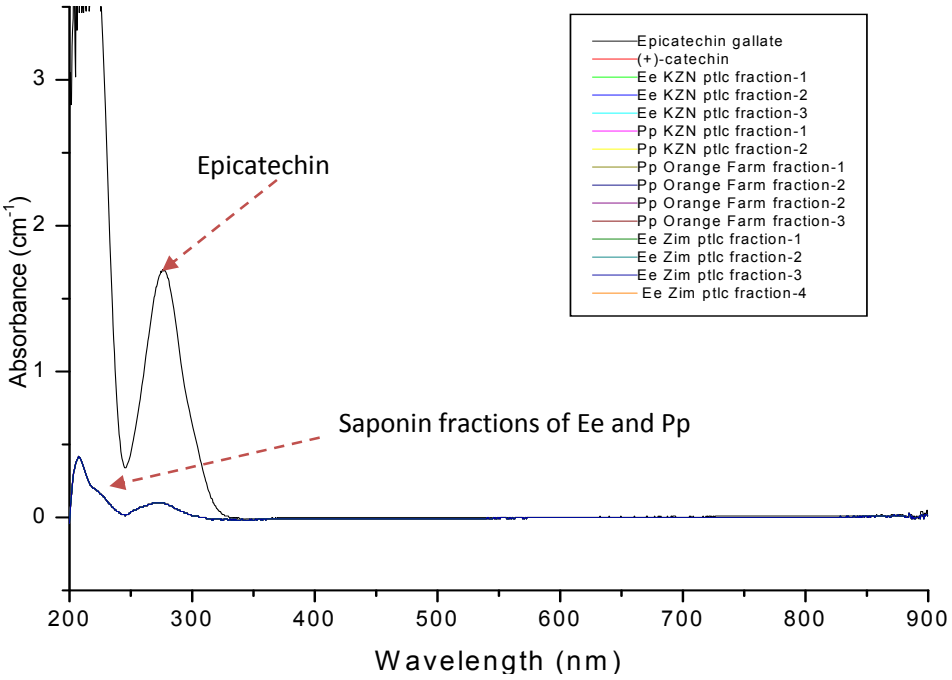


Figure 1. The UV-Vis spectra of *E. elephantina* (Ee) and *P. prunelloides* (Pp) fractions from different geographical locations (Kwazulu Natal, KZN; Zimbabwe, Zim; Orange Farm, OF).

scribed by traditional healers. Furthermore, both groups of secondary metabolites are good candidates for the formation of phytosomes which augers well for the application of these two plants in the phytopharmaceutical industry. The health benefits of saponins include the reduction of cholesterol levels in the intestinal tract; hence, mitigating obesity and antimutagenicity thus preventing cancer cells from growing. The non sugar parts of saponins have a direct antioxidant activity; hence, reducing the risk of cardiovascular disorders. It is most likely that the antioxidant capacities exhibited by extracts of these two medicinal plants emanates partly from this group of phytochemicals apart from the flavonoids and other secondary metabolites yet to be discovered. The relatively high content of saponins suggests that both *E. elephantina* and *P. prunelloides* have cytotoxic effects such as intestinal cell membrane permeabilization.

Saponins are reported to mimic the sex hormone involved in controlling the onset of labour in women and the subsequent release of milk (Okwu and Okwu, 2004). It is most likely that the efficacy of concoctions of *P. prunelloides* administered to expectorant women and those that deliver without the release of the placenta for the enhancement of ease delivery (Okwu, 2003) emanates partly from this class of compounds.

TLC for the fractions of *E. elephantina* and *P. prunelloides* against saponin standards

The green colour after spraying with vanillin sulphuric acid and baking confirmed the presence of saponins in the methanol extracts of *E. elephantina* (Ee) and *P. prunelloides* (Pp) (Figure 2). Furthermore, TLC analysis of saponin fraction against digitonin afforded sub-fraction 61 of *E. elephantina* which exhibited the presence of saponins and triterpenoids as depicted by the green and purple colours, respectively. The prepapratory TLC for fraction 59 afforded five sub-fractions that exhibited the purple colour for triterpenoid after spraying with vanillin sulphuric acid and baking. Both fractions 61 and 59 of *E. elephantina* gave positive results from Liebermann-Burchard and Molish reactions which suggested that they were trietepenoid saponins.

Frontier transmission infra-red (FT-IR) spectra of saponins from *E. elephantina*

Some functional groups in the saponin fractions of *E. elephantina* and *P. prunelloides* were examined using FTIR spectrometry and the results are shown in Figures 3 and 4. A peak at 3459.16 cm^{-1} was displayed from the spectrum indicative of the presence of hydroxyl group. The peaks ranging from 3016.75 to 2925.56 cm^{-1} sug-gested the presence of C-H while the intense peak at 1738.93 to 1739.00 cm^{-1} were indicative of the presence of carbonyl groups (C=O) and asymmetric C=O stret-ching in the carbohydrates were also suggested by the peak at 1435.19 cm^{-1} (Yim et al., 2007). Oligosaccharide linkage to sapogenins absorption bands were evidenced by the C-O-C bands, between 1052.75 and 1092.63 cm^{-1} (Zheng et al., 2008). The peak at 1235.54 cm^{-1} may be indicative of C-O stretching in ether or alcohols. The peaks 904 and 727

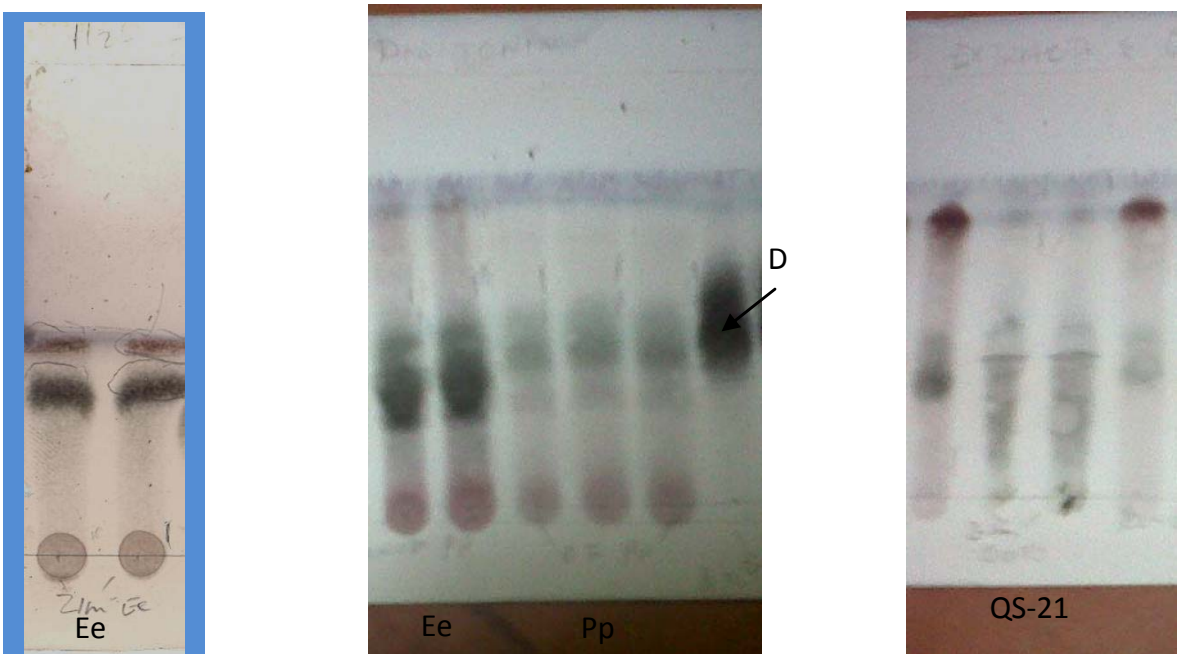


Figure 2. TLC for *E. elephantina* (*Ee*) and *P. prunelloides* (*Pp*) saponin fractions after baking at 110°C against digitonin (D) and quillaja saponin (QS-21) standards.

were assigned as characteristic absorption bands of carbohydrates and their derivatives according to Nakanishi and Solomon (1977) and Deng et al. (2003). The stronger the relative intensity of the absorption bands between 1150 and 700 cm^{-1}, the higher the starch content (Nakanishi et al., 1977; Li et al., 2004; Hua et al., 2003; Woo et al., 1999). The difference in sugar chain of fraction 59-2 and 59-4 could therefore be explained by the fact that the former fraction eluted earlier than the latter due to stearic reasons. The –OH, C-H, C=C, C=O and C-O-C absorption bands found in *P. prunelloides* and *E. elephantina* are suggestive of Oleonane-type triterpenoid saponins as confirmed by the Liebermann-Burchard and Molish reactions as well as liquid-chromatogragphy-electronspray-ionisation-mass spectrometry (LC-ESI-MS). These oleonane-type triterpenoid saponins are characterized by the C=O infrared absorbance due to acid/ester linkages. Such triterpenoid saponins are also likely to be bidesmosides since they have two attachments for glycones (that is, glycosidic and ester groups) to the sapogenin.

The aforementioned referred infrared functional group absorptions characteristic of saponins are also cited in these literature (Kirmizigul and Anil, 2002; Natori et al., 1981; Da Silver Bernadete et al., 2002).

Particle size and zeta potential for *E. elephantina* and *P. prunelloides* nanoparticles

Zeta potential is the measure of the magnitude of the repulsive or attractive electrical forces that exists between atoms, molecules, particles and cells in a fluid. Its measurement relates to the overall charge of particles to the same extent but it also relates to the stability of particles in dispersion. An increase in the 'zeta potential' in solution allows it to dissolve and hold more material. When 'zeta potential' is too low, blood begins to coagulate making it difficult for the heart to pump it through the circulatory system. This is a condition known as intravascular coagulation which may lead to many cardiovascular problems. There are numerous causes of cardiovascular problems for example, processed foods which have a tendency of reversing nature's ratios of K^+ to Na^+, artificial farming methods applying cationic herbicides and pesticides that have a tendency of reversing the zeta potential, increased use of pharmaceutical drugs, over 90% of which are strongly cationic and the use of strongly cationic cans of food and drinks (Riddick, 1968). These different causes of cardiovascular problems result in the ionic content of blood being skewed towards cationic species; hence, lowering the blood 'zeta potential' ultimately creating cardiovascular stress that causes intravascular coagulation. The higher the 'zeta potential' for example (-100 to -60 mV), the greater the stability and the lower the 'zeta potential' for example (-5 to +5 mV), the lower the stability of solution (Riddick, 1968).

A further analysis of the causes of cardiovascular diseases shows that they are associated with developed worlds in which most food materials are processed. The 'zeta potential' (mV) for the nanoparticles/phytosomes produced in this study are summarized in Table 5. Generally, all 'zeta potential' values for the phytosomes formed fell between -5 and +5 mV indicating that they are relatively unstable; and hence, have a high potential for

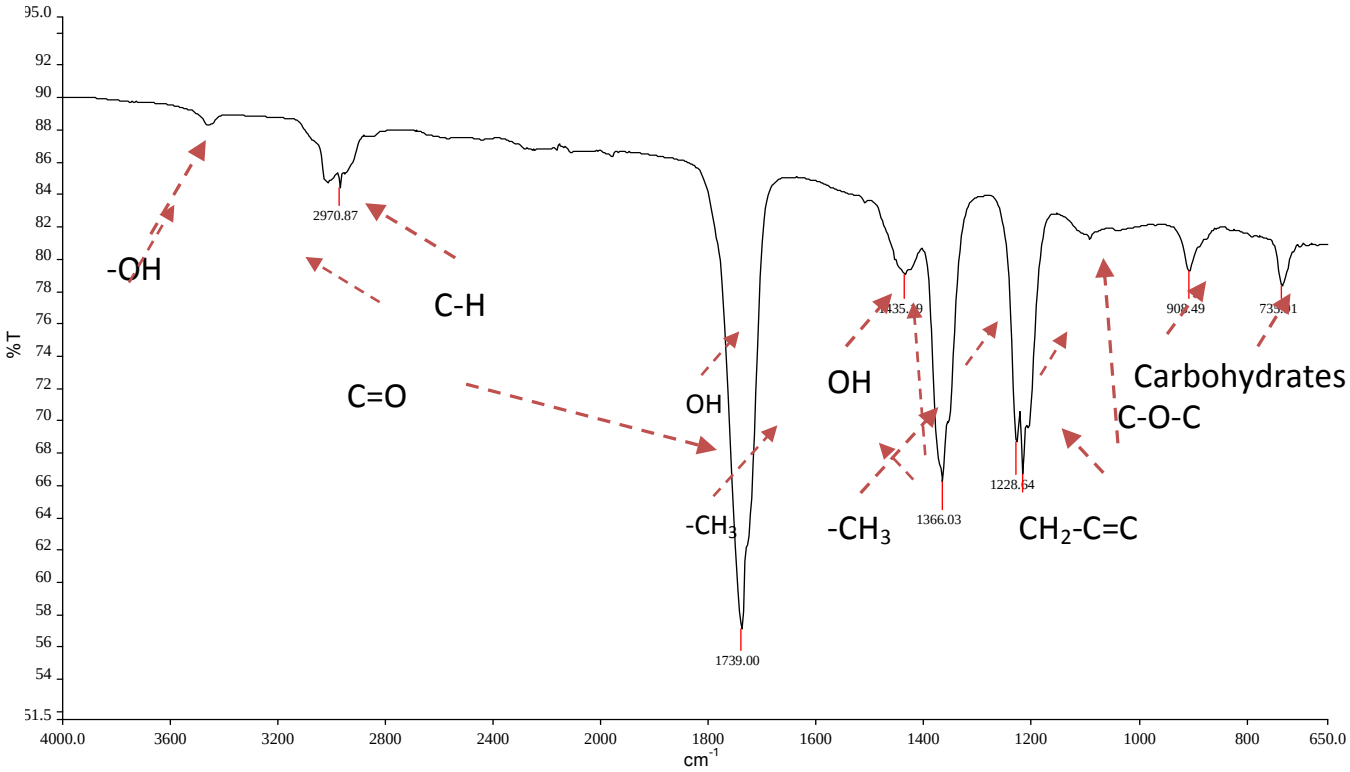


Figure 3. FT-IR spectra of *E. elephantina* isolate 59-2.

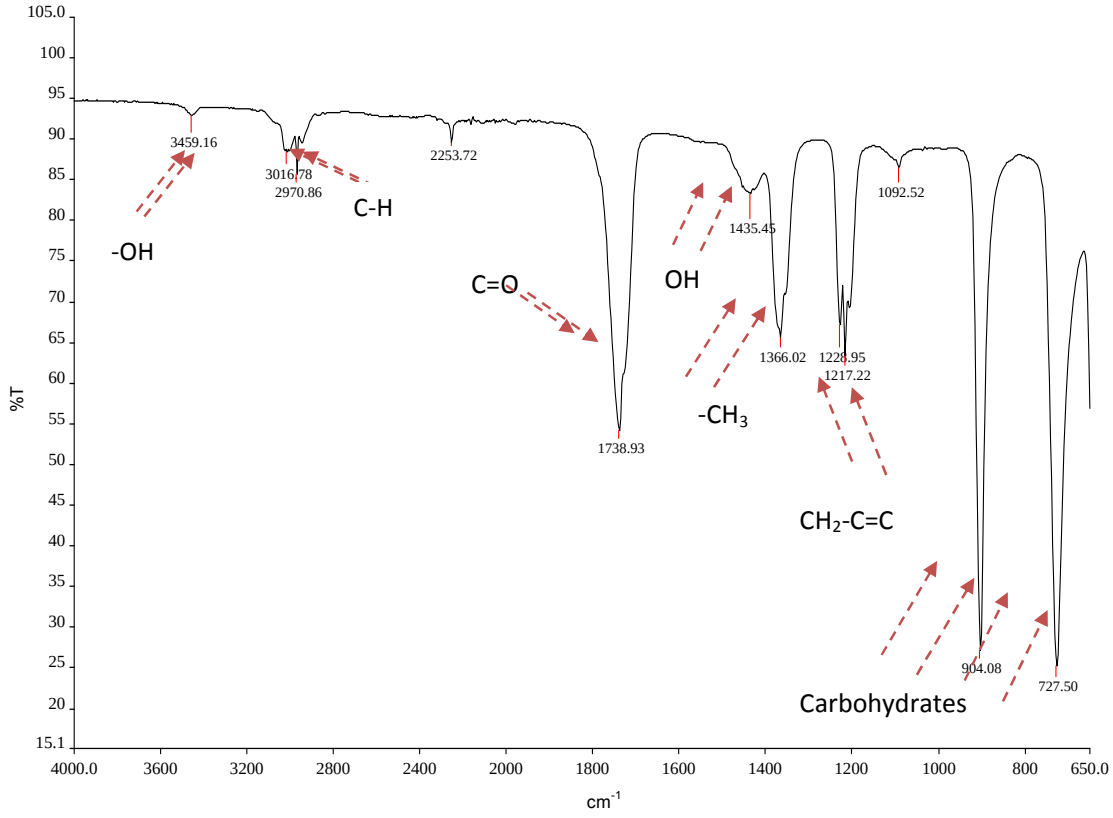


Figure 4. FT-IR spectra of *E. elephantina* isolate 59.4.

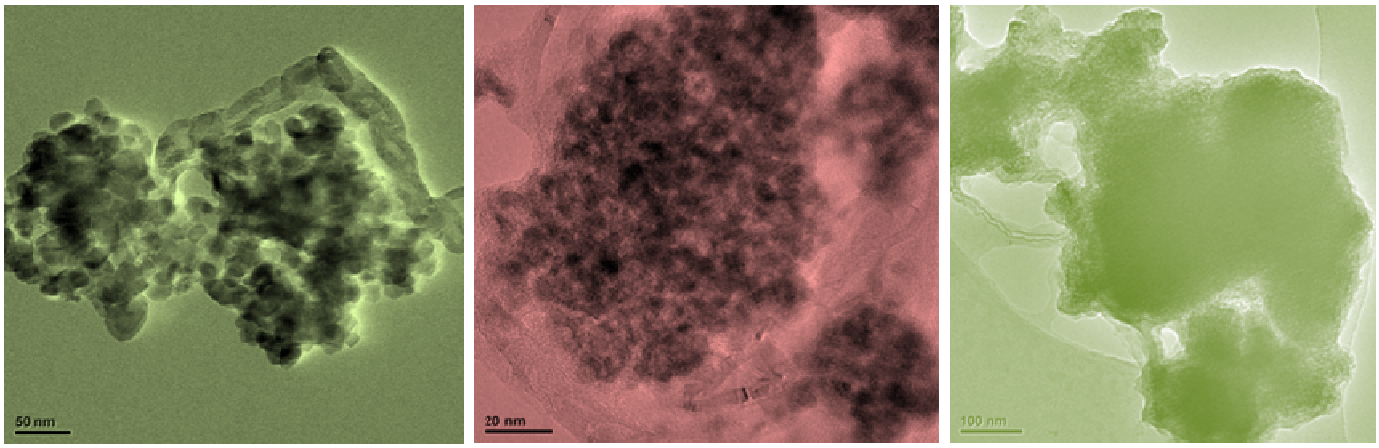


Figure 5. TEM: (a) Ee + cholesterol, (b) Pp + cholesterol, (c) cholesterol alone.

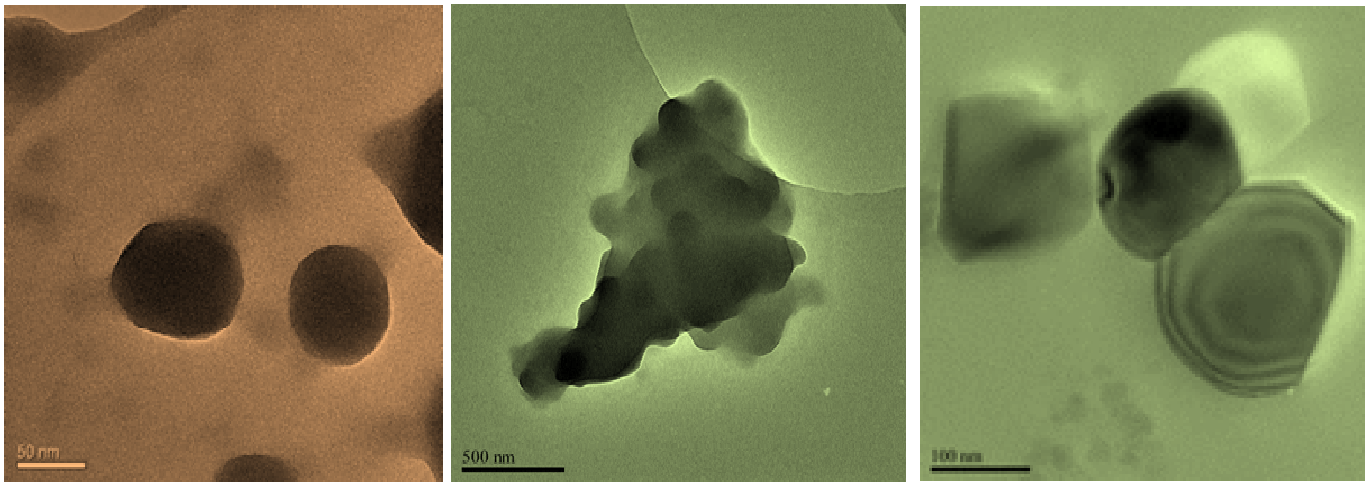
aggregation over time.

Morphology of nanoparticles

The particle size (nm) for samples of the phytosomes produced in this study are shown in Figures 5 to 7. The essence of L-α-phosphotidycholine in the formation of phytosomes was clearly demonstrated by the TEM images in Figure 5a to 5b. In both cases, only cholesterol was added to either *E. Elephantorrhiza* (Ee) or *P. prunelloides* (Pp) resulting in an insignificant formation of distinct phytosomes. A close observation on Figure 5 suggests that the Pp fraction exhibits a greater potential to form phytosomes in the nano scale (20 nm) relative to Ee fractions (50 nm). Spherical monolayer nanoparticles/ phytosomes were clearly observed in the TEM images soon after the addition of L-α-phosphotidycholine to the mixture (Figure 6). The size of the nanoparticles varied between tens to hundreds of nanometers in diameter that is, 50, 500 and 100 nm (Figure 6a, 6b and 6c, respectively). It should be noted that the TEM image (Figure 6b) for which nanoparticle size was 500 nm represented an aggregate of particles implying that the discrete particles had a far smaller size than 500 nm. This difference in the size of particles could be due to the difference in the nature of the saponins or secondary metabolites since they were derived from two different plants that is, 6a from Ee and 6b from Pp. Long sonication periods combined with nanofiltration in a microextruder system enabled to control the vesicle size and to produce relatively uniform vesicles (Figure 6a and 6c in particular). In order to evaluate the vesicle's stabi-lity, the saponin nanoparticles were left at room (tempera-ture).

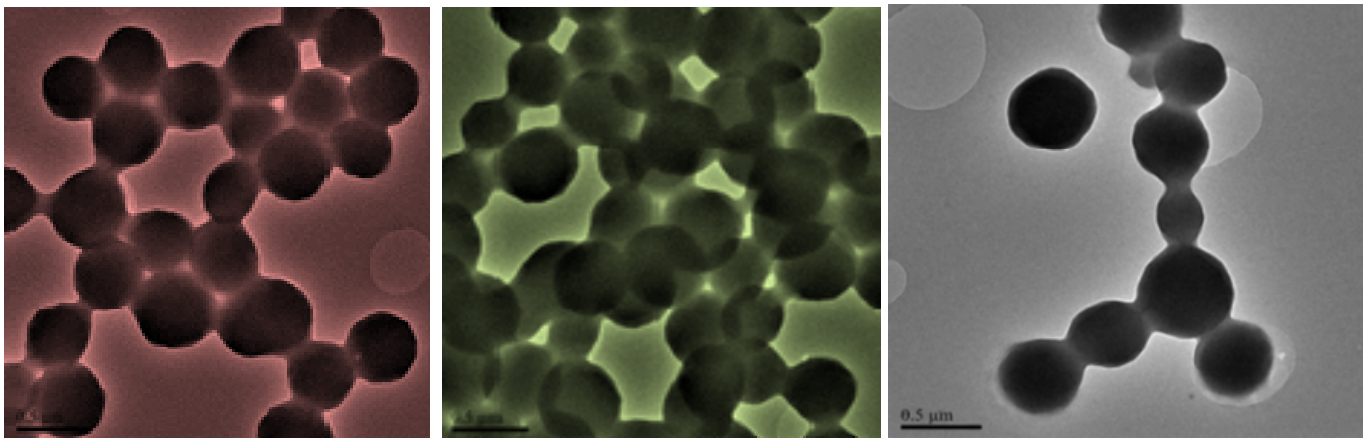
After a period of two weeks, some fusion and aggregation of vescicles appeared, indicating a relatively strong aggregation and precipitation capacity of the nanoparticles as depicted in Figure 7. The particle size rose from the nano scale to the micro scale that is, from about 50 nm to 0.5 µm (Figure 7a to 7c). This clearly demonstrated a high degree of aggregation of the phytosomes formed from these two medicinal plants. Oleanolic acid is one of the most common aglycones which has been reported to possess anti-viral, (anti-HIV), anti-inflammatory, hepatoprotective, anti-ulcer, anti-bacterial, hypoglycaemic, antifertility and anti-carcinogenic activity (Liu, 1995, 2005). *E. elephantina* and *P. prunelloides* are used to remedy some of the aforementioned referred anomalies and the presence of the oleanolic acid aglycone may account for the pharmacological benefits of these two medicainal plants. The agglomeration of the nanoparticles of *E. elephantina* and *P. prunelloides* saponins as they were mixed with α-phosphotidycholine may justify the use of the two plants for the reduction of body weight as it is anticipated that they accomplish this by lowering gastrointestinal cholesterol absorption. Recent studies report that vytorin and zetia, two major synthetic drugs administered in the treatment of high cholesterol levels failed to decrease the incidence of heart disease (Mitka, 2008). On the other hand, the use of anti-obesity drugs is severely restricted due to the accompanying side effects (Wasan and Looije, 2005). Hence, there is need to explore more efficient, safe and economic alternatives to combat dyslipidemia and associated metabolic disorders.

Saponin extracts from *E. elephantina* and *P. prunelloides* may also be exploited as hypocholesterodemic as well as antioxidative alternatives to the synthetic drugs that are implicated for a number of side effects. The binding of saponins to bile acids may have important implications in the mitigation of carcinogenesis. Bile acids excreted in the liver are termed primary bile acids. They are metabolized in the colon thereby producing secondary bile acids (Pollak et al., 1985). Some of the so formed secondary bile acids promote colon cancer. By binding to primary bile acids resulting in the formation of phytosomes, it may be proposed that saponins from *E.*



(a) (b) (c)

Figure 6. TEM saponin + cholesterol + L-α-phosphotidycholine (freshly prepared).



(a) (b) (c)

Figure 7. TEM saponin + cholesterol + L-α-phosphotidycholine (after 3 weeks).

elephantina and *P. prunelloides* may reduce the formation of the secondary bile acids; hence, reducing chances of colon cancer. A variety of natural products (Pollak, 1985; Price et al., 1987; Malinow, 1984; Miettinen et al., 1976; Cayen 1971) have been shown to inhibit cholesterol absorption from intestinal lumen in experimental animals, andconsequently reduce the concentration of cholesterol in the plasma. Although, there is controversy on the mechanism of cholesterol reduction by saponins, there is a consensus on its ultimate reduction (Temel et al., 2009).

Mortality from cardiovascular disease is ranked the second highest cause of death worldwide (Malach and Imperato, 2006). This anomaly is generally attributed to the elevated levels of plasma cholesterol (hypercholesterolemia) which results in coronary heart disease (Altman, 2003; Jalali-khanabadi et al., 2006). Elevated lipid levels can be decreased by controlled diet to avert hyperlipidemia. The use of anti-hyperlipidemic drugs is more practical but these are not always satisfactory due to the side effects of chemically synthesized drugs (Chiang et al., 2007). Phytopharmaceuticals are gaining importance in allopathic as well as traditional medicine due to their non-additive and non-toxic nature (Jenkins et al., 1983; Raskin et al., 2002). The presence of a significant amount of saponins in both *E. elephantina* and *P. prunelloides* lends support to the wide application of these two medicinal plants for the various ailments for which they are administered in southern Africa. Diosgenin that was also identified in both *P. prunelloides* and *E. elephantina* has been implicated for having favourable effects on glucose lowering (McAnuff et al., 2005) and antioxidant activity (Son et al., 2007; Jayachandra et al., 2009).

Conclusion

This study demonstrates for the first time that *P. prunelloides* and *E. elephantina* saponin extracts and fractions have the potential to bind cholesterol *in vitro* resulting in the formation of phytosomes with particle sizes ranging between tens and hundreds nanometers. Spherical nanoparticles/phytosomes/liposomes were clearly identified in the TEM images with particle sizes ranging between 10 and 500 nm. The zeta potential for the particles characterized by the 'zeta sizer' fell between -5 and +5 mV. This clearly demonstrates that the particles so formed have a high degree of aggregation, a characteristic feature that may be proposed for the reduction of the gastrointestinal absorption of saponins. Since *P. prunelloides* and *E. elephantina* exhibited a high triterpenoid saponin content, these two medicinal plants could be used as alternative sources of saponins in the manufacture of adjuvants or phytosomes in place of synthetic as well as Quillaja saponins that have their shortcomings.

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