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OPTIMIZATION, FORMULATION, CHARACTERIZATION AND ANTIBACTERIAL ACTIVITY OF PHYTOSOMES CONTAINING AQUEOUS EXTRACT OF *SOLANUM XANTHOCARPUM* FRUIT

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ABSTRACT

Purpose: To prepare a phytosomal suspension of Solanum xanthocarpum fruit aqueous extract. The method applied in extracting powdered leaf was the maceration method with the use of distilled water with a rate of 5.78. The analysis results revealed the existence of alkaloids and flavonoids where total phenolic content was 21.7 + 0.804 w/w in the extract, presence of phytosomes with lecithin as lipid component was prepared using solvent evaporation method. Phytosome surrogate size was discovered to be between 569nm and 2018nm with polydispersity index of 0.169-0.531. SEM image revealed that the phytosomes were strong, almost perfect round, vesicles. The phytosome vesicles had a flat and continuous surface morphology. The concentration of fat at level 3 (0.3 g [v]) and extract at level 2 (0.2 g [v]) were suggested as what led to the smallest particle size and was determined by predictive analysis. The DSC analysis revealed that distinct differences and endothermic maxims were achieved, which were used to indicate the production of stable phytosomes as a result of a molecular interaction between extract and lecithin. Also, the phytosomes showed high activity against Staphylococcus, but they were not effective against E. coli.

KEYWORDS: Phytosystem, Antibacterial, Solanum xanthocarpum, Lecithin, Solvent evaporation.

INTRODUCTION

The majority of the detrimental pathophysiological diseases have a strong correlation with free radicals such as tension pains, neoplasm diseases, diabetes and other people care disease system. It implies that a free radical is an atom, or a molecule that has a free electron and is therefore unstable. Antioxidants are referred to as free radical scavengers, the substances that guards and lessens destruction of the free radicals by supplying electrons to a cell with a free radical. The antioxidant transforms the free radicals to waste products that are then removed out of the body. India has been among the highest levels of natural medicines. Their use in modern medicine has been sparse though as a result of their complicated structures, large dosage, lack of absorption and multiple dosage. Lipid-protected bioactive phytoconstituents of herbal extracts Phytosomensing Phytosoms are known to be produced by mixing soluble bioactive plant components or standardised plant extracts with phospholipids to form lipid-compatible molecular complexes that maximise absorption, and bioavailability. Phytosomes as another approach to these limitations Over the past few years, there has been an increasing trend in the attention of pharmaceutical experts to the exploitation of plant extracts or phytoconstituents in combination with phospholipids to generate a new generation of herbal medicines to enhance bioavailability of herbal drugs and patient compliance. More than fifteen articles on phytosomal formulations of diosgenin, curcumin, quercetin, chrysin, Brassica nigra extract and Diospyros kaki extract were published in 2019 in peer-reviewed journals. There were published 4–6 Research on phytosomes in a year (2020) most of them refer to improved formulations containing phytosomes. Notable studies were Centella asiatica phytosomes with improved memory performance, Vasaka phytosome-based nanoformulations for better bioavailability and Icariin phytosomes having potent anticancer property. Seven or nine Research was displayed in the course of phytoseme surface modification (2021). The dominant element of major studies on phytosome was functionalisation of quercetin and snake venom loaded phytosomes.

Other studies that have been published in journals, but are not available with high index databases, also mention the importance of phytosomes in the promotion of standardised extracts (or phytoconsituents) which are optimally developed. 1012.

Solanum xanthocarpum in its flavonoid and phenolic compounds that are effective free radical scavengers, contains anti-oxidants that protect the cells in the tissues, as well. The treatment of throat infection makes use of the fruit plant. The current research was aimed at

producing Solanum xanthocarpum fruit phytosume such that the bioavailability of phytosume can be maximized after oral dose of aqueous extract has been taken.

MATERIAL AND METHODS

Preparation and Collection of Plant Material

The Solanum xanthocarpum dried fruits presented on the site, indianjadibooti. com. On site, low-speed blender was used on the bought fruit. The powdered fruit was stored in a small air tight jar until used.

Physicochemical Evaluation

Establishment of foreign matter

Plant material was carefully measured and a thin layer of it was mixed and examined using a magnifying glass. Rest of the materials that were not necessary were cleaned up manually. The leaves were again weighed to determine the percentage of foreign matter against the air-dried medicine. Fourteen

Ash values Determination of ash values[15]

Total Ash

The finely ground plant material (4 g) was weighed and put into the silica crucible that had been pre-weighed by setting it on the scale to 4 g. Then they were heated gradually to 250 o C till they become white - no carbon. The content was cooled using a desiccator and weighed. Ratio of percentage of fat in ash material to air-dried material was determined.

Acid-Insoluble Ash

All the ash in the crucible was put into the crucible along with 25ml of concentrated hydrochloric acid. A watch glass was now placed on the top of the beaker and it was boiled five minutes in a soft way. Single feeding (ten millilitres of the hot water) was added on the feedings, and the watch glass was rinsed into the crucible. The insoluble product was obtained on ashless filter paper [Wathman (no.42)], and shaken with hot water until the filtrate became neutral, then burned at constant weight at a temperature between 600298C. The concentration of ash that had been air-dried/material was calculated.

Water-Soluble Ash

The crucible containing all the ash was put in twenty and five millilitres of distilled water and heated within five minutes. The sintered glass crucibles or ashless filter paper were weighed,

washed with hot water and then incinerated 15min without exceeding the temperature of 450 o C; The weight of the resultant ash was taken away and added to the weight of all the ash. Calculation of the ash was performed using the ratio of the weight of ash at 550 o C/weight of air-dried waste.

Calculation of loss on drying[15]

The weighing vial being tare and dry weighed before the dry powdered plant material was added in accurate amounts. One sample was placed in the oven to be dry at 100105 o C. This would be done until two weigh-ins are within a difference of 5mg. Drying weight of the air-dried material (mg)/weight of the material.

Alcohol Soluble Value and Water Soluble Value

Dried plant material in 5g (powdered) was then weighed to a glass stopper Erlenmeyer flask and soaked in either 100 mL ethanol or water and then the sample extracted by maceration over more than 24 hours, stirring at every third hour during the initial six hours then settling the rest of the 18-hour period. The solution was filtered quickly after which 25 mL of the filtrate was put in a preweighed and water bath dried petri dish. The weight of residue was taken after drying at 105 o C in six hours and refrigeration (30 min) in desiccator. The concentrations of alcohol or water soluble compounds were determined in terms of milligrams per gram of air dried matter.

Extraction of Plant Material[16]

The plant material was weighed in 50 g into a glass jar of 2 L. A charge of 1000 mL of distilled water was added to the jar and macerated in 24 hours. Subsequently the first 6 hours were spent in shaking jar up and down, while the remaining 18 hours standing. The macerating was filtered and the filtrate dried in a water bath. Rotary evaporation was used to remove any solvent of the sticky syrupy residue. S further analyses were made on the dried extract in a desiccator.

Primary qualitative phytochemical screening[17]

Proper dilution of the extract in required Solvent and other alkaloids, glycosides, phenolics, tannins, sterols etc. tests were done as per laid down procedures.

Total Phenolic content[18]

The nutraceutical powder (1g) was macerated with 15ml of methanol (50% v/v in water) and

filtered and the filtrate diluted to an end volume of 100 ml using methanol (50% v/v in water). 1 ml of distilled water was added with the help of a test tube to 1 ml of the substance. Folin-Ciocalteu reagent (1.5ml) was then added and the mixture allowed to stand in the room temperature (5min). Four millilitres of 20% (w/v) Na2CO3 was introduced and the mixture stirred and allowed to stand at room temperature of 30 minutes then the mixture was diluted to 25 millilitres of distilled water. The absorbance of the sample was then recorded at765 nm at a blank of distilled water.

Similar experimental conditions were followed to prepare and dilute the stock solutions to become the analytical curve of gallic acid (10100 ppm). The control was made ready and incubated similar to the rest, using 200 µL of methanol and relevant reagents.

Preparation and optimization of echinacea-phytosomes by solvent evaporation process[19]:
The phytosome particle size was also researched on in relation to the extract concentration and lipid content using a classical factorial design; results are presented in Table 1.

Then, heat was applied to a solution of the respective quantity of Solanum xanthocarpum and soy lecithin (Table 2) in a 100 mL round-bottom flask additional 30 mL ethanol for four hours between at a temperature range of (40-50 o C.) to obtain viscous precipitate. It was then put into lyophilisation to produce the phytosomes. It is an amber glass vial containing the dehydrated phytosome complex and blizzarding it.

Table 1: Design table.

Levels	Factor 1	Factor 2
	Lipid concentration	Extract concentration
-1	0.1 g	0.1 g
0	0.2 g	-
+1	0.3g	0.2g

Table 2: Batch processing for phytosome preparation.

Formulation Code	Lecithin	Extract	Ethanol (mL)
XP 1	0.2	0.2	30
XP 2	0.1	0.2	30
XP 3	0.1	0.1	30
XP 4	0.3	0.2	30
XP 5	0.3	0.1	30
XP 6	0.2	0.1	30

Assessment of phytosomes[20]

Visualisation

Scanning electron microscopy was used to visualise phytosomes. Both the surface morphology of the complex and the spatial distribution of particles, in terms of size, have been characterized by scanning electron microscopy. In the case of auxiliary electron emissive scanning electron microscope (Hitachi-S 3400N), samples were sputtered using 14 mA of gold/palladium at an argon atmosphere during 120 seconds and examined at a voltage of 15.0 kV in morphological assessment.

Differentiated scanning calorimetry

Thermograms of lecithin and phytosome were acquired in order to verify the compatibility. All the samples were heated at a rate of 5 o C/min between 25o C and 300 o C and the resulting thermograms were analyzed qualitatively on the basis of variances in peak onset time, relative area and shape and quantitative variances in enthalpy and the presence or absence of peaks.

RESULTS AND DISCUSSION

Figure 1 Solanum xanthocarpum dried specimen was preserved in an air tight pouch and the colour of the dry fruit varied between pale green to yellow(Figure 1). The yellow fruit was coarsely ground to obtain the powder.

Figure 1: (A) Received fruit pack (B) Crude dried fruit (C) Powdered fruit

Physicochemical Investigation

The leaf, l analyses were conducted mainly The procedures described in section 2 were followed and the results are summarised in table 3.

Table 3: Results of preliminary investigation of the leaf.

Plant Part	Parameter	Value Obtained (%)
Solanum xanthocarpum Fruit Powder	Foreign Matter	1.1
	Total Ash	8.3
	Water Soluble Ash	4.1
	Acid Insoluble Ash	3.4
	Ethanolic Soluble extractives	9.0
	Water Soluble Extractives	6.1
	Loss on Drying	1.96

Extraction and Phytochemical Screening

The dark brown extract had a yield of 5.78%. The phytochemical investigation indicated that the extract had flavonoids and alkaloids. The positive results using extract were also on alkaloids, phenolics, flavonoids.

Total Phenolic Content

The linear equation of gallic acid is y = 0.0046x + 0.0024 and the standard curve of gallic acid was plotted and displayed in distilled water to calculate the statistics of absorption. Percentage (w/w) of the phenolics in extracts is displayed. Solanum xanthocarpum aqueous extract total phenolic content was determined to be of 21.7 ± 0.804% w/w.

Preparation of phytosomes

Solvents evaporation method was used to prepare phytosomes of the Solanum xanthocarpum fruit extract.[34] It involves the combination of the lipid (lecithin) and phytoconstituents or extract in an organic solvent in a flask. The drug is entrapped at optimal temperature (usually within 40 o C) and time in the reaction mixture to form phytosomes. The evaporator was then rotated and the organic solvent was removed.

The optimization of the phytosomes

To determine the ideal fat: extract ratio in preparation of phytosomes, we took the particle size as a reponse variable. It was meant to get the minimum size. The sizes of the particles in the six formulations developed were measured and analysed through ANOVA that yielded an equation on which the optimal particle size could be predicted.

A zeta sizer was used in measuring the size distribution and particle size of the phytosomes per batch. The polydispersity index of the formulations was between 0.169 and 0.531 and they varied in size based on the formulation employed (569-2018 nm) (Table 4).

Table 4: Particle size and size distribution of various batches of phytosomes.

Formulation Code	Particle Size (nm)	Polydisperisty Index (PDI)
XP 1	817	0.317
XP 2	1311	0.296
XP 3	1456	0.531
XP 4	569	0.169
XP 5	1053	0.386
XP 6	2018	0.429

The lipid and extract concentration had a huge impact on the phytosome size. Higher lipids phytosome were found to be of smaller size than lesser lipids Phytosomes as seen through particle size.

The trial version Design Expert 7.0.0 was utilized in the case to test the existing correlation existing between fat and extract concentration and the particle size. The ANOVA resulted in the following equation that gives the correct factorial model to predict particle size:

Particle size = 1204 + (179.5 * A1) + (213.5 * A2) - 305 0*B

Its model standard deviation is 381.24 with an estimated R-squared of 0.9920.

Table 5 provides a summary of the expected particle size estimating on the formula above.

Table 5: Diagnostics Case Statistics.

Standard Order	Actual Value	Predicted Value	Residual	Leverage	Studentized Residual	Fitted Value / DFFITS	Run Order
1	1456	1688.5	-232.5	0.667	-1.056	-1.589	3
2	2018	1722.5	295.5	0.667	1.343	* 4.27	6
3	1053	1116	-63	0.667	-0.286	-0.292	5
4	1311	1078.5	232.5	0.667	1.056	1.589	2
5	817	1112.5	-295.5	0.667	-1.343	* -4.27	1
6	569	506	63	0.667	0.286	0.292	4

*Exceeds Limit

Fig. 2: Presents a graph of predicted and actual particle size.

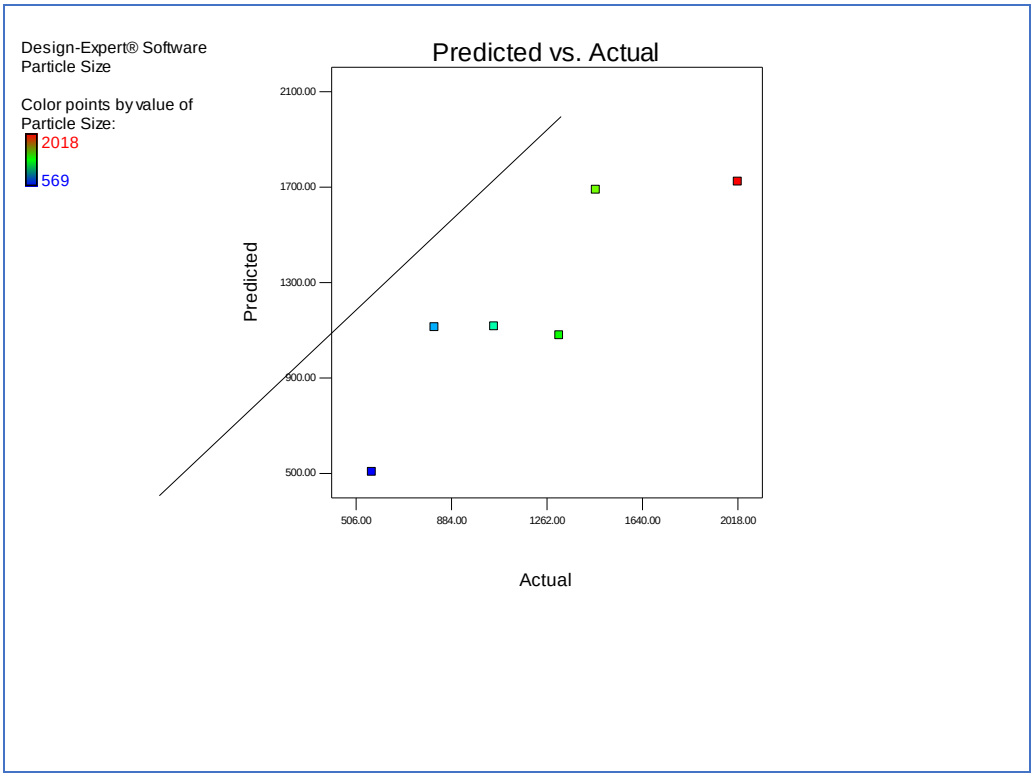

Figure 2: Predicted vs. Actual.

These were answers of numerical solving and lipid quantitative values the model can provide. This resulted in the generation of six combinations (which were viable), and the most suitable was considered graphically (Figure 3).

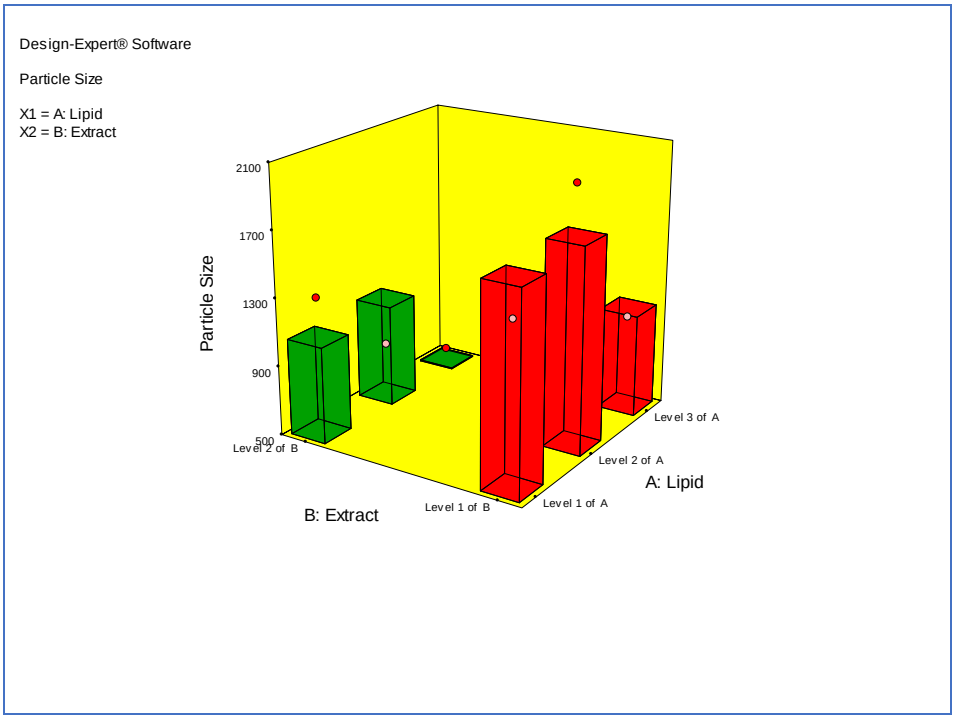

Figure 3: The size of particles that are going to be optimized.

Extract concentration (level 2: 0.2 g) and fat content (level 3: 0.3 g), were able to provide the smallest particle size with a point forecast.

Evaluation of phytosomes

Surface morphology (visualization)

An electron microscope was used to determine the lowest particle size phytosomes. The picture of SEM depicted rigid, near spherical particles which were consistent with vesicles. From the According to the scanning electron microscopy (SEM) images, it is evident that the phytosome vesicles were smooth and uniform on their surface as in Figure 4.

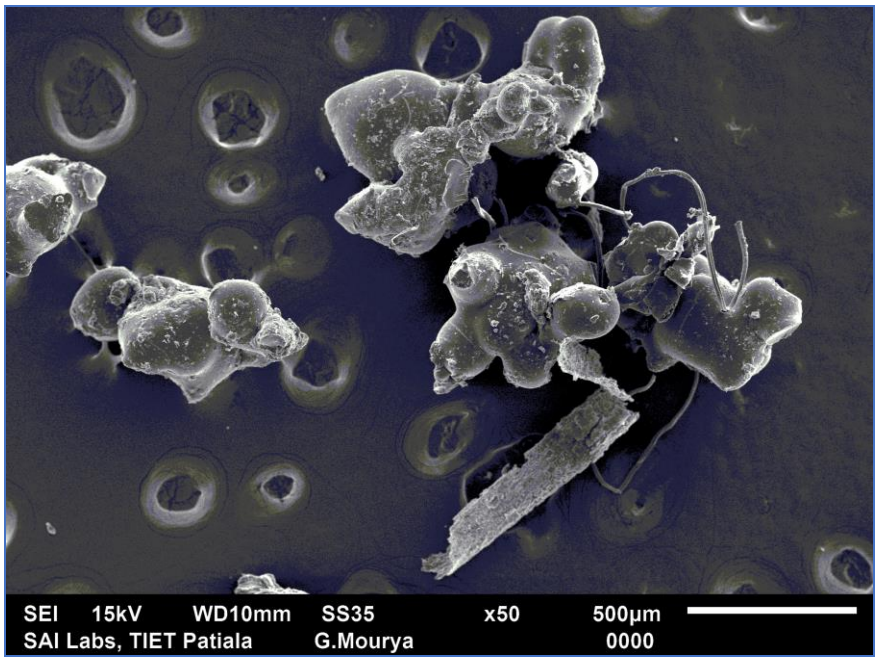

Figure 4: SEM Image of XP 4.

Differential Scanning Calorimetry

Those who have a high peak of 57o C when melted are shown in the thermogram of the soy lecithin, whereas the thermogram of the phytosystem showed a distinct endothermal peak of 257 C, indicating that there was sufficient interaction between extract and phospholipids in the formulated structure as determined by DSC, that is, hydrogen bonds or van der Waals forces between molecules and.

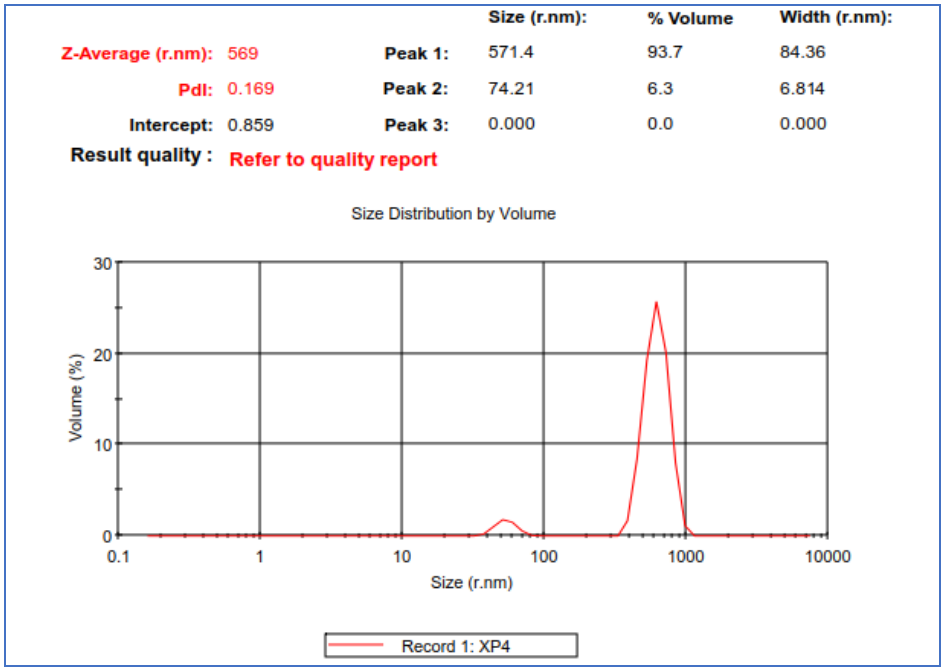

Figure 5: Particle size and size distribution of XP 4.

Antibacterial Study

The zone-of-inhibition was used to determine the antibacterial activity of the phytosomes against Staphylococcus aureus and Escherichia coli using zone-of-inhibition. The phytosomes exhibited no notable impact on E. coli but great antibacterial impact on Staphylococcus (Fig 6a and 6b).

Figure 6 (a) E.coli and phytosomes bacterial culture plate; (b) S. aureus and phytosomes bacterial culture plate.

CONCLUSION

The above thesis demonstrates the enormous potential of a plant extract to enhancein vitro antibacterial activity by a phytosome technique of drugdelivery. Phytosomes are applicable in both plant extracts and phytoconstituents to alleviate therapy, reduce dosage and increase the concentrations of its treatment programs.

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