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Extraction, Phytochemical Analysis and Development of Phospholipid Complex of Alhagi Camelorum

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This paper explained that Diabetes Mellitus is a serious metabolic disorder caused by insulin deficiency or resistance, preventing the proper utilization of glucose in the body and leading to various health complications. The currently available antidiabetic drugs often have side effects, making the search for natural alternatives essential. This paper focuses on the phytochemical properties of Alhagi camelorum and the development of its phospholipid complex to enhance bioavailability and therapeutic potential. Phytochemicals such as flavonoids, terpenoids, tannins, and alkaloids play a crucial role in diabetes management through different mechanisms. However, their poor solubility and absorption limit their effectiveness. To address this, various methods for preparing phospholipids complexes, such as thin-layer rotary evaporation, solvent evaporation, and reflux techniques, have been explored. Additionally, techniques like DSC, zeta potential analysis, and spectroscopic evaluation have been used to assess the quality and efficacy of the complex. This research highlights that phyto-phospholipids complexes offer a promising approach to improving the therapeutic potential of herbal compounds. Various preparation methods, including thin-layer rotary evaporation, solvent evaporation, and reflux, have been discussed. Characterization techniques such as DSC, zeta potential analysis, and spectroscopic evaluation help assess the complex's stability and effectiveness. This research demonstrates that phospholipid complexes can significantly improve the absorption and therapeutic potential of herbal compounds, offering a promising strategy for diabetes treatment with enhanced efficacy and reduced side effects.

Keywords: Alhagi camelorum.

1. Introduction

Diabetes Mellitus

Diabetes mellitus, belongs to the class of metabolic diseases which the main symptom associated with this disease is the high sugar levels in blood for a long period. It can be categorized to the world's major diseases considering that affects high population in earth. Diabetes mellitus is a disorder that affects the body's ability to make or use insulin. Insulin is a hormone produced in the pancreas that helps transport glucose (blood sugar) from the bloodstream into the cells so they can break it down and use it for fuel. People cannot live without insulin. Diabetes results in abnormal levels of glucose in the bloodstream.

This can cause severe short-term and long term consequences ranging from brain damage to amputations and heart disease 1,2.

Causes of Diabetes

- Living a sedentary lifestyle
- Obesity
- Increasing age
- Family history of gestational diabetes
- Overweight or obese
- Suffer from polycystic ovary syndrome
- Cushing's syndrome. This syndrome increases production of the cortisol hormone, which serves to increased blood glucose levels. An over-abundance of cortisol can cause diabetes.
- Glucagonoma. Patients with glucagonoma may experience diabetes because of a lack of equilibrium between levels of insulin production and glucagon production.
- Steroid induced diabetes (steroid diabetes) is a rare form of diabetes that occurs due to prolonged use of glucocorticoid therapy 3,4.

Symptoms

The main symptoms of Diabetes mellitus marked hyperglycemia combined with polyuria, polydipsia, polyphagia also known as the 3 P's signs. The presence of the 3 P's could indicate that the blood sugar level is high. In type I, 3 P's can be observed in higher rate while they can be developed quickly. In type the 3 signs are nearly undetectable and develop more gradually. Not so often, weight loss, blurred vision as well as susceptibility to infections could also be aroused by chronic hyperglycemia.

Diabetic patients may also have high blood pressure and anomaly of lipoprotein metabolism. Among other long-term symptoms, retinopathy with possible vision loss, nephropathy inducing kidney failure, peripheral neuropathy related with the presence of foot lesions, amputations 5.

Etiologic Classification

Type 1 Diabetes Mellitus

Type 1 diabetes mellitus (T1DM) comprises several diseases of the pancreatic ß cells which lead to an absolute insulin deficiency. This is usually considered to be the result of an autoimmune destruction of the pancreatic ß cells (type 1A). Some patients with T1DM with no evidence of ß cell autoimmunity have underlying defects in insulin secretion often from inherited defects in pancreatic ß cell glucose sensing and from other genetic or acquired diseases.

Type 2 Diabetes Mellitus

Type 2 diabetes mellitus (T2DM) is by far the more common type of diabetes and is characterized by insulin resistance resulting from defects in the action of insulin on its target tissues (muscle, liver, and fat), but complicated by varying and usually progressive failure of beta cells' insulin secretary capacity. Most patients with T2DM in the US and Europe are overweight or obese, however in India and China, most T2DM patients have a lean body mass index (BMI), albeit with increased visceral and hepatic fat.

Classification of Diabetes based on insulin insufficiency

Type I Diabetes

It is as a result of β-cell destruction which customarily provokes complete insulin insufficiency. It was formerly known as insulin-dependent, juvenile or childhood-onset diabetes and it is occasioned by an autoimmune reaction, in which the immune system invaded against the insulin-producing pancreatic beta cells. Type I diabetes is distinguished by deficient insulin production in the body. In such type of Diabetes mellitus the patients require daily administration of insulin so as to normalize the glucose level in the blood. Have not taken the insulin, their life is being threatened and can be fatal 6.

Type II Diabetes

Type 2 diabetes is characterized by insulin resistance and, at least initially, a relative deficiency of insulin secretion. In absolute terms, the plasma insulin concentration (both fasting and meal-stimulated) usually is increased, although "relative" to the severity of insulin resistance, the plasma insulin concentration is insufficient to maintain normal glucose homeostasis. A large number of genes have been associated with type 2 DM, but they explain a low percentage of the disease heritability 6,7.

Gestational Diabetes Mellitus

Gestational diabetes mellitus (GDM) is a condition in which a hormone made by the placenta prevents the body from using insulin effectively. Glucose builds up in the blood instead of being absorbed by the cells. The placenta supplies a growing fetus with nutrients and water, and also produces a variety of hormones to maintain the pregnancy. Some of these hormones (estrogen, cortisol, and human placental lactogen) can have a blocking effect on insulin. Normally, the pancreas is able to make additional insulin to overcome insulin resistance, but when the production of insulin is not enough to overcome the effect of the placental hormones, gestational diabetes results. Gestational diabetic symptoms disappear following delivery 8.

Different categories of antidiabetic medications are there in the market for the remedial action, which includes insulin analogues, sulphonylureas, biguanides, dipeptidyl peptidase-4 inhibitors, thiazolidiones, α-glucosidase inhibitors, etc, where the mechanism of counteracting this increased glucose level is different for different categories.

However, long term treatment and side effect of the available hypoglycemic medications leading towards huge demand for efficacious, decreased side effects and affordable agents for the treatment of diabetic condition 9.

Medicinal Plants used for Treatment of Diabetes

Traditional medicines are usually the first choice for primary healthcare of patients in developing countries because of better cultural acceptability, better compatibility with the human body and lesser side effects than modern therapies. The use of plants for healing purposes predates human history and forms the origin of much modern medicine. Clinical, pharmacological, and chemical studies of these traditional medicines, which were derived predominantly from plants, were the basis of most early medicines. Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies. More than 400 plant species having hypoglycemic activity have been available in the literature, however, investigating new antidiabetic drugs from natural plants has still been attractive because they contain phytoconstituents that demonstrate alternative and safe effects on the treatment of diabetes mellitus 10,11.

Phytochemicals Involved in Anti -Diabetic Activity

Flavonoids like anthocyanidins, flavan-3-ols, flavonol, flavones, flavanones and isoflavones show the anti-diabetic properties due to their ability to modulate some cell signaling 12.

Terpenoid, andrographolide-lipoeic acid conjugate has demonstrated hypoglycemic potentials. Benzofuran-2-carboxaldehyde is a hypoglycaemic diterpene isolated from Globba pendula 13.

Tannin is a polyphenolic biomolecule present in natural products such as berries, nuts, legumes, chocolate, spices and herbs. Three major classes of tannins are distinguished viz: Hydrolyzable tannins e.g. gallic acid, Non-hydrolyzable or condensed tannins e.g. flavones and Phlorotannins e.g. phloroglucinol. Tannins have been reported as the bioactive antidiabetic principles of some medicinal plants 14.

Alkaloids are classified as true alkaloids e.g. nicotine, atropine, morphine; protoalkaloids e.g. mescaline, adrenaline, ephedrine; polyamine alkaloids e.g. putrescine, spermidine and spermine; pseudoalkaloids e.g. caffeine, theobromine, theophylline etc, peptide and cyclopeptide alkaloids. Alkaloids have been implicated as the active principles in some anti-diabetic medicinal plants 15.

Saponins are major plant metabolites that naturally occur as surface-active glycoside. They are composed of sugar moieties linked to a hydrophobic aglycone known as sapogenin which could also be a triterpenoid or a steroids. Several anti-diabetic medicinal plants owe their activities to saponins. The anti-diabetic properties of Anabasis articulate are as a result of saponin present in it. The saponin induces insulin production 16.

Other compounds with anti-diabetic activities such as polyunsaturated fatty acids and glycols have also been reported. In another study of ours entitled “Bioassay-guided isolation and structural elucidation of the anti-diabetic principle of methanol leaf extract of Newbouldia leavis (P. Beauv)”, we isolated 9-(4-nonyl-phenyl)-non-8-enoic acid, a long chain unsaturated fatty acid from Newbouldia laevis as the anti-diabetic principle of the plant. The polyunsaturated fatty acid exhibited hypoglycemic activities comparable to that achieved by the reference drug, glibenclamide, in the alloxan-induced diabetic rat. Pinitol, a sugar alcohol isolated from Sutherlandia frutescens leaves has shown great anti-diabetic activities. The anti-diabetic activities of pinitol were likened to that of insulin 17.

Mechanism Action of Phytochemicals

Some of the mechanisms involved include increase in insulin secretion, decreases in hepatic glucose output, regulation of certain enzymes involved in carbohydrate metabolism such as αglucosidase inhibitors, modulation of certain regulation molecules such as PPARγ, hypolipidaemic activities, antioxidant effects, interference with the activities of some glycolytic enzymes such as phosphoenolpyruvate carboxykinase activities, amelioration of glycosylated haemoglobin, enhancement of the expression of glucose transporters and others 18.

Novel Drug Delivery System

New vesicular drug delivery systems aim to deliver the drug at a rate directed by need of body during the period of treatment and carry the active entry to the site of action. Many novel vesicular drug delivery systems have been arrived encompassing various routes of administration, to achieve targeted and controlled drug delivery. Targeted drug delivery is a mode of delivering the therapeutic agent to the tissues that improve the therapeutic efficacy and reduces the side effects. Drug targeting means the delivery of drugs to receptors, organs or any other specific part of the body 19,20 .

Classification

Sustained- or controlled- drug delivery systems provides drug action at a pre-determined rate by providing a prolonged or constant (Zero-order) release respectively, at the therapeutically effective levels in the circulation.

Localized drug delivery devices provide drug action through rate limiting drug release in the vicinity of the target.

Pre-determine rate of drug delivery provide drug action by change the release of drug molecules by system design which control the molecular diffusion of drug molecules in systemic circulation.

Targeted drug delivery provides drug action by using carries either for passive or active diffusion or one base or self programmed approach, usually used with suitable sensory devices, which recognize their receptor at the targeted site.

Phospholipid Complex: A Novel Approach of Phytochemical Delivery

From ancient times, phyto-chemical and phyto-pharmacological studies have been well establish the various creations in natural behavior and their numerous health promising advantages of botanical herbs.

Some of the biologically active phyto-constituents are consist in the nature of polar solvents (water). However, water-soluble phyto-constituents are weakly absorbed when it takes orally or after applied topically.

Owing to their big molecular mass and reduced lipid solubility profile it cannot be wrapped by passive distribution. Thus, there are many phytoconstituents which possibly will have various rings system and therefore, they could not be immersed from the intestinal fluid into the blood through basic dispersion course. Also, a small number of phyto-molecules are originated in broke environment which is soluble in lipids along with added oils as well as and it repeatedly show the inhibition to pass the small intestine owing to its diploid characters. The efficiency of every natural product is based on release of complexes. So it resultant shows the lower bioavailability over the herbal drugs 21,22.

Structure of Phospholipids Complex

The term ‘phyto’ means plant, while ‘some’ means cell-like. Phyto-phospholipid complexes are formed by interactions between active constituents and the polar head of phospholipids. Interactions between active constituents and phospholipids permit phospholipid complexes to be an essential part in which the phospholipids head group is attached, but the two long fatty acid chains do not participate in complex formation. The two long fatty acid chains can move and encapsulate the polar part of complexes to form a lipophilic surface. Phyto-phospholipid complexes form agglomerates when diluted in water, which resembles a small cell that shows some similarity to liposomes 22,23.

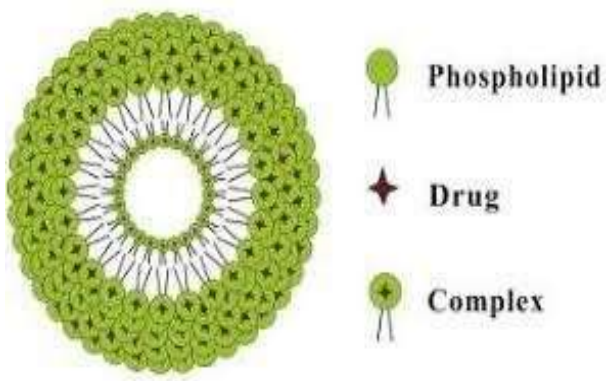

FIG 1: STRUCTURE OF PHOSPHOLIPID COMPLEX

Properties of Phospholipids Complex

Biological Properties

Phytosome increases the active absorption of active ingredients and also increase the systemically bioavailability when administered orally.

These are the advance form of herbal products and having better efficacy as per compare to conventional herbal extract.

Phytosome has better pharmacokinetic as compare to simple herbal drugs 24.

Physicochemical Properties

Phytosomes are the complex between phytoconstituents and natural phospholipid, and the complex is obtained by reacting an appropriate amount of phospholipid and chief constituents in particular solvent.

The interaction between phospholipid and substrate is due to the development of hydrogen bonds between the polar head of phospholipid and the polar functionalities of the chief constituents.

On treatment with hydrophilic environment phytosome shows a cell-like structure like liposomes, but in a liposome, the chief constituent interacts within the internal pocket while in phytosome the chief active constituents are enveloped the polar head of phospholipid and becoming an integral part of the membrane 25.

Advantages of Phospholipid Complex

There is a greatly increase the bioavailability and reforms the absorption of herbal drugs owing to their combination with phospholipids and botanical herbs in the intestinal tract

Phytosomes have been used to convey the hepatic caring flavonoids moiety since they can also be capable of become bioavailable in the biological environment by acting as liver protective substances

Phytosomes may be too make the improvement of the distribution of drug in the course of skin via transdermal route at the same time they can act as bridge for the release of enormous mixed collection of drugs such as peptides and protein Phytosomes can be adopted as systemic targeting agents to the transition of biological material from the hydrophilic nature into lipophilic nature of enterocyte cell and from nearby into cells

Phytosomes have low risk report over the toxicological outline of the phospholipids are maintain in well form in the systematic text.

Phytosomes do not have the difficulty with drug entrapment throughout formulation development. In addition, the entrapment effectiveness is elevated besides predetermined form, for the reason that the drug itself forms vesicles subsequent to conjugation with lip 26, 27.

Preparation of Phospholipid Complex by Different Methods

Thin Layer Rotary Evaporator Vacuum Method

Phytosome vesicles can be made by thin layer rotary evaporator vacuum method. The phytosomal complex was mixed in anhydrous ethanol in 250 ml round bottom flask. The flask was attached to a rotary evaporator. The solvent will evaporate at a temperature about 60 ° C forming thin layer films around the flask. The film is hydrated by phosphate buffer having pH 7.4, and the lipid layer will peel off in phosphate buffer forming vesicle suspension. The phytosomal suspension was subjected to probe sonication with 60% amplitude. Phytosomal suspension will be stored in the refrigerator for 24 hrs, before characterization 28.

Solvent Evaporation Method

Accurately, weight the quantity of phospholipid and cholesterol in round bottom flask and dissolve it in 10 mL of chloroform followed by sonication for 10 minutes using bath sonicator. Organic solvent removal can be done by subjecting it under reduced pressure in a rotary evaporator (40°C). After

complete removal of the solvent thin layer is formed which is hydrated with polyphenolic extract of the drug in a rotary evaporator. Phospholipids mixture was sonicated in an ice bath for heat dissipation. Prepared phytosome were stored in an amber colored bottle 29.

Reflux Method

Phytosomes can be prepared by reflux method. Polyphenolic extract and phospholipid were placed in 100 mL round bottom flask and refluxed in DCM for 1 hr not exceeding 40°C. The clear solution was evaporated and add 15 mL of n-hexane until a precipitate was obtained. The precipitate was taken and placed in a desiccators 30.

Anti-solvent Precipitation Method

Specific quantities of the medication and soy lecithin were taken in a 100 ml round bottom flask and refluxed for 2 h at a temperature not exceeding 60oC with 20 ml of dichloromethane. The concentration of this mixture is 5-10 ml. Hexane (20 ml) was carefully added with continuous stirring to get the precipitate filtered and collected and stored overnight in vacuum desiccators. In mortar, the dried precipitate is crushed and sifted through # 100 mesh 31.

Characterization of Phospholipid Complex

Transition Temperature: Differential Scanning Calorimetry (DSC) can determine the transition temperature of vesicular lipid system 32.

Entrapment Efficiency: The entrapment efficiency of a phytosomal formulation can be determined by subjecting the formulation to ultra-centrifugation technique 33.

Vesicle size and Zeta potential: The particle size and zeta potential of phytosomes can be determined by dynamic light scattering, which uses a computerized inspection system and photon correlation spectroscopy 34.

Surface Tension Activity Measurement: The surface tension activity of the drug in aqueous solution will be measured by Du Nouy ring tensinometer 35.

Spectroscopic Evaluation: The spectroscopic evaluations are widely employed in order to confirm the formation of the complex between phytoconstituents and the phospholipid moiety as well as to study the corresponding interaction between the two components High Performance Liquid Chromatography (HPLC) or UV-Visible Spectroscopy method is used to determine the percentage drug entrapment by extracting the phytosomes with suitable solvent system by centrifugation and estimating its supernatant. The widely employed methods are H1NMR or C13NMR or FTIR 36.

In Vivo Evaluation: Experimental models are chosen on the basis of anticipated therapeutic activity of the plant constituent in phytosome for in vivo and in vitro examination. For example, examination of antihepatotoxic activity can be assessed by antioxidant or free radical scavenging property of phytosome. The in vivo anti-hepatotoxic studies on animals through the effect of phytosome on alcohol induced or paracetamol-induced hepatotoxicity 37, 38.

Therapeutic Applications of Phospholipid Complex

Curcumin Phytosomes-

Curcumin phytosome is unique curcumin extract that is significantly better absorbed than other curcumin extracts (Huang et al., 2015). Phytosomes are plant extracts bound to phosphatidylcholine (PC), an essential component of human cells. Our bodies can make PC but we can also get it from food or supplements. Phytosomes of curcumin having better antioxidant property 39.

Phytosomes of Grape Seed-

Procyanidine from grape seed extract is the main phytoconstituents which having better antioxidant property. Green tea rich in polyphenols and phytosome process binds the green tea extract to phosphatidylcholine for rapid and effective absorption, increasing bioavailability.

Phytosomes of Gingko biloba-

Gingko biloba phytosomes contains 24% gingko flavones glycoside and 6% terpenes lactones. Its improved oral bioavailability and good tolerabilitymakes it the ideal gingko products even for long term treatment. Antioxidant capacity the improved efficacy of gingko phytosomes in combating the allergen induced bronchospam.

Phytosomes of Arjuna Bark –

Phytosomes of Arjuna bark shows antiproliferative activity on human breast cancer cell line MCF-7by MTT assay by comparing its activities with Quercetin and its phytosomes.

Panax ginseng Phytosomes-

Phytosomal complex of panax ginseng helpful in preparation of dermatological and cosmetic pharmaceutical formulations shows moisturizing effect. Also it more active in vasal protection, capillary permeability, protection against UV radiation 40, 41.

The phytophospholipid complexation technique has offered a great opportunity and hope in improving the in vivo bioavailability of herbal drugs. The formulation methodology for phytosomes is simple that can be easily upgraded to a commercial scale. The characterization methodologies are well established for this type of novel formulation. Flavonoids are the most important group of phytochemicals. Different flavonoids which have shown antioxidant activity fifty to two hundred times more potent than vitamin C or E43. Many marketed formulations have already approved for innovative formulations, processes and applications of phytosomes. Up to the potential of phytosomes technique was concerned, it has an excellent future for use in the formulation technique and applications of hydrophilic herbal compounds.

2. MATERIAL AND METHOD

MATERIAL Numerous relevant chemicals, were used. This chapter deals with the material and methods used for phytochemical extraction, their preliminary chemical screening, formulation and evaluation of phospholipids complex of Alhagi camelorum.

Chemicals:

Chemicals used in the investigation are listed in Table 1.

TABLE 1: CHEMICALS USED FOR STUDY

Sr. No.	Chemicals	Supplier
1.	Potassium Mercuric Iodide	Thomas Baker, Mumbai
2.	Iodine	Loba chemie Pvt. Ltd., Mumbai
3.	Potassium Iodide	Loba chemie Pvt. Ltd., Mumbai
4.	Potassium Bismuth Iodide	S. D. Fine Chem. Ltd., Mumbai
5.	Picric acid	Thomas Baker, Mumbai
6.	Sodium nitropruside	Loba chemie Pvt. Ltd., Mumbai
7.	Sodium hydroxide	Loba chemie Pvt. Ltd., Mumbai
8.	Pyridine	S. D. Fine Chem. Ltd., Mumbai

9.	Ferric chloride	Thomas Baker, Mumbai
10.	Gelatin	S. D. Fine Chem. Ltd., Mumbai
11.	Lead acetate	Loba chemie Pvt. Ltd., Mumbai
12.	Nitric acid	S. D. Fine Chem. Ltd., Mumbai
13.	Copper acetate	S. D. Fine Chem. Ltd., Mumbai
14.	Sodium Chloride	S. D. Fine Chem. Ltd., Mumbai
15.	Methanol	Qualigens Fine Chemicals, Mumbai
16.	Ethanol	Qualigens Fine Chemicals, Mumbai
17.	Chloroform	Qualigens Fine Chemicals, Mumbai
18.	Folin-Ciocalteu reagent	Loba chemie Pvt. Ltd., Mumbai
19.	Fehling's solution	Central drug house ltd new Delhi

Instruments:

Instruments used in the investigation are listed Table 2.

TABLE 2: INSTRUMENTS USED FOR STUDY

Sr. No.	Instruments	Supplier
1.	UV -Visible Spectrophotometer	Labindia 3000+
2.	Micro Centrifuge	REMI laboratory, Mumbai
3.	pH Meter	Accumax India, New Delhi
4.	Electronic Balance	Contech Instruments Ltd. , Mumbai
5.	Hot Air Oven	Oracle Equipments, New Delhi
6.	Vortex Apparatus	Ambros Lab Equipments, Ambala
7.	Rotary Vaccum Evaporator	Microtech Scientific Instruments, New Delhi
8.	Sonicator	Athena Technology, Thane

METHODS:

Collection of Plant Material

Seeds of Alhagi camelorum were collected from ruler area of Bhopal (M.P), India in the months of March, 2023. The plant was authenticated by the taxonomist Dr. J. Mehta, Department of Botany, Career College, Bhopal (M.P.) and Voucher specimen with corresponding Herbarium number BOT/ Career /2023/15.

Cleaning

After procurement of plant material, they were cleaned properly. The cleaning process involved the following steps. Very first the decayed or deteriorated plant material was removed. This was followed by washing with tap water and distilled water. The washed plant material was wrapped in blotting paper in order to remove extra water 42.

Drying

Soon after cleaning, plant material was kept for drying sun but under the shade. The main purpose of drying is to remove the water content from plant so that the plant material can be stored. The dried plant part was finely powdered using electric grinder, sieved and packaged in polyethylene bags until when

needed 43.

Organoleptic Characters

Dried Plant materials were crushed in mortar pestle to obtain a powdered form and then

subsequent used for organoleptic characters. A small amount of powdered drug was spread on a white tile and physically examined for general appearance i.e. color, taste, texture etc 44.

Physicochemical Parameters

Loss on Drying

2 g of plant material was placed (without preliminary drying) after accurately weighing it in a tarred evaporated dish. This was dried at 105°C for 5 h and weighed. Drying and weighing was continued at 1 h interval until we got the constant weight. Constant weight was reached when two consecutive weights, after drying for 30 min. and cooling for 30 min. in a desiccator, showed not more than 0.1 g 45.

Total Ash Value

About 2-3 g of ground plant material was incinerated in a tarred platinum/silica crucible at a temperature not exceeding 450°C until free from carbon. Then it was cooled and weighed. The percentage of ash with reference to the air dried plant material was calculated 46.

Acid Insoluble Ash Value

To the crucible containing total ash, 25 mL of dilute HCl was added. The insoluble matter was collected on an ashless filter paper (Whatmann number 41) and washed with hot water until the filtrate was neutral. The filter paper containing insoluble matter was transferred to the original crucible and dried on a hot plate and ignited to constant weight. The residue was allowed to cool in a suitable desiccator for 30 min. and weighed without delay. The content of the insoluble ash was calculated with reference to the air dried plant material 47.

Water Soluble Ash Value

The ash was boiled for 5 min with 25 mL of water; insoluble matter was collected in a Gooch crucible or on an ash less filter paper, washed with hot water, and ignited for 15 min. at a temperature not exceeding 450°C. The difference in the weight of the insoluble matter and the weight of ash represented the water soluble ash. The percentage of water soluble ash was calculated with reference to the air dried plant material 48.

Fluorescence Analysis

Fluorescence analysis of the drug was observed under day and UV light using various solvent extracts as well as acids and alkaline treated with solutions of the drug. The powder was treated

with neutral, acids and alkaline solvents solutions like acetic acid, picric acid, FeCl3, 1 N hydrochloric acid, 1% sulphuric acid 1 N NaoH nitric acid, aqueous and KoH.

Extraction Procedure

Following procedure was adopted for the preparation of hydroalcoholic extract from the shade dried and powdered herbs 49.

Defatting of Plant Material

50 gram plant material of Alhagi camelorum were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using

maceration. The extraction was continued till the defatting of the material had taken place50.

Extraction by Maceration Process

Defatted dried plant material of Alhagi camelorum were extracted with hydroalcoholic solvent (ethanol: water: 80:20 v/v) using maceration. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extract 51.

Determination of Percentage Yield

The percentage yield of yield of each extract was calculated by using formula (Ahmad 2015):

$$\text{Percentage yield} = \frac{\text{Weight of extract} \times 100}{\text{Weight of powdered drug taken}}$$

Qualitative Phytochemical Tests

Phytochemical examinations were carried out extracts as per the following standard methods.

Detection of Alkaloids: Extract were dissolved individually in dilute Hydrochloric acid and filtered.

Mayer's Test: Filtrates was treated with Mayer's reagent (Potassium Mercuric Iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids 52.

Wagner's Test: Filtrates was treated with Wagner's reagent (Iodine in Potassium Iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids 53.

Dragendroff's Test: Filtrates was treated with Dragendroff's reagent (solution of Potassium Bismuth Iodide). Formation of red precipitate indicates the presence of alkaloids 54.

Hager's Test: Filtrates was treated with Hager's reagent (saturated picric acid solution). Presence of alkaloids confirmed by the formation of yellow coloured precipitate 55.

Detection of Carbohydrates: Extract was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

Fehling's Test: Filtrates was hydrolysed with dil. HCl, neutralized with alkali and heated with Fehling's A & B solutions. Formation of red precipitate indicates the presence of reducing sugars56.

Detection of Glycosides: Extract was hydrolysed with dil. HCl, and then subjected to test for glycosides.

Legal's Test: Extract was treated with sodium nitropruside in pyridine and sodium hydroxide. Formation of pink to blood red colour indicates the presence of cardiac glycosides 57.

Detection of Saponins

Froth Test: Extract was diluted with distilled water to 20ml and this was shaken in a graduated cylinder for 15 minutes. Formation of 1 cm layer of foam indicates the presence of saponins 58.

Foam Test: 0.5 gm of extract was shaken with 2 ml of water. If foam produced persists for ten minutes it indicates the presence of saponins 59.

Detection of Phenols

Ferric Chloride Test: Extract was treated with 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols 60.

Detection of Tannins

Gelatin Test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins 61.

Detection of Flavonoids

Alkaline Reagent Test: Extract was treated with few drops of sodium hydroxide solution. Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids 62.

Lead acetate Test: Extract was treated with few drops of lead acetate solution. Formation of yellow colour precipitate indicates the presence of flavonoids 63.

Detection of Proteins

Xanthoproteic Test: The extract was treated with few drops of conc. nitric acid. Formation of yellow colour indicates the presence of proteins 64.

Detection of Diterpenes

Copper acetate Test: Extract was dissolved in water and treated with 3-4 drops of copper acetate solution. Formation of emerald green colour indicates the presence of diterpenes 65.

Quantitative Studies of Bioactive Constituents

Estimation of Total Phenolic Content

The total phenolic content of the extract was determined by the modified Folin-Ciocalteu method66 10 mg Gallic acid was dissolved in 10 ml methanol, various aliquots of 10- 50µg/ml was prepared in methanol. 1gm of dried powder of drug was extracted with 100 ml methanol, filter, and make up the volume up to 100 ml. One ml (1mg/ml) of this extract was for the estimation of Phenol. 1 ml of extract or standard was mixed with 5 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 4 ml (7.5g/l) of sodium carbonate. The mixture was vortexed for 15s and allowed to stand for 30min for colour development. The absorbance was measured at 765 nm using a spectrophotometer 67.

Estimation of Total Flavonoids Content

Determination of total flavonoids content was based on aluminium chloride method 66. 10 mg quercetin was dissolved in 10 ml methanol, and various aliquots of 5- 25µg/ml were prepared in methanol. 10 mg of dried extract was dissolved in 10 ml methanol and filter. Three ml (1mg/ml) of this extract was for the estimation of flavonoids.1 ml of 2% AlCl3 solution was added to 3 ml of extract or each standard and allowed to stand for 15min at room temperature; absorbance was measured at 420 nm 68.

Formulation Development of Phospholipids Complex

Preparation of Phospholipids Complex

The complex was prepared with phospholipids: cholesterol and Alhagi camelorum in the ratio of 1:1:1, 1:2:1, 2:1:1, 2:3:1 respectively using solvent evaporation method. Weight amount of extract and phospholipids and cholesterol were placed in a 100ml round-bottom flask and 25ml of dichloromethane was added as reaction medium 69. The mixture was refluxed and the reaction temperature of the complex was controlled to 50°C for 3 h. The resultant clear

mixture was evaporated and 20 ml of n-hexane was added to it with stirring. The precipitated was filtered and dried under vacuum to remove the traces amount of solvents. The dried residues were gathered and placed in desiccators overnight and stored at room temperature in an amber colored glass bottle 70.

TABLE 3: DIFFERENT FORMULATIONS OF PHOSPHOLIPIDS COMPLEX

Formulation	Ratio of Phospholipids and Cholesterol	Extract Concentration (%)	Dichloromethane Concentration
Optimization of polymer (Phospholipids and Cholesterol)			
F1	1:1	1	20 ml
F2	1:2	1	20 ml
F3	2:1	1	20 ml
F4	2:3	1	20 ml
Optimization of Extract Concentration			
F5	2:1	0.5	20 ml
F6	2:1	1.0	20 ml
F7	2:1	1.5	20 ml
F8	2:1	2.0	20 ml
Optimization of Dichloromethane concentration			
F9	2:1	1.0	5 ml
F10	2:1	1.0	10 ml
F11	2:1	1.0	15 ml
F12	2:1	1.0	20 ml

After thorough optimization and evaluation of various formulation parameters such as the phospholipid to cholesterol ratio, extract concentration, and dichloromethane concentration, Formulation F10 was selected as the optimized formulation. This formulation exhibited the most favorable characteristics for the development of phospholipid complexes, based on its particle size and entrapment efficiency.

In the optimization of the phospholipid to cholesterol ratio, Formulation F3 (2:1 ratio) showed a favorable particle size and entrapment efficiency. However, when considering subsequent stages of optimization, Formulation F10, which maintained the 2:1 phospholipid to

cholesterol ratio, showed further improvements. Specifically, F10 demonstrated an optimal particle size of 310.32 nm and an entrapment efficiency of 74.65%, which was higher than other formulations in this category, indicating better encapsulation of the active ingredient. For the optimization of extract concentration, Formulation F10 was formulated with 1.0% extract concentration, which offered a balanced and efficient result. The particle size of 310.32 nm and entrapment efficiency of 74.65% indicated that this extract concentration provided a stable and efficient complex, allowing for high drug loading and improved therapeutic potential. This formulation demonstrated an excellent trade-off between high encapsulation and desirable particle size, which is essential for the effective delivery of the active compound.

In the final optimization stage, the dichloromethane concentration was varied, and Formulation F10, with 10 ml dichloromethane, exhibited the most favorable results. With a particle size of 310.32 nm and a high entrapment efficiency of 74.65%, F10 demonstrated superior characteristics in terms of drug encapsulation and particle uniformity compared to other formulations with higher dichloromethane volumes.

Considering the results from the optimization of all three parameters phospholipid and cholesterol ratio, extract concentration, and dichloromethane concentration Formulation F10 stood out as the best formulation. With a 2:1 ratio of phospholipids to cholesterol, 1.0% extract concentration, and 10 ml dichloromethane, F10 provided the optimal particle size of 310.32 nm and a high entrapment efficiency

of 74.65%. These attributes indicate that F10 offers the best balance of stability, encapsulation efficiency, and particle size, making it the most suitable formulation for further development.

Characterization of Prepared Phospholipids Complex

Microscopic Observation of Prepared Phospholipids Complex

An optical microscope (Cippon, Japan) with a camera attachment was used to observe the shape of the optimized phospholipids complex formulation.

Entrapment Efficiency

Phospholipids complex preparation was taken and subjected to centrifugation using cooling centrifuge (Remi) at 12000 rpm for an hour at 40C 22..

The clear supernatant was siphoned off carefully to separate the non entrapped flavonoids and the absorbance of supernatant for non entrapped Alhagi camelorum was recorded at λmax 420.0 nm using UV/visible spectrophotometer (Labindia 3000+). Sediment was treated with 1ml of 0.1 % Triton x 100 to lyse the vesicles and diluted to 100 ml with 0.1 N HCl and absorbance taken at 420.0 nm. Amount of quercetin in supernatant and sediment gave a total amount of Alhagi camelorum in 1 ml dispersion. The percent entrapment was calculated by following formula.

Percent Entrapment=(Amount of drug in sediment)/(Total amount of drug added) X 100

Particle Size and Size Distribution

The particle size, size distribution of optimized phospholipids complex formulation were determined by dynamic light scattering (DLS) using a computerized inspection system (Malvern Zetamaster ZEM 5002, Malvern, UK). The electric potential of the phospholipids complex, including its Stern layer (zeta potential) was determined by injecting the diluted system into a zeta potential measurement cell 71.

Transmission Electron Microscopy

Surface morphology was determined by TEM, for TEM a drop of the sample was placed on a carbon-coated copper grid and after 15 min it was negatively stained with 1% aqueous solution of Phosphortungustic acid 72. The grid was allowed to air dry thoroughly and samples were viewed on a transmission electron microscopy (TEM Hitachi, H-7500 Tokyo, Japan).

In vitro Dissolution Rate Studies

In vitro drug release of the sample was carried out using USP- type II dissolution apparatus (Paddle type) 73. The dissolution medium, 900 ml 0.1N HCl was placed into the dissolution flask maintaining the temperature of 37±0.50C and 75 rpm. 10 mg of prepared phospholipids complex was placed in each basket of dissolution apparatus. The apparatus was allowed to run for 8 hours. Sample measuring 3 ml were withdrawn after every interval (30 min, 1 hrs, 2 hrs, 4 hrs, 6 hrs, 8 hrs, and 12 hrs.) up to 12 hours using 10 ml pipette. The fresh dissolution medium (370C) was replaced every time with the same quantity of the sample and takes the absorbance at 256.0 nm using spectroscopy.

Mathematical Treatment of In-vitro Release Data: The quantitative analysis of the values obtained in dissolution/release tests is easier when mathematical formulas that express the dissolution results as a function of some of the dosage forms characteristics are used.

Zero Order kinetics: The pharmaceutical dosage forms following this profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action. The following relation can, in a simple way, express this model:

$Qt = Qo + Ko\ t$

Where Qt is the amount of drug dissolved in time t, Qo is the initial amount of drug in the solution (most times, Qo=0) and Ko is the zero order release constant (Singhvi and Singh, 2011).

First Order Kinetics: The following relation expresses this model:

log Qt = log Qo +K1t/2.303

Where Qt is the amount of drug dissolved in time t, Qo is the initial amount of drug in the solution and K1 is the zero order release constant

In this way a graphic of the decimal logarithm of the released amount of drug versus time will be linear. The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminish 74.

Higuchi Model: Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs in semi-solid and/or solid matrixes. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media. The simplified Higuchi model is expressed as:

Q= KH.t1/2

Where Q is the amount of drug released in time t and KH is the Higuchi dissolution constant. Higuchi model describes drug release as a diffusion process based in the Fick's law, square root time dependent. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms such as transdermal systems and matrix tablets with water-soluble drugs 75.

Korsmeyer Peppas Model: Korsmeyer et al. used a simple empirical equation to describe general solute release behaviour from controlled release polymer matrices:

Mt/M∞= atn

Where Mt/M ⌋is fraction of drug released, a is kinetic constant, t is release time and n is the diffusional exponent for drug release. 'n' is the slope value of log Mt/M ⌋ versus log time curve. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. concluding for values for a slab, of n =0.5 for fickian diffusion and higher values of n, between 0.5 and 1.0, or n =1.0,

Mtl

M ⌋

for mass transfer following a non-fickian model. In case of a cylinder n =0.45 instead of 0.5, and 0.89 instead of 1.0. This equation can only be used in systems with a drug diffusion coefficient fairly concentration independent. To the determination of the exponent n the portion of the release curve where Mt/M ⌋< 0.6 should only be used. To use this equation it is also necessary that release occurs in a one-dimensional way and that the system width-thickness or length-thickness relation be at least 10. A modified form of this equation was developed to accommodate the lag time (l) in the beginning of the drug release from the pharmaceutical dosage form:

When there is the possibility of a burst effect, b, this equation becomes:

Mt

M ⌋

In the absence of lag time or burst effect, l and b value would be zero and only atn is used. This mathematical model, also known as Power Law, has been used very frequently to describe release from several different pharmaceutical modified release dosage forms 76.

TABLE 4: INTERPRETATION OF DIFFUSIONAL RELEASE MECHANISMS

Release exponent (n)	Drug transport mechanism	Rate as a function of time
0.5	Fickian diffusion	$t^{-0.5}$
0.5<n<1.0	Anomalous transport	t^{n-1}
1.0	Case-II transport	Zero-order release
Higher than 1.0	Super Case-II transport	t^{n-1}

In vitro Anti-Diabetic Activity using Alpha Amylase Inhibition Assay

10 mg acarbose was dissolved in 10 ml methanol and various aliquots of 100- 500µg/ml were prepared in methanol. 10 mg of phospholipids complex was extracted with 10 ml methanol, filter and make up the volume up to 10 ml. 500 µl of each extract was for the estimation of enzyme inhibition. A total of 500 µl of test samples and standard drug (100-500µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase (0.5mg/ml) solution and were incubated at 25°C for 10 min (Bernfeld, 1955). After these, 500 µl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added to each tube. The reaction mixtures were

then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then incubated in a boiling water bath for 5 min, cooled to room temperature.

The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle 77.

Stability Studies of Optimize Phospholipids Complex Formulation

Stability study was carried out for optimize phospholipids complex formulation at two different temperatures i.e. refrigeration temperature (4.0±0.2°C) and at room temperature (25-28 ± 2°C) for 3 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid any interaction between the formulation and glass of container. The formulations were analyzed for any viscosity and % assay 78.

3. RESULTS AND DISCUSSION

Results of Organoleptic characters

TABLE 5: ORGANOLEPTIC CHARACTERS OF ALHAGI CAMELORUM

Plant	Color	Odour	Taste	Texture
Alhagi camelorum	Brown	No characteristic	Slightly sweet	Brown and foliaceous

Results of physicochemical parameter of Alhagi camelorum

TABLE 6: RESULTS OF PHYSICOCHEMICAL PARAMETER

S. No.	Test parameters	% (w/w)
1.	Loss on drying at 105°C	3.8 %
2.	Total ash	4.2%

3.	Acid insoluble ash	1.7 %
4.	Water soluble ash	7.9 %

Table 6 presents the results of various physicochemical parameters like Loss on drying, total ash, acid insoluble ash, water soluble ash for Alhagi camelorum. These parameters provide insights into the quality and characteristics of the plant materials.

Results of fluorescence analysis

TABLE 7: FLUORESCENCE ANALYSIS OF SEEDS OF ALHAGI CAMELORUM

S. No.	Treatment with chemical reagent	Observation		
		Normal light	Short UV	Long UV
1	Acetic acid	Black	Black	Black
2	Picric acid	Brown	Black	Black
3	$FeCl_3$	Black	Bluish black	Bluish black
4	1 N hydrochloric acid	Brown	Black	Black
5	1% sulphuric acid	Brown	Black	Black
6	1 N NaoH	Yellowish brown	Bluish black	Bluish black
7	Nitric acid	Brown	Greenish black	Bluish black
8	Aqueous	Brown	Brown	Black
9	KoH	Brown	Black	Black

The seeds samples of Alhagi camelorum were subjected to fluorescence analysis which revealed various different colour pattern of fluorescence. The samples are trated with chemical agents like Acetic acid, Picric acid, FeCl3, 1 N hydrochloric acid, 1% sulphuric acid, 1 N NaoH, Nitric acid, Water & KoH and the colour of theor fluorescence in normal light (Fig 2), short UV (Fig 3) & long UV light (Fig 4) was recorded.

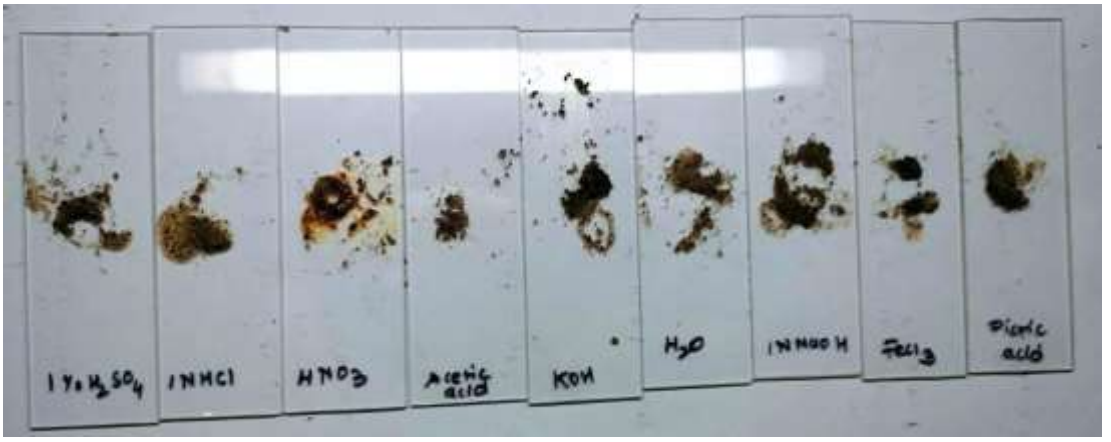

FIG 2: FLUORESCENCE ANALYSIS IN NORMAL LIGHT

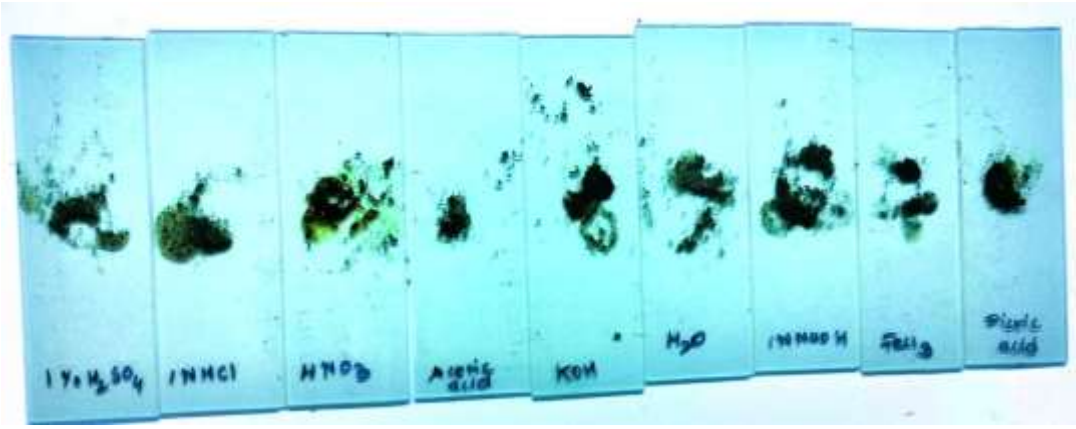

FIG 3: FLUORESCENCE ANALYSIS IN SHORT UV

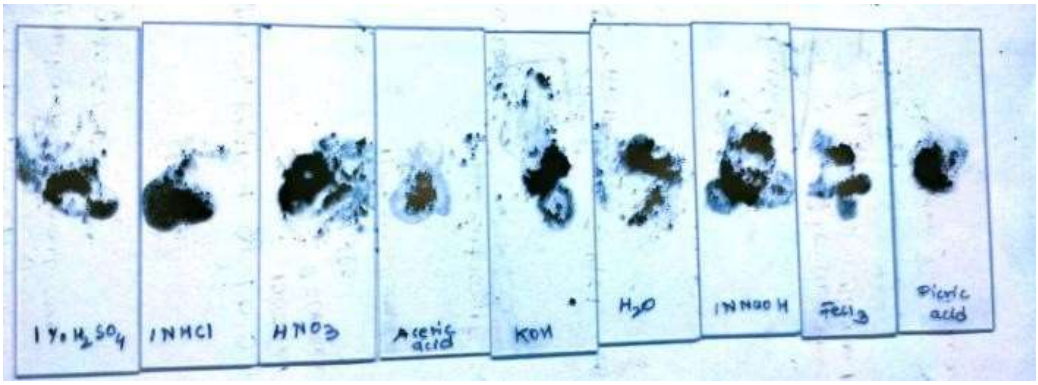

FIG 4: FLUORESCENCE ANALYSIS IN LONG UV

Result of percentage yield of extract

The crude extract so obtained after the maceration process, each extracts were further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction (Table 8) is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used.

TABLE 8 : RESULT OF PERCENTAGE YIELD OF EXTRACTS

S. No.	Extracts	Percentage Yield (%)
1.	Pet. Ether	1.4%
2.	Hydroalcoholic	6.7%

Results of phytochemical testing

A small portion of the dried extracts were subjected to the phytochemical test using Kokate (1994) methods to test for alkaloids, glycosides, tannins, saponins, flavonoids and steroids separately for extracts of all samples. Small amount of each extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml.

TABLE 9 : RESULT OF PHYTOCHEMICAL SCREENING OF ALHAGI CAMELORUM EXTRACT

S. No.	Constituents	Hydroalcoholic extract
1.	Alkaloids	
	Mayer's Test	-ve
	Hager's Test	-ve
	Dragendroff's Test	-ve
	Wagner's Test	-ve
2.	Glycosides	
	Legal's test	+ve
3.	Flavonoids	
	Lead acetate	+ve
	Alkaline reagent test	+ve
4.	Phenol	
	Ferric chloride test	+ve
5.	Proteins	
	Xanthoproteic test	-ve
6.	Carbohydrates	
	Fehling's test	+ve
7.	Saponins	
	Froth Test	+ve
	Foam test	+ve
8.	Diterpenes	
	Copper acetate test	+ve
9.	Tannins	
	Gelatin Test	+ve

Abbreviation: +ve indicate presence, -ve indicate absence of phytochemicals.

From the (Table 9) results obtained and it is clear that the Alhagi camelorum plant shows the presence of flavonoids, carbohydrates, diterpenes, tannins, glycosides, phenol and saponins were found in Alhagi camelorum when extracted using maceration procedure. The phytochemical analysis of Alhagi camelorum plant indicates the presence of phenols and flavonoids present in sufficiently enough quantity according to preliminary phytochemical analysis.

Estimation of total phenolic content (TPC)

Total phenolic compounds (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve:

$Y = 0.011X+0.011$, $R2= 0.998$,

where X is the gallic acid equivalent (GAE) and Y is the absorbance.

TABLE 10: PREPARATION OF CALIBRATION CURVE OF GALLIC ACID

S. No.	Concentration (µg/ml)	Mean Absorbance
1	10	0.135±0.001
2	20	0.247±0.003
3	30	0.364±0.001
4	40	0.474±0.002
5	50	0.581±0.001

*Average of three determination, Mean ± SD

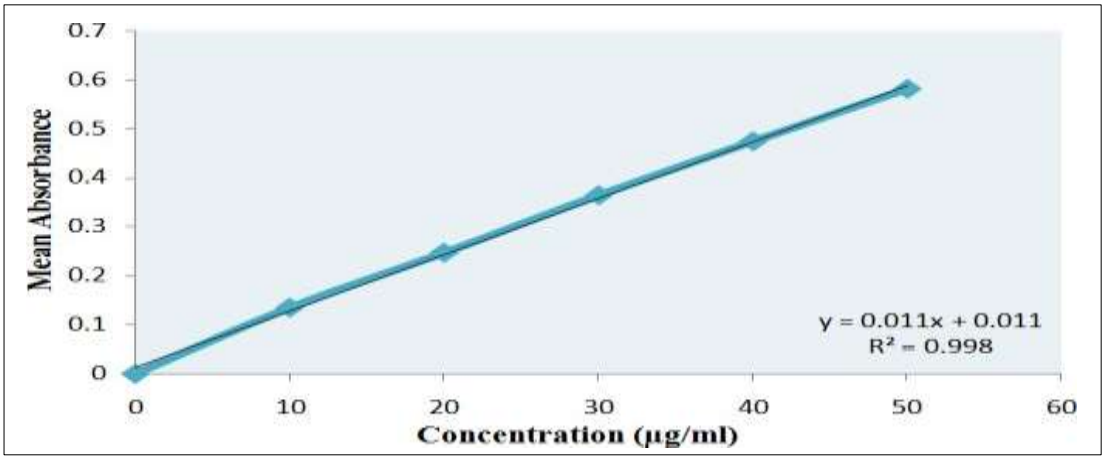

FIG 5: GRAPH OF CALIBRATION CURVE OF GALLIC ACID

Total flavonoid content estimation (TFC)

The total flavonoid content (TFC) was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.040X + 0.012, R2= 0.999, where X is the quercetin equivalent (QE) and Y is the absorbance.

Calibration Curve of Quercetin

TABLE 11: PREPARATION OF CALIBRATION CURVE OF QUERCETIN

S. No.	Concentration (µg/ml)	Mean Absorbance
1	5	0.232±0.002
2	10	0.415±0.005
3	15	0.617±0.001
4	20	0.815±0.003
5	25	1.021±0.002

*Average of three determination, Mean ± SD

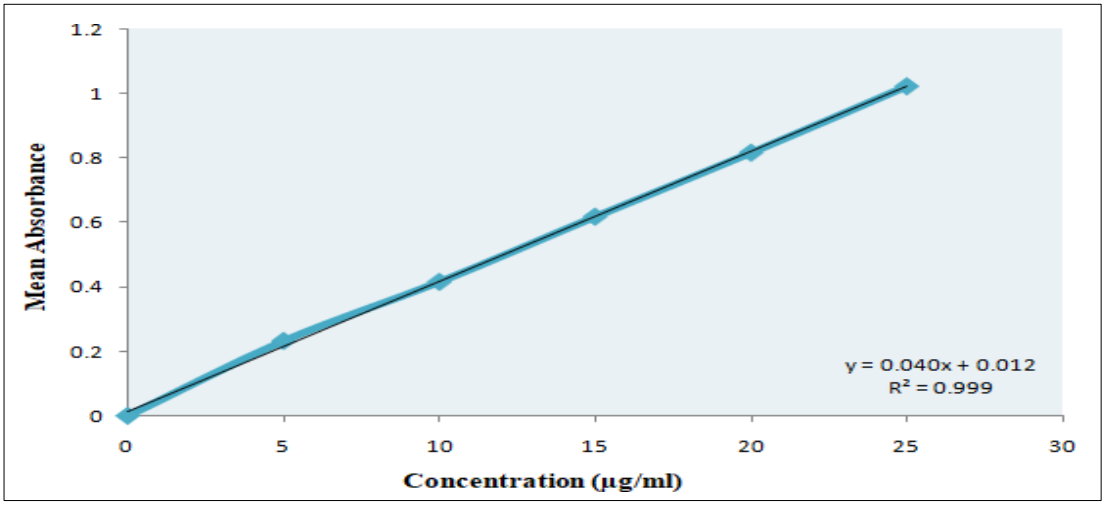

FIG 6: CALIBRATION CURVE OF QUERCETIN

TABLE 12: TOTAL BIOACTIVE CONSTITUENTS CONTENT OF ALHAGI CAMELORUM

S. No.	Extract	Total phenol (mg/100mg)	Total Flavonoid (mg/100mg)
1.	Hydroalcoholic extract	0.357 mg/100mg	0.646 mg/100mg

Characterization of phospholipids complex of hydroalcoholic extract of Alhagi camelorum

Microscopic observation of prepared phospholipids complex

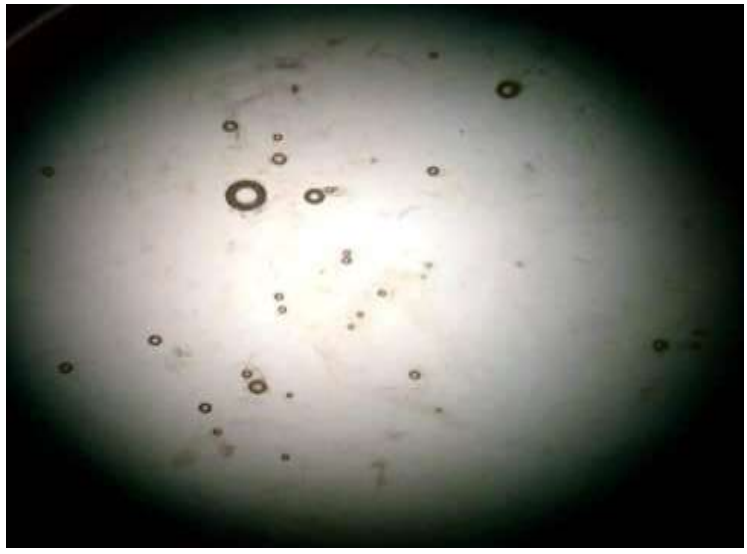

FIG 7: MICROSCOPIC OBSERVATION OF OPTIMIZED BATCH F10

Entrapment efficiency and particle size analysis

The particle size of the phospholipid complexes ranged from 310.32 nm (F10) to 385.45 nm (F1). Generally, particle sizes showed a slight decrease in formulations with increased phospholipid content (F10, F12), suggesting that a higher concentration of phospholipids may facilitate the formation of

smaller nanoparticles.

Smaller particles, such as in F10 (310.32 nm), are desirable for enhanced bioavailability and improved penetration through biological membranes, especially for topical or oral drug delivery. These smaller particles are typically associated with better dispersibility and faster dissolution rates, which could lead to faster therapeutic effects.

Entrapment efficiency ranged from 55.85% (F1) to 74.65% (F10). Higher entrapment efficiency is typically observed in formulations with a more stable phospholipid structure that can encapsulate a larger proportion of the drug.

F10 showed the highest entrapment efficiency (74.65%), indicating that the optimized formulation with the right concentration of phospholipid may be more effective at encapsulating the bioactive compound, reducing leakage during storage or application. This is a desirable characteristic, as it leads to more controlled release and prolonged therapeutic action. Formulations with lower EE, such as F1 (55.85%), may benefit from further optimization, particularly in the choice of phospholipid, solvent system, and processing method to increase encapsulation efficiency.

The optimal formulation, based on particle size and EE, appears to be F10 with a 310.32 nm particle size and 74.65% entrapment efficiency. This formulation could be considered for further studies, such as in-vitro drug release and stability testing, as it demonstrates the best balance between particle size and drug encapsulation.

The formulations show significant variation in both particle size and entrapment efficiency. F10 appears to be the most promising formulation, with the smallest particle size and the highest entrapment efficiency, making it suitable for further development and evaluation for therapeutic applications 79.

TABLE 13: PARTICLE SIZE AND ENTRAPMENT EFFICIENCY OF PHOSPHOLIPIDS COMPLEX

Formulation Code	Particle size (nm)	Entrapment Efficiency (%)
F1	385.45	55.85
F2	362.25	62.23
F3	347.85	68.86
F4	358.85	63.32
F5	347.74	65.74
F6	347.85	68.86
F7	345.78	67.85
F8	326.65	64.78
F9	325.65	61.25
F10	310.32	74.65
F11	314.56	65.45
F12	347.85	68.86

Average of three determinations (n=3)

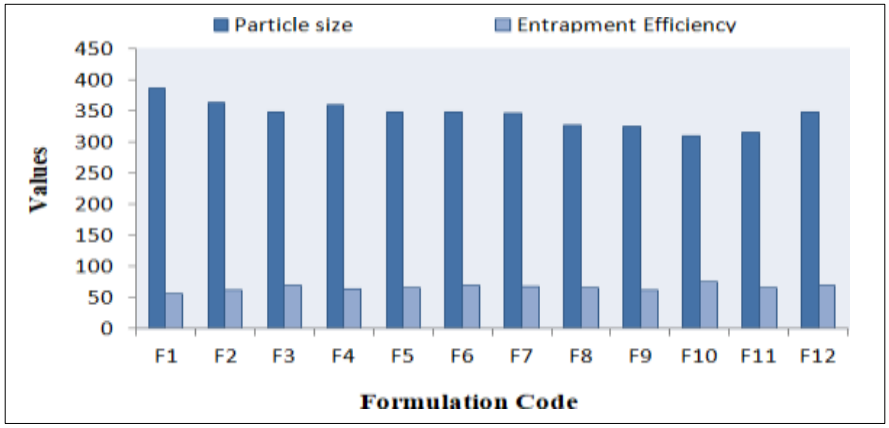

FIG 8: GRAPH OF PARTICLE SIZE AND ENTRAPMENT EFFICIENCY

Transmission electron microscopy (TEM)

TEM is a microscopy technique in which a beam of electrons is transmitted through an ultra-thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device, such as a fluorescent screen, on a layer of photographic film, or to be detected by a sensor such as a CCD camera. TEMs are capable of imaging at a significantly higher resolution than light microscopes, owing to the small de Broglie wavelength of electrons. This enables the instrument's user to examine fine detail even as small as a single column of atoms, which is thousands of times smaller than the smallest resolvable object in a light microscope. TEM forms a major analysis method in a range of scientific fields, in both physical and biological sciences. TEM characterization revealed that the phospholipids complexes are spherical in shape. However, some variation in size distribution was observed in the TEM image, which might be attributed to an uncontrolled charge neutralization process involved between oppositely charged chains occurring during the formation of phospholipids complex.

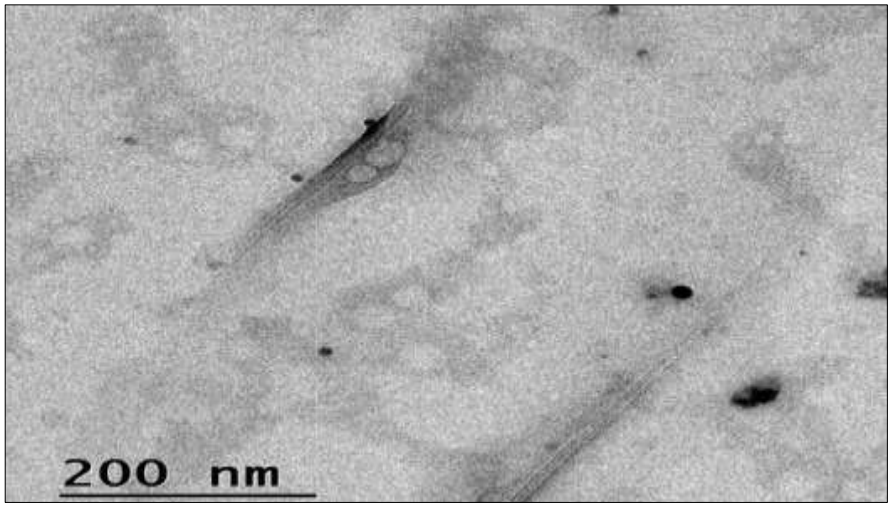

FIG 9: TEM IMAGE OF PHOSPHOLIPIDS COMPLEX

In vitro drug release study of phospholipids complex formulation

TABLE 14: IN-VITRO DRUG RELEASE DATA FOR OPTIMIZED FORMULATION F10

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative*% Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	20.23	1.306	79.77	1.902
1	1	0	35.65	1.552	64.35	1.809
2	1.414	0.301	47.78	1.679	52.22	1.718
4	2	0.602	68.98	1.839	31.02	1.492
6	2.449	0.778	79.95	1.903	20.05	1.302
8	2.828	0.903	86.32	1.936	13.68	1.136
12	3.464	1.079	98.78	1.995	1.22	0.086

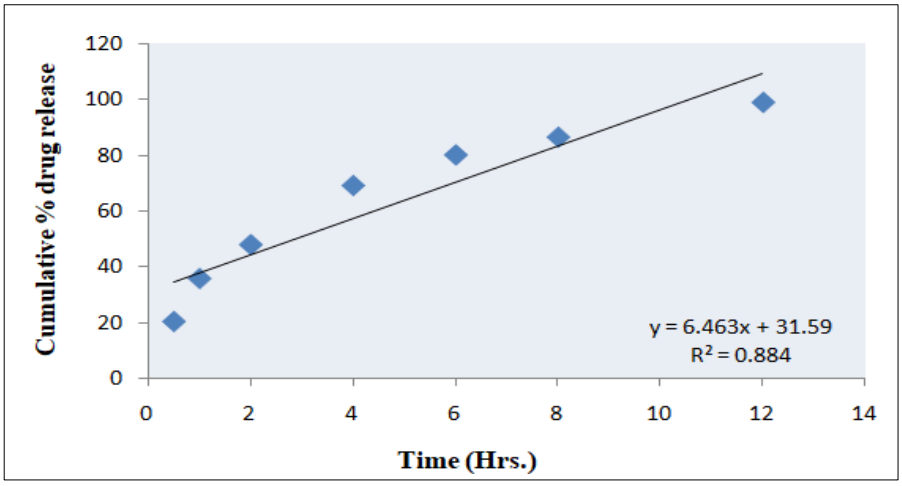

FIG 10: CUMULATIVE % DRUG RELEASED VS TIME(Zero Order Kinetics)

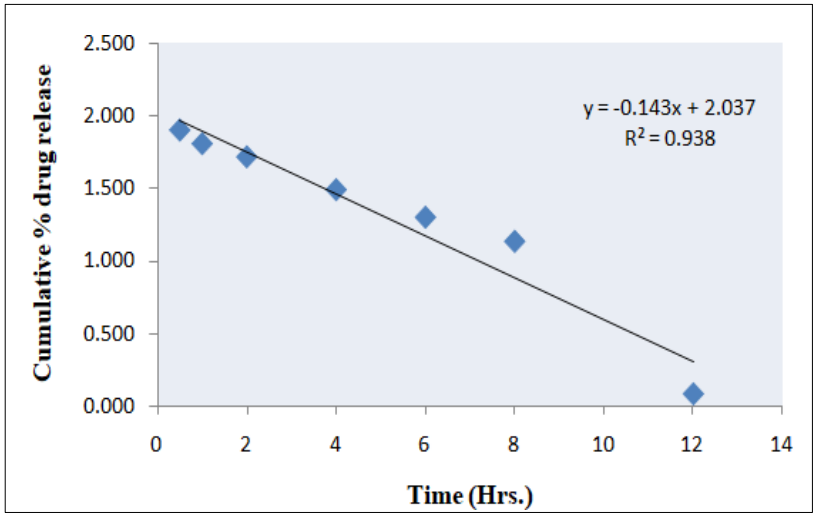

FIG 11: LOG CUMULATIVE % DRUG REMAINING VS TIME

(First Order Kinetics)

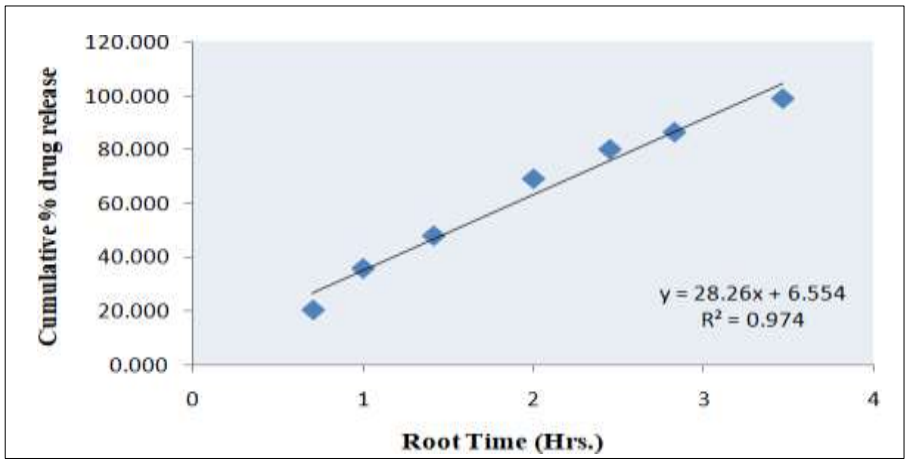

FIG 12: CUMULATIVE % DRUG RELEASE VS ROOT TIME

(Higuchi Release Kinetics)

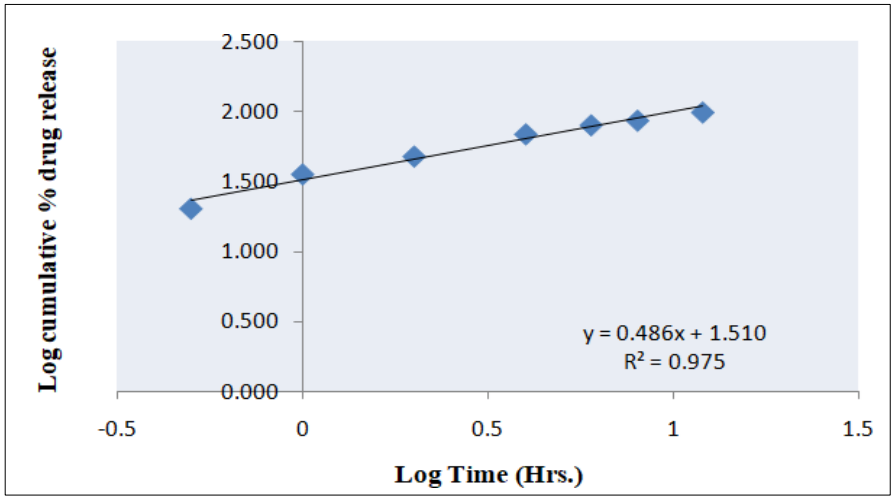

FIG 13: LOG CUMULATIVE % DRUG RELEASE VS LOG TIME

(Korsmeyer Peppas Model)

TABLE 15: REGRESSION ANALYSIS DATA OF OPTIMIZED FORMULATION F10

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
F10	0.884	0.938	0.974	0.975

When the regression coefficient values of were compared, it was observed that ‘r2’ values of Korsmeyer Peppas was maximum i.e. 0.975 hence indicating drug release from formulations was found to follow Korsmeyer Peppas kinetics.

Results of in vitro antidiabetic studies of phospholipids complex

TABLE 16: RESULTS OF IN VITRO ANTIDIABETIC STUDIES

S. No.	Concentration (µg/ml)	Acarbose (% Inhibition)	Phospholipids complex (% Inhibition)
1.	100	43.32	28.37
2.	200	69.98	37.02
3.	300	73.65	43.76
4.	400	78.89	51.59
5.	500	85.45	64.14
IC_{50} Value	105.11	358.83	

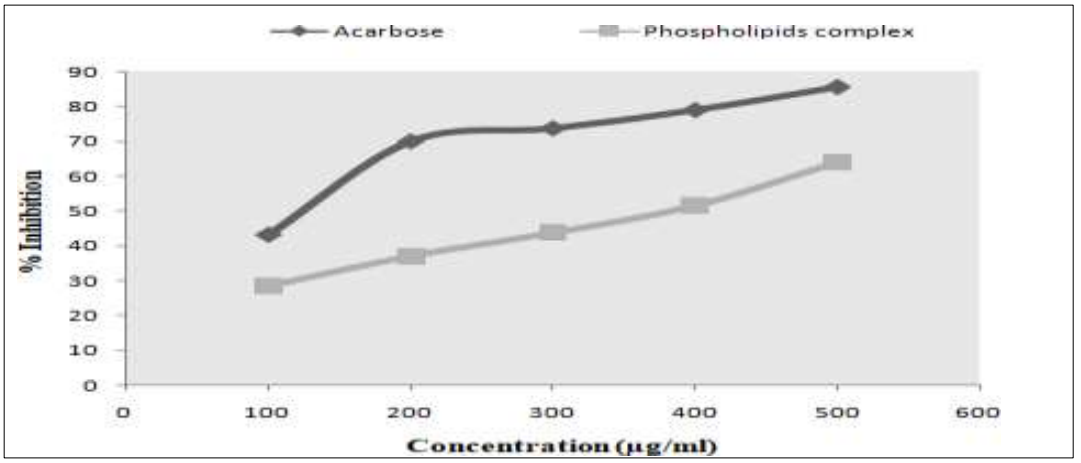

FIG 14: GRAPH OF % INHIBITION OF ACARBOSE AND PHOSPHOLIPIDS COMPLEX

Results of stability studies

Results of stability studies clearly indicates that optimized batches of phospholipids complex were stable over the chosen temperature and humidity conditions up to 3 months as were found no significant variation in physical appearance and % drug content.

TABLE1 17: STABILITY OF OPTIMIZED FORMULATION

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temp.	4.0 ± 0. 2°C	25-28 ± 2°C	4.0 ± 0. 2°C	25-28 ± 2°C	4.0 ± 0. 2°C	25-28 ± 2°C
Particle Size (nm)	310.32	325.89	322.45	345.85	326.85	355.87
Entrapment Efficiency (%)	74.65	70.65	72.85	68.98	71.65	65.77
Physical Appearance	Normal	Normal	Normal	Normal	Normal	Normal

The stability studies of the optimized phospholipid complex formulations indicate that they remain stable over a three-month period under both 4.0 ± 0.2°C and 25-28 ± 2°C conditions. Particle size showed minimal fluctuation, ranging from 315.45 nm to 355.87 nm, and entrapment efficiency decreased slightly from 73.65% to 65.77%. The physical appearance of the formulations remained

normal throughout, with no significant changes. These results suggest that the formulations are stable and suitable for extended use under the tested conditions.

4. DISCUSSION

The study of Alhagi Camelorum in the context of pharmaceutical research holds significant potential due to its bioactive compounds and medicinal properties. The extraction and phytochemical analysis of this plant are crucial steps in identifying its therapeutic applications, particularly in managing diseases like diabetes mellitus. Medicinal plants have been historically significant in healthcare, and Alhagi Camelorum is no exception, as it contains bioactive compounds such as flavonoids, alkaloids, and tannins, which contribute to its pharmacological effects. Phytochemicals from medicinal plants often suffer from poor bioavailability due to their large molecular size and low lipid solubility. To overcome this challenge, novel drug delivery systems such as phospholipid complexes (phytosomes) have emerged. These complexes enhance the bioavailability of plant-based compounds by improving their absorption and systemic circulation. The phospholipid complex of Alhagi Camelorum aims to optimize the therapeutic potential of its bioactive constituents by increasing their solubility and stability in the body.

Developing such complexes involves various techniques, including solvent evaporation, thin-layer rotary evaporation, and anti-solvent precipitation, each contributing to improved drug delivery. The advantages of phytosomes include better absorption, increased therapeutic efficacy, and reduced side effects, making them a promising approach in modern herbal medicines.

5. CONCLUSION

By formulating a phospholipid complex, the absorption and stability of Alhagi Camelorum's phytochemicals can be significantly improved, leading to better therapeutic efficacy. This novel drug delivery system not only enhances bioavailability but also ensures targeted and controlled drug release, minimizing potential side effects. Various preparation methods, including solvent evaporation and thin-layer rotary evaporation, provide effective ways to develop these complexes for pharmaceutical applications.

In conclusion, integrating Alhagi Camelorum into a phospholipid complex can open new avenues for natural drug development. This innovative approach aligns with the need for safer and more effective herbal formulations, making it a valuable contribution to modern medicine. Further research and clinical studies will be essential to fully establish its pharmacological benefits and therapeutic applications.

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References

1. Blair M. Diabetes mellitus review. Urologic nursing. 2016 Jan 1;36(1).
2. Asmat U, Abad K, Ismail K. Diabetes mellitus and oxidative stress-A concise review. Saudi pharmaceutical journal. 2016 Sep 1; 24(5):547-53.
3. Mitra A. Diabetes and stress: a review. Studies on Ethno-Medicine. 2008; 2(2):131-5.
4. Choudhury H, Pandey M, Hua CK, Mun CS, Jing JK, Kong L et al. An update on natural compounds in the remedy of diabetes mellitus: A systematic review. Journal of traditional and complementary medicine. 2018;8(3):361-76.
5. Bolen S, Feldman L, Vassy J, Wilson L, Yeh HC, Marinopoulos S, Wiley C, Selvin E, Wilson R, Bass EB, Brancati FL. Systematic review: comparative effectiveness and safety of oral medications for type 2 diabetes mellitus. Annals of internal medicine. 2007 Sep 18;147(6):386-99.
6. Atkinson MA, Eisenbarth GS, Michels AW. Type 1 diabetes. The Lancet. 2014 Jan 4;383(9911):69-82.
7. Chatterjee S, Khunti K, Davies MJ. Type 2 diabetes. The lancet. 2017 Jun 3;389(10085):2239-51.
8. Buchanan TA, Xiang AH. Gestational diabetes mellitus. The Journal of clinical investigation. 2005 Mar 1;115(3):485-91.
9. Khan HM, Murtaza G, Usman M, Rasool F, Akhtar M, Qureshi MI, Farzana K. Evidence based study of side effects of drugs used in the treatment of diabetes mellitus. African Journal of Pharmacy and Pharmacology. 2012 Jun 29;6(24):1805-8.
10. Kooti W, Farokhipour M, Asadzadeh Z, Ashtary-Larky D, Asadi-Samani M. The role of medicinal plants in the treatment of diabetes: a systematic review. Electronic physician. 2016 Jan;8(1):1832.
11. Shukia R, Sharma SB, Puri D, Prabhu KM, Murthy PS. Medicinal plants for treatment of diabetes mellitus. Indian Journal of Clinical Biochemistry. 2000 Aug;15(1):169-77.
12. Chen J, Mangelinckx S, Adams A, Wang ZT, Li WL, De Kimpe N. Natural flavonoids as potential herbal medication for the treatment of diabetes mellitus and its complications. Natural product communications. 2015 Jan; 10(1).
13. Maulidiani S K, Paetz C, Stanslas J, Abas F and Lajis, N H, Naturally occurring labdane diterpene and benzofuran from Globba pendula, Nat Prod Commun, 2009, 4(8), 1031-1036
14. Velayutham R, Sankaradoss N, Ahamed KN. Protective effect of tannins from Ficus racemosa in hypercholesterolemia and diabetes induced vascular tissue damage in rats. Asian Pacific Journal of Tropical Medicine. 2012 May 1;5(5):367-73.
15. Rasouli H, Yarani R, Pociot F, Popovic-Djordjevic J. Anti-diabetic potential of plant alkaloids: Revisiting current findings and future perspectives. Pharmacological research. 2020 May 1;155:104723.
16. Elekofehinti OO. Saponins: Anti-diabetic principles from medicinal plants–A review. Pathophysiology. 2015 Jun 1;22(2):95-103.
17. Bosha A, Anaga A O and Asuzu I U, Bioassay-guided isolation and structural elucidation of anti-diabetic principle of methanol leaf extract of Newbouldia laevis (P.Beauv), J Pharm Pharmacol, 2015, 3, 516-526.
18. Aba PE, Asuzu IU. Mechanisms of actions of some bioactive anti-diabetic principles from phytochemicals of medicinal plants: A review. Indian Journal of Natural Products and Resources Vol. 9(2), June 2018, pp 85-96
19. Gupta S, Singh RP, Lokwani P, Yadav S, Gupta SK, Vesicular system as a targeted drug delivery system: An overview, International Journal of Pharmaceutical Technology, 3, 2011, 987-1021.
20. Rathore P, Planterosomes: potential Phyto-phospholipid carriers for the bioavailability enhancement of herbal extracts, International journal of pharmaceutical science and research,3, 2012, 737-75
21. Semalty A, Semalty M, Rawat MS. The phyto-phospholipid complexes-phytosomes: A potential therapeutic approach for herbal hepatoprotective drug delivery. Pharmacognosy Reviews. 2007;1(2).
22. Gnananath K, Nataraj KS, Rao BG. Phospholipid complex technique for superior bioavailability of phytoconstituents. Advanced pharmaceutical bulletin. 2017 Apr;7(1):35.
23. Sharma S, Sikarwar M, Phytosome: A review, Plant indica, 1(2), 2005, 1-3.
24. Li J, Wang X, Zhang T, Wang C, Huang Z, Luo X, Deng Y. A review on phospholipids and their main applications in drug delivery systems. Asian journal of pharmaceutical sciences. 2015 Apr 1;10(2):81-98.
25. Pawar HA, Bhangale BD. Phytosome as a novel biomedicine: a microencapsulated drug delivery system. J Bioanal Biomed. 2015 Jan 1;7(1):6-12.
26. Tripathy S, Patel DK, Barob L, Naira SK. A review on phytosomes, their characterization, advancement & potential for transdermal application. Journal of Drug Delivery and Therapeutics. 2013 May 13;3(3):147-52.

27. Patel J, Patel R, Khambholja K, Patel N. An overview of phytosomes as an advanced herbal drug delivery system. Asian J Pharm Sci. 2009 Apr;4(6):363-71.
28. Maryana W, Rachmawati H, Mudhakir D. Formation of phytosome containing silymarin using thin layer hydration technique aimed for oral delivery. Mater Today Proc 2016;3:857-8
29. Dhase SA, Saboo SS. Preparation and evaluation of phytosome containing methanolic extract of leaves of Aegle marmelos (Bael). Int J Pharm Technol Res 2015;8(6):232-3.
30. Keerthi B, Pingali SP, Srinivas P. Formulation and evaluation of capsule of Ashwagandha phytosome. Int J Pharm Sci Rev Res 2014;29(2):140.
31. Jan Husch, Janine Bohnet, Gert Fricker, Carsten Skarke, Christian Artaria, et al. (2013) Enhanced absorption of boswellic acids by a lecithin delivery form (Phytosome®) of Boswellia extract. Fitoterapia 84: 89-98.
32. Sikarwar, Mukesh S., et al. "Preparation, characterization and evaluation of marsupsin–phospholipid complex." Aaps Pharmscitech 9 (2008): 129-137.
33. Pathan, Rahila Ahmad, and Uma Bhandari. "Preparation & characterization of embelin–phospholipid complex as effective drug delivery tool." Journal of Inclusion Phenomena and Macrocyclic Chemistry 69 (2011): 139-147.
34. Li, Ying, et al. "Comparative physicochemical characterization of phospholipids complex of puerarin formulated by conventional and supercritical methods." Pharmaceutical research 25 (2008): 563-577.
35. Semalty, Ajay, et al. "Preparation and characterization of phospholipid complexes of naringenin for effective drug delivery." Journal of inclusion phenomena and macrocyclic chemistry 67 (2010): 253-260.
36. Tan Q, Liu S, Chen X, Wu M, Wang H, Yin H, He D, Xiong H, Zhang J. Design and evaluation of a novel evodiamine-phospholipid complex for improved oral bioavailability. Aaps Pharmscitech. 2012 Jun;13:534-47.
37. Cevc G, Schatzlein, Transdermal Drug Carriers: Basic Properties, Optimization and Transfer Efficiency in Case of Epicutaneously Applied Peptides, Journal Control Release, 36, 1995, 3-16.
38. GMM Maghraby E, Williams AC, Barry BW, Oestrodiol Skin Delivery from Ultra deformable Liposomes: Refinement of Surfactant Concentration, International Journal of Pharmacy, 196, 2000, 63-74
39. Kumar A, Kumar B, Singh SK, Kaur B, Singh S. A review on phytosomes: novel approach for herbal phytochemicals. Asian J Pharm Clin Res. 2017;10(10):41-7.
40. Chivte Prajkta, Pardhi Vinal, Joshi Vineeta, Ajitha Rani R, A Review on Therapeutic Applications of Phytosomes; Journal of Drug Delivery and Therapeutics. 2017; 7 (5):17-21
41. Amrita I. Jadhav, Amol A. Wadhav, Vilas A. Arsul, H. S. Sawarkar; Phytosomes: A novel approach in herbal drug delivery system; Int.J.Pharm Anal Vol: 2 Issue:5 Page:478-486
42. Saiki, Hiroshi, and Osamu Maeda. "Cleaning procedures for removal of external deposits from plant samples." Environmental Science & Technology 16.8 (1982): 536-539.
43. Poos, T., and E. Varju. "Drying characteristics of medicinal plants." International Review of Applied Sciences and Engineering 8.1 (2017): 83-91.
44. Koroma, Lahai, Jonathan Kpaka, and Augustine Kpaka. "Organoleptic Evaluation and Physiochemical Characteristics of Powdered Plant Organs of the Traditional Medicinal Plant Caloncoba Echinata." International Journal of Innovation in Engineering 2.3 (2022): 14-22.
45. Nasreen S, Radha R. Assessment of Quality of Withania somnifera Dunal (Solanaceae) Pharmacognostical and physicochemical profile. International Journal of Pharmacy and Pharmaceutical Sciences. 2011; 3(2): 152-155.
46. Vijayan S, Seethalakshmi B, Jayanthi J, Ragunathan MG. Quantitative analysis of physicochemical parameters and estimation of terpenoids in the flower extract of Butea superb (Roxb.). Journal of Science. 2016; 6 (11):497-500.
47. Vinotha S, Ira T, Sri R S. Phyto, Physicochemical Standardization of Medicinal Plant Enicostemma littorale, Blume 2013; 3(2):52-58
48. Nivedithadevi D and Somasundaram R. Pharmacognostical and Qualitative Phytochemical Studies on the aerial parts of Tephrosla purpurea (L). International Journal of Research in Biological Sciences. 2012; 2 (2):48-53.
49. Mukherjee PK. Quality Control of Herbal Drugs, 2nd Edition, Business Horizons, 2007; 2-14.
50. Handa SS, Khanuja SPS, Longo G, Rakesh DD (2008) Extraction Technologies for Medicinal and Aromatic Plants, (1stedn), no. 66. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology.

51. Yung OH, Maskat MY, Wan Mustapha WA (2010) Effect of extraction on polyphenol content, antioxidant activity and pH in pegaga (Centella asiatica). Sains Malaysiana39: 747-752.
52. Ayetree Rai Basumatary, 2016. Preliminary Phytochemical Screening of some compounds from plant stem bark extracts of Tabernaemontana divaricata Linn. used by Bodo Community at Kokrajhar District, Assam, India. Archives of Applied Science Research, 8 (8):47-52.
53. Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and Extraction: A Review. Internationale Pharmaceutica Sciencia. 2011; 1(1):98-106.
54. Auwal MS, Saka S, Mairiga IA, Sanda KA, Shuaibu A and Ibrahim A. Preliminary phytochemical and elemental analysis of aqueous and fractionated pod extracts of Acacia nilotica (Thorn mimosa). Veterinary Research Forum. 2014; 5(2):95-100.
55. Baghel, PS. and Sudip Ray, 2017. Preliminary phytochemical screening of certain aphrodisiac plants used in traditional system of medicine. International Journal of Botany Studies, 2(5): 33-36.
56. Bhatt, M. Gahlot, M. Juyal, V. and Singh, A. 2011. Phytochemical investigation and antidiabetic activity of Adhatoda zeylanica. Asian J Pharm Clin, 4(2):27-30.
57. Laghari AH, Memon S, Nelofar A, Khan KM. Determination of volatile constituents and antimicrobial activity of camel thorn (Alhagi camelorum) flowers. Analytical Letters. 2014 Feb 11;47(3):413-21.
58. Iqbal MO, Manzoor M, Mumtaz A, Riaz R, Arshad S, Khan IA, Javaid U, Manzoor Z, Munawar SH, Andleeb S, Ahmed MM. Evaluation of the hepatoprotective activity of hydroalcoholic extract of Alhagi camelorum against valproic acid-induced hepatotoxicity in rats. Biomedicine & Pharmacotherapy. 2022 Jun 1; 150:112953.
59. Bimala Subba, Anjana Sharma and Anupa Budhathoki, 2016. Assessment of phytochemical content, antioxidant and antibacterial activities of three medicinal plants of Nepal. J. Med. Plants Res, 10(45): 829-837.
60. Geetha, N. and Geetha, TS. 2014. Phytochemical Screening, Quantitative Analysis of Primary and Secondary Metabolites of Cymbopogan citratus (DC) stapf. leaves from Kodaikanal hills, Tamilnadu. Int.J.PharmTech Res, 6(2): 521-529.
61. Joseph, BS. Kumbhare, PH. and Kale, MC. 2013. Preliminary phytochemical screening of selected Medicinal Plants. Int. Res. J. of Science & Engineering, 1(2): 55-62.
62. Kumar, A. and Sunita Patra, 2017. Qualitative and Quantitative Analysis of Secondary Phytochemical In Gymnema Sylvestre. Indian J.Sci.Res. 12 (2): 150-156.
63. Kocabas A. Ease of Phytochemical Extraction and Analysis from Plants. Anatolian Journal of Botany. 2017; 1(2):26-31
64. Subroto, Edy, et al. "The analysis techniques of amino acid and protein in food and agricultural products." Int. J. Sci. Technol. Res 9.10 (2020): 29-36.
65. Emran TB, Mir MN, Rahman A, Zia Uddin, Islam M. Phytochemical, Antimicrobial, Cytotoxic, Analgesic and Anti-inflammatory Properties of Azadirachta indiaca: A Therapeutic Study. Journal of Bioanalysis and Biomedicine. 2015; 12:1-7
66. Geeta Parkhe, Deepak Bharti. Phytochemical investigation and determination of total phenols and flavonoid concentration in leaves extract of Vitex trifolia Linn. Journal of drug delivery & therapeutics. 2019; 9(4-A):705-707.
67. Ainsworth, Elizabeth A., and Kelly M. Gillespie. "Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent." Nature protocols 2.4 (2007): 875-877.
68. Kalita, Pallab, et al. "Estimation of total flavonoids content (TFC) and anti oxidant activities of methanolic whole plant extract of Biophytum sensitivum Linn." Journal of Drug delivery and Therapeutics 3.4 (2013): 33-37.
69. Xia, Hai-jian, et al. "A novel drug–phospholipid complex enriched with micelles: preparation and evaluation in vitro and in vivo." International Journal of Nanomedicine (2013): 545-554.
70. Guo, B.; Liu, H.; Li, Y.; Zhao, J.; Yang, D.; Wang, X.; Zhang, T. Application of phospholipid complex technique to improve the dissolution and pharmacokinetic of probucol by solvent-evaporation and co-grinding methods. Int. J. Pharm. 2014, 474, 50–56.
71. Huang, J.; Chen, P.X.; Rogers, M.A.; Wettig, S.D. Investigating the phospholipid effect on the bioaccessibility of rosmarinic acid-phospholipid complex through a dynamic gastrointestinal in vitro model. Pharmaceutics 2019, 11, 156.
72. Ruan, J.; Liu, J.; Zhu, D.; Gong, T.; Yang, F.; Hao, X.; Zhang, Z. Preparation and evaluation of self-nanoemulsified drug delivery systems (SNEDDSs) of matrine based on drug–phospholipid complex

technique. Int. J. Pharm. 2010, 386, 282–290.
73. P. Costa and J. M. Sousa Lobo, “Modeling and comparison of dissolution profiles,” Eur. J. Pharm. Sci., vol. 13, no. 2, pp. 123–133, 2001.
74. Paarakh, M. Padmaa, et al. "Release kinetics–concepts and applications." International Journal of Pharmacy Research & Technology (IJPRT) 8.1 (2018): 12-20.
75. Dash S, Murthy PN, Nath L, Chowdhury P. Kinetic modeling on drug release from controlled drug delivery systems. Acta Pol Pharm. 2010 May 1;67(3):217-23.
76. Talevi, Alan, and María Esperanza Ruiz. "Korsmeyer-Peppas, Peppas-Sahlin, and Brazel-Peppas: Models of Drug Release." The ADME Encyclopedia: A Comprehensive Guide on Biopharmacy and Pharmacokinetics. Cham: Springer International Publishing, 2022. 613-621.
77. Aljarah, Ali Kadhim, and Imad Hadi Hameed. "In vitro anti-diabetic properties of Methanolic extract of Thymus vulgaris using α-glucosidase and α-amylase inhibition assay and determination of its bioactive chemical compounds." Indian Journal of Public Health Research and Development 9.3 (2018): 388-392.
78. Saoji, Suprit D., et al. "The study of the influence of formulation and process variables on the functional attributes of simvastatin–phospholipid complex." Journal of Pharmaceutical Innovation 11 (2016): 264-278.
79. Dong, Wujun, et al. "Self-assembled lecithin/chitosan nanoparticles based on phospholipid complex: a feasible strategy to improve entrapment efficiency and transdermal delivery of poorly lipophilic drug." International Journal of Nanomedicine (2020): 5629-5643.