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Research Article

Green Synthesis of Liposomes of *Oxalis corniculata* Linn. for Bioavailability Enhancement

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Abstract

Liposomes are spherical vesicles composed of cholesterol or phospholipids and resemble to cell wall. They are used as nano-drug delivery vehicle for drug administration. Extract loaded liposomes of *Oxalis corniculata* Linn. (ELL) were studied for microscopic features, evaluated and characterized. The *in-vitro* drug release profile showed initial burst (< 10% in 1 hour) followed by consistent release (> 90 % in 24 hours).
Keywords: Liposomes, NDDS, *Oxalis corniculata* Linn, Bioavailability enhancement, Phospolipid.

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1. Introduction

Most of plant extracts show poor lipid solubility and low absorption through biological membranes, hence becomes the major regulating factor for their bioavailability. Since, this is only the un-metabolized drug which is responsible for therapeutic action, development of a new drug delivery system which offer a carrier of desired characteristics and hence deliver maximum drug in the controlled manner could be a solution. Liposomal encapsulation of a medication can completely change the pharmacokinetic properties of a drug, hence can target the medication to specific organs and increase the bioavailability as well as the efficacy of the encapsulated drug.

Oxalis corniculata L. or Changeri elevates kapha and vata and is a good appetizer which can also cure many diseases. The major phyto-constituents present in the plant are hydrophilic in nature. Microscopic vesicle are bilayer of phospholipid or amphipathic lipids like phosphatidyl choline which encapsulates compound effectively and delivers hydrophilic and lipophillic substance [1-2]. Pharmacokinetics of drugs are completely changed by liposomal encapsulation which increases bioavailability, efficacy of the drug at target organs [3].

Literature revealed the importance of plant in Ayurveda. Most of the preparations are lipid based like, Changeri Ghrita (Gavyamrut, Planet Ayurveda, Parmanand Ayurveda etc). Hence, this is a small step to dispense the plant in a novel formulation for medicinal use.

2. Material & Method

Leaves of OCL were collected from local area, identified by comparison with in-house herbarium (A.No.8931) and confirmed on the basis of morphological and microscopic characteristics. All the chemicals used were of laboratory/analytical grade. Collected leaves were extracted with methanol by maceration and the extract was stored in air tight container until. Physicochemical, phytochemical and quantitative phytochemical analysis of extract was performed followed by formulation of liposomes.

In-vitro antioxidant activity, anti-inflammatory & protein binding assay, anti-diabetic assay of the extract was performed using standard protocol. Further the extract was used for preparation of liposomes. Liposomes loaded with ME of OCL i.e. ELL were formulated by dehydration and rehydration technique. Small polymeric particles with slight odour were produced and characterized on the basis of their shape, size, lamellarity, *In-vitro* skin permeation and *In-vitro* drug release study.

3. Results & Discussion

3.1 Physicochemical Parameters

The observed pH value for the sample of OCL was 4.70±0.12. It is well documented that pH is a significant factor in the medicinal activities of phytoconstituents. Furthermore, the pH values from 4 to 7 exhibited a relatively high antioxidant activity. Total ash value recorded for the OCL was 9.17±1.14 %., means that the mineral content are higher. The total ash is a measure of purity which represents physiological and non- physiological index. Physiological ash of the plant denotes to biochemical make-up while non-physiological ash denotes contaminants from environments, such as carbonates, phosphates, nitrates, sulphates, chlorides and silicates of various metals which were pulled up from the soil. The observed acid insoluble ash of OCL was 3.87±0.47% and LOD was 0124±0.05 %. The observed water and alcohol extractive values for were 25.71±2.05 % and 8.33±1.05 % respectively; this suggests the presence of polar compounds in the plant under study.

3.2 Qualitative Analysis of Phytoconstituents

Qualitative phytochemical tests were performed on ME of OCL to check the presence or absence of important phytochemocal including protein, glycosides, flavonoids, terpenoids, amino acid, alkaloids, phenols, carbohydrates, saponins, tannins, and steroids. In the ME of OCL, Carbohydrates, alkaloids, phenols, flavonoids, tannins and steroids were observed (Table: 1).

Phytoconstituents are biologically active compounds responsible for various activities of that plant, like antioxidant, antimicrobial, antifungal anticancer and hence could be used in the treatment of various ailments. Plants have two types of metabolites primary and secondary. Primary metabolites are synthesized from simple element and are necessary for vitality of plant, while secondary metabolites are the by-products of primary metabolites utilization. They are less used by plants themselves, hence remains stored. These secondary metabolites are very active therapeutically on animals and hence are used in the treatment of various diseases of humans as well as other animals. [4].

OCL contains tannins, mixture of 8 oleic, linoleic, linoleic and stearic acid and palmitic acid. Tartaric, citric acid and malic acid present in stem whereas citric and tartaric acid present in leaves [5-7]. Flavonoids, phenols, glycosides, carbohydrates, phytosterols, protein, amino acids and volatile oil are available in methanolic and ethanolic extracts of OCL. [8].

Table 1: Qualitative analysis of phytochemical present in the ME of OCL

Phytoconstituents	Present/Absent
Glycosides	+
Terpenoids	+
Protein	-
Amino acids	-
Alkaloids	+
Carbohydrates	+
Flavonoids	+
Phenols	+
Saponnins	+
Steroids	+
Tannins	+

Above phytoconstituents have shown various pharmacological activities like, cardiotonic, antibacterial and insecticidal activities etc. Saponins from some plants have shown antidiabetic and hypochlesterolemic activities, whereas triterpenoids from some plants are used as anticancer and analgesics [4].

3.3 *In-vitro* Antioxidant Activity

Total antioxidant capacity of ME of plant was determined using the Phosphomolybdenum Assay with reference to standards. The observed antioxidant activity (at 50 µL concentration) for BHT (standards) and ME was 97.31±4.51 and 94.55±3.27 % respectively, which supports very good total antioxidant capacity of ME of OCL.

Through test sample the bleaching of DPPH absorption represent its capacity to scavenge free radicals, and transition metal based system [7]. In DPPH assay, concentration depended effect was noticed (Figure-1). It has noticed that ME is more effective at 100 µL or may at higher concentration(s). The observed IC_{50} values of the ME and standards (ascorbic acid, AA; and butylated hydroxyl toluene, BHT) were 56.71±2.18, 48.49±2.42 (AA), and 22.93±2.49 % (BHT) mg/ml respectively.

Metal chelating scavenging activity is one of the most significant parts of antioxidant assay. The observed values of MC activity for sample and EDTA (control) were checked at different concentrations (25, 50, 75, and 100 µL). The effect was found to be concentration depended, where inhibition increased from 25 to 100 µL similarly as of standard. The ME has shown highest activity at 100 µL with values 53.35±3.94 % as compared to 95.32±5.88% of standard (EDTA). The values were found to be significantly different and the low value for ME may be attributed to the presence of chelating agents with multiple metal ions within (figure-1). The observed IC_{50} values of the ME and EDTA were 84.58±5.32 and 51.21±2.61 mg/ml respectively.

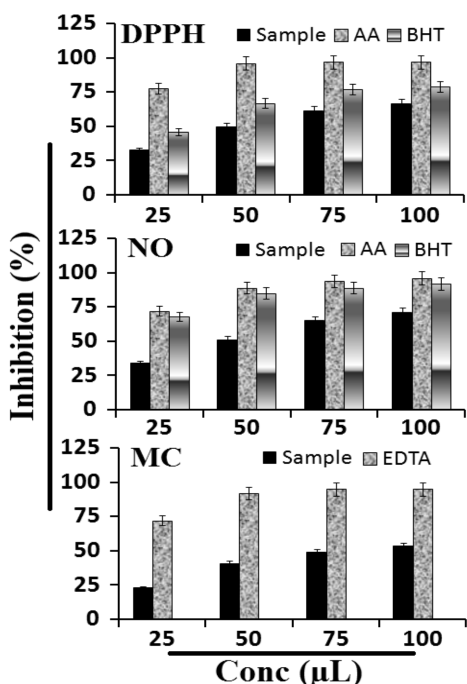


Figure 1: Antioxidant studies (DPPH, NO, and MC) of ME. Value are expressed as mean ±SD (n =).

Nitric oxide (NO) play important role in different inflammatory process but their sustained productions are toxic to tissues and contribute to vascular collapse associated with septic shock. Chronic use of NO causes carcinomas and various inflammatory conditions including ulcerative colitis, multiple sclerosis, arthritis and diabetes [9-10].

The observed values NO activity for samples and standard was checked at four concentrations (25, 50, 75, and 100 µL). It was noticed that ME and standards (AA and BHT) have similar trend which was concentration dependent, and maximum values were noticed at maximum concentration i.e.at 100 µL. The observed highest activity at 100 µL with values 71.31±6.48, 98.44±5.11 and 92.12±3.74 % for the ME, ascorbic acid, and BHT. The observed IC_{50} values for the ME, ascorbic acid, and BHT was 52.16±3.22, 51.33±2.22 and 58.92±4.14 mg/ml respectively.

Antioxidant is one of the most powerful anti-aging, lifesaving and most importantly anticancer substances. Before vital molecules are damaged antioxidants securely interact with free radical and terminate the oxygen chain reaction. Antioxidants are available in various types of fruits and plant materials. Vitamin C, vitamin B_3 and vitamin A are highly found in leaves of OCL and have antioxidant, anti-inflammatory, refrigerant and anti-scorbutic [11-12].

3.3.1 Total Phenol and Flavonoids Contents

To calculate the TPC (GAE)/g and TFC (QE)/g, standard curves of gallic acid and quercetin was plotted by taking concentration on X-axis and absorbance at Y-axis. The observed equation of line for TPC and TFC was Y = 0.009X + 0.005 with R^2 = 0.999 and Y = 0.011X + 0.028 with R^2 = 0.998 respectively. In case of ME, the observed value of TPC and TFC was 46.94±2.11 mg GAE/µg and 28.24±1.33 mg QE/µg respectively. To treat various disease pharmacists mainly target the plant with high phenolic content and these high amount of phenolic content determines the ability of plant to treat inflammatory disease and can be used to improve wound healing [4,9,13,14].

The correlation between antioxidant activities to TPC and TFC of ME was analysis to analyze by simple linear correlation. Phenols and flavonoids compounds as reducing agents had strong positive relationships indicated the role of reducing agents thus contributing to antioxidant activity.

3.3.2 Anti-Inflammatory & Protein Binding Assay

Proteins denaturation by heat is the major cause inflammation. In present study (at 50 µL), as compared to control (acetyl salicylic acid), ME was ~ 4 times ((21.88±1.35) less effective than standard (95.12±4.35). Hence, the ME is more effective against the heat induced recovery of BSA protein, but not up to the good level.

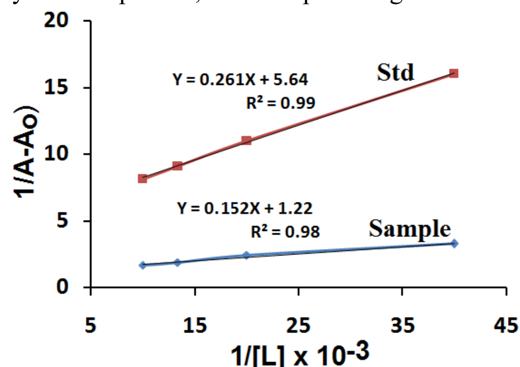


Figure-2: Quantitative analysis of rate constants in protein (BSA) binding study.

In BSA protein binding experiments, slight increase in the concentrations protein resulted in an increase in light absorption and shifting of BSA band a little higher end (295 nm) This explains the complex formation. The average values of protein binding constants (k) for the standard (acetyl salicylic acid), and ME was $2.60\pm0.05\times10^{-4}$ μM^{-1}, and $1.52\pm0.04\times10^{-4}$ μM^{-1} respectively. Results suggest that standard and ME have almost similar interactions with BSA protein. As compared to standard HI have almost half value of binding constant indicated the lesser interactions than standard. Generally, strong interactions of any medicine with proteins with binding constant (k) ranging from 10^6 -10^{11} M^{-1} is not a good thing for the proper drug delivery.

3.4 Anti-Diabetic Assay

The α-amylase inhibition different concentrations of ME were determined as compared to Acarbose as a standard. The ME has shown very good activity in all concentrations and it was found to be concentration dependent (Figure-2). Observed values were comparable with standard acarbose.

The observed IC_{50} values of the ME and Acarbose was 97.51±8.33 and 54.62±3.51 mg/ml respectively.

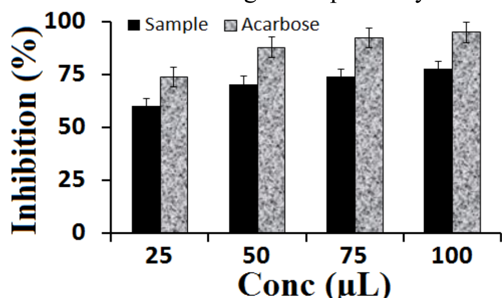
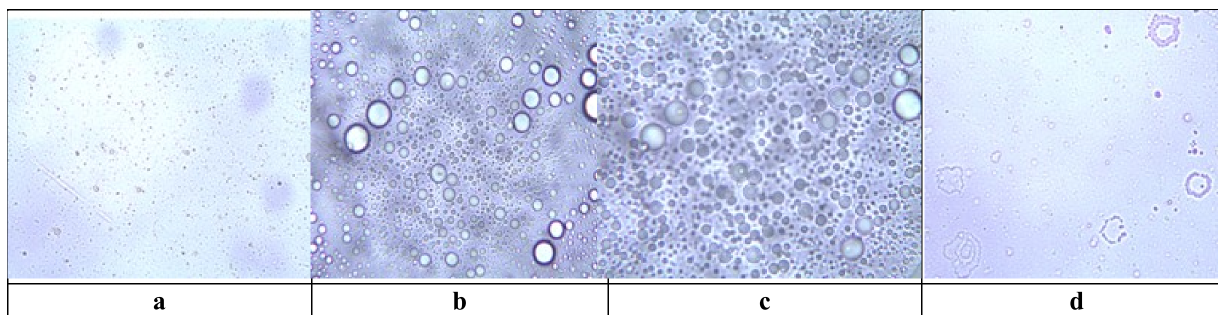


Figure-3: Antidiabetic studies of ME and Acarbose [expressed as mean ± SD (n = 3)].

Diabetes mellitus (increase in glucose level in blood which is not absorbed by the body) is a non-communicable disease mostly hereditical in nature but sometimes may occur due to disturbed the life style. It is a complex disorder caused by raised hepatic glucose production, impaired insulin action production or suppression of Alpha-amylase enzyme. Maintenance of blood glucose level of diabetic patients could prevent further associated complications. [15-18]. Allopathic system of medicine does not cure the disease completely, however, can control up to a significant extent. These medicines are expensive and some may have side effects on lifelong administration [15-25]. Herbs and medicinal plants possessing anti-diabetic properties could be used as effective substitute. Approximately forty seven plants belonging to 29 different families has been review by Benalla *et al* (2010) with alpha glucosidase inhibition excluding OCL [18]. In the present study ME of OCL at varying concentrations was subjected to determine anti-diabetic activity *in-vitro* using yeast as model [24] and % increase in glucose uptake in yeast cells by ME of OCL was determined as compared to standard and a proportional relationship was observed. The highest percentage of inhibition is 77.94±3.25 % (at 100 µL), which was in very good range as compared to standard (97.11±3.66 %) (Figure-3). This significant anti-diabetic activity was comparable to the standard drug inhibition. In most of the cases proper management of blood glucose level could be effective in the prevention of associated complication. These *in-vitro* results were promising but could be validated *in-vivo*. [20-24]

3.5 Liposomes and Characterization

Liposomes loaded with ME of OCL were formulated by dehydration and rehydration technique. Small polymeric particles with slight odour were produced and characterized on the basis of their shape, size, lamellarity, *In-vitro* skin permeation and *In-vitro* drug release study.



a	b	c	d

Figure-4: Microscopic images of liposomes (a-100x, b,c-400x, d-1000x).

Methanolic extract was obtained was a semisolid mass with dark green color, characteristic odour and sour herbaceous taste. The unsonicated liposomal formulation (ELL) was an opaque liquid.

ELL vesicles were visualized using Binocular microscope (LMI, BM-400). A drop of unsonicated liposomal formulation was put on glass slide covered with cover slip, sealed and examined at magnification 100x, 400x and 1000x. The Binocular microscopic images are shown in Figure - 6. Unilammelar liposomes were seen to be dispersed with opaque color, spherical in shape with an average size of 0.57 µ and size distribution between 0.25- 90 µ (Figure-4).

3.6 Drug Release Study of Liposomes

Spectrophotometric analysis was done at 294 nm. The absorbance (i.e. concentration) of test solutions was found to be proportional with time with very slow release initially during first hour (Figure-5).

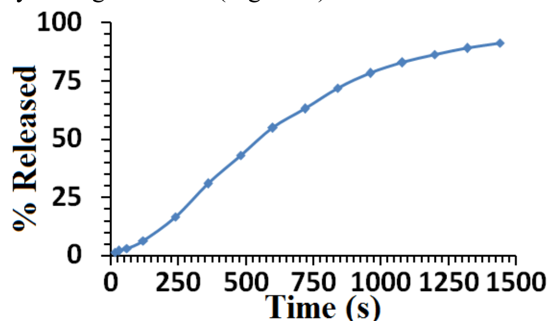


Figure-5: Kinetics of drug release study of liposomes.

The data of drug release study showed that the percentage of drug released after 4 hours is 17.88 % which indicates sustained release. After first hour rapid and linear

release was noticed till the end of a day (24 hrs.) (Figure-6). Drug loading efficiency and *in-vitro* drug release are important factor for such type of loaded dosage forms because it affects the extent of total drug delivered to the site of action. [26-27]

Novel dosage form development is a result of customized and précised physical, chemical, microbial and stability studies, and so for the liposomes too. Fusion and breakage of liposomes on storage causes critical problems that lead to drug leakage from the vesicles, hence visual inspection and size distribution are important parameters for evaluation. [28].

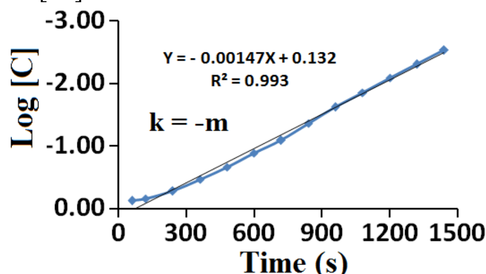


Figure-6: Kinetics of drug release study of liposomes.

To find out the rate constant (k), various kinetic plots (zero, first and second order) were plotted. Data was found linear with first order kinetics. The rate constant was calculated from the equation of line, Y = - 0.00147X + 0.132 by plotting Log [C] vs Time (s). The value of rate constant was 6.11×10^{-3} s^{-1}. First order kinetics is well documented for sustained release.

Liposomes are microscopic spherical vesicles of phospholipid bilayer which encapsulate a variety of compounds effectively and delivers hydrophilic and lipophillic substance, while are non-toxic at the same time. In addition, liposome properties differ with respect to lipid arrangement, particle size, surface charge and the method of liposome preparation, the rigidity or fluidity and the charge of the bilayer were by the choice of bilayer components for instance, saturated or saturated and unsaturated phosphatidyl choline [29-31]. These liposomes can be used to deliver drug inside desired therapeutic range to abnormal cells without influencing normal cells and hence lesser adverse effects [32-38]

4. Conclusion

The current piece of research deals with the fabrication of liposomes loaded with methanolic extract of *Oxalis corniculata* Linn. (OCL). Liposomes were prepared by thin film dehydration and rehydration method. Unilammelar tiny liposomes were seen dispersed with opaque color, spherical in shape with an average size of 0.57 μ and size distribution between 0.25- 90 μ.

The *in-vitro* drug release profile showed initial burst (< 10% in 1 hour) followed by consistent release (> 90 % in 24 hours).

The aim of the formulation of liposomes loaded with methanolic extract of OCL was achieved. As mentioned above the drug/ extract was found to be released from liposomes satisfactorily, hence a better way could be designed with liposomal formulations of hydrophilic drugs/ extracts for effective administration with optimum bioavailability and minimum possible dose. Moreover, the sustained release characteristics of the liposomes will lead to the design of a précised dose regimen.

When compared with the conventional dosage forms novel drug delivery systems offer more patient compliance and palatability. The prepared formulation has these properties. Further the liposomes could be incorporated into a gel or could be converted into any other dosage forms as required.

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