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Research Article

DEVELOPMENT AND *IN VITRO* EVALUATION OF PHYTOSOMES OF ELLAGIC ACID

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ABSTRACT

Objectives: The main objective of the present work is to enhance the bioavailability of ellagic acid (EA) by increasing its dissolution there by allowing for the exploitation of its therapeutic effects.

Methods: Phytosomes containing EA were prepared by anti-solvent precipitation method. The prepared phytosomes were evaluated for drug entrapment efficiency, *in vitro* drug release, and drug excipient interaction studies.

Results: Formulation F2 containing EA and soya lecithin in the ratio (1:2) showed highest percentage of drug release as 85.40% in 60 min and 95.86% in 120 min. The drug entrapment efficiency values were satisfactory. There were no interactions between the drug and the excipients used in its preparation according to Fourier-transform infrared spectra of pure EA and EA phytosomes.

Conclusion: Phytosomes of EA were successfully produced by anti-solvent precipitation method and the percentage drug entrapment efficiency was satisfactory in almost all formulations. Formulation F2 exhibited highest percent of drug release as 85.40% in 60 min and 95.86% in 120 min to possess optimum bioavailability.

Keywords: Phytosomes, Bioavailability, Anti-solvent precipitation, Dissolution.

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INTRODUCTION

Ellagic acid (EA), a polyphenolic compound belonging to the family of ellagitannins, chemically named as per IUPAC as 6,7,13,14 – tetrahydroxy – 2,9 – dioxatetracyclo hexadeca 1,4,6,8,11,13 hexane – 3,10 – dione is an organic hetero tetracyclic compound [1]. It exhibits lipophilic nature due to 6-member hydrocarbon rings and hydroxyl groups; lactone rings are responsible for its hydrophilic property [2]. Natural sources of EA include pomegranates, berries such as blackberries, blackcurrants, raspberries, strawberries, grapes, and dried fruits such as walnuts and almonds [2].

EA has been reported to show a wide variety of pharmacological activities such as antioxidant, antimalarial, anti-inflammatory, antifibrogenic, and antiviral. It also exhibits cytotoxic, anticancer, and antitumor effects [3,4]. EA shows significant neuro [5] and cardioprotective effects [6]. Wound healing activity has also been reported in studies [7]. Although EA has various pharmacological activities, its oral bioavailability and short plasma half-life are significant limitations. Individual intake from dietary sources is reported to be as low as 343 mg/year, which is insufficient to achieve the plasma concentration required for eliciting pharmacological action [8]. EA is available commercially in form of pomegranate extract. However, these extracts could not overcome the pharmacokinetic limitations of the EA and its bioavailability issues were not focused [9]. In recent years, several strategies based on the nanoparticle approach have been employed to enhance the bioavailability of EA. Polysaccharide-based systems, polyester-based systems, PEG acrylate-based systems, and molecular dispersion in polymer matrices were studied [10-12].

Phytosomes also known as herbosomes are novel vesicular drug delivery systems that are considered promising drug delivery candidates for the delivery of phytochemicals which are having poor solubility [13]. Phytosomes are complex of phospholipids and phytochemicals. The phospholipids that are used in the preparation of phytosomes have an affinity for polyphenols. The nitrate or phosphate group of the former interact with the hydroxyl groups of the latter and also the formation of hydrogen bond and hydrophobic interactions between the two has also been reported [13,14]. There are no formulations of EA as phytosomes, according to the literature. Therefore, the aim of the current research is to prepare phytosomes of EA with enhanced dissolution properties, which would help to overcome pharmacokinetic and bioavailability constraints.

METHODS

EA was procured from Marigold bio extracts private limited, Warangal, Telangana, India. Soya lecithin was purchased from Shiva Biochem Industries, Amaravati, Maharashtra, India. Dichloromethane was obtained from Siri chemicals, Rajahmundry, Andhra Pradesh, India and all other chemicals used were of analytical grade.

Preparation of phytosomes

Phytosomes of EA were prepared by anti-solvent precipitation method. Different ratios of EA and soya lecithin, namely, 1:1, 1:2, 1:3, 1:4, 2:1, 2:3, 3:1, 3:2, and 3:4 were taken in the compositions, as shown in Table 1. In a 100 mL round bottom flask, EA and soy lecithin were placed, and 60 mL of dichloromethane was added which was refluxed for 2 h. To obtain the complex as a precipitate, 60 mL of n-hexane was added after the solution had been concentrated to 5–10 mL. The precipitate was collected and stored for 24 h in a desiccator. Through #100 mesh, the dried precipitate was sieved. Powdered complex was stored in ambered colored bottles [15].

In vitro evaluation of prepared phytosomes

Drug entrapment efficiency

The percentage of drug entrapment efficiency was determined for all the EA phytosomes prepared (F1–F9). 100 mg of the product was weighed and transferred into 100 mL volumetric flask containing 100 mL of phosphate buffer (pH=7.4) and was kept aside. After 24 h, the flask's contents were stirred continuously for 2 h at 35°C. The solution was then filtered using a Whatman filter paper, and 1 mL of the

Table 1: Composition of ellagic acid phytosomes

Formulation	Drug EA (mg)	Lipid (soya lecithin) (mg)	Solvent (dichloromethane) (mL)	Anti-solvent (n-hexane) (mL)
F1 (1:1)	1000	1000	60	60
F2 (1:2)	1000	2000	60	60
F3 (1:3)	1000	3000	60	60
F4 (1:4)	1000	4000	60	60
F5 (2:1)	2000	1000	60	60
F6 (2:3)	2000	3000	60	60
F7 (3:1)	3000	1000	60	60
F8 (3:2)	3000	2000	60	60
F9 (3:4)	3000	4000	60	60

EA: Ellagic acid

sample was diluted to 10 mL before being tested for drug entrapment efficiency using a UV visible spectrophotometer (LABINDIA UV 1700) at a wavelength of 254 nm. Drug entrapment efficiency was calculated using the following formula [15].

$$\text{Percentage drug entrapment efficency}=\frac{\text{Actual drug content}}{\text{Theoretical content}}\times 100$$

In vitro drug release studies

In vitro drug release studies were carried out for pure EA and also for prepared EA phytosomes using USP type II dissolution apparatus (LABINDIA DISSO 8000). The dissolution was performed in 900 mL of phosphate buffer pH 7.4 at 37±0.5°C temperature and 100 rpm. Phytosomes equivalent to 100 mg of EA was placed in the medium. 5 mL of dissolution fluid was withdrawn through a filter at various time intervals (5, 10, 15, 30, 45, 60, 90, and 120 min) over a period of 2 h. Every time equal volume of fresh medium was replaced and the sample which was withdrawn was suitably diluted and analyzed using UV visible spectrophotometer (LABINDIA UV 1700) at 254 nm.

Assessment of drug release kinetics

The order of drug release from the phytosomes was determined by fitting the data into zero-order kinetics ($Q= K_0t$) and first-order kinetics ($\log Q = \log Q_0 + K_t t/2.303$). The mechanism of drug release was studied from Higuchi model ($Q =KHt^{1/2}$) and Korsmeyer–peppas model ($F = (M_t/M)= Kt^n$) [16].

Fourier-transform infrared spectroscopy (FTIR) of EA phytosomes

FTIR was used to investigate the drug excipient interaction between EA and other ingredients used to prepare the phytosomes. FTIR spectrum of pure EA and also of the phytosomes prepared was obtained in transmittance mode in the range of 400–4000 cm^{-1}.

RESULTS

Phytosomes of EA were successfully prepared by anti-solvent precipitation method. A total of 9 formulations, taking different concentrations of EA and soya lecithin, were prepared. All the formulations prepared were subjected to *in vitro* evaluation of percentage entrapment efficiency, drug release studies, and release kinetics. The promising formulation of the nine formulations was further studied for drug-excipient interaction by FTIR.

Drug entrapment efficiency

The drug entrapment efficiency values of the prepared phytosomes of EA (F1–F9) are shown in Table 2.

In vitro drug release study

The *in vitro* release studies of EA from the phytosomes were studied in phosphate buffer pH 7.4. The results are shown in Fig. 1 and Table 3. The drug release studies were performed in triplicate to minimize the errors. Pure EA evidenced only 15.70% in 120 min, indicating poor solubility of EA. Of all the formulations of phytosomes, formulation F2 (1:2 EA: Soya lecithin) showed a 95.86% drug release at the end of 120 min.

Table 2: Entrapment efficiency of ellagic acid phytosomes

Formulation code	Percentage entrapment efficiency (mg)*
F1 (1:1)	80.04±0.47
F2 (1:2)	93.03±0.76
F3 (1:3)	79.55±0.61
F4 (1:4)	80.18±0.29
F5 (2:1)	86.14±1.59
F6 (2:3)	89.54±0.89
F7 (3:1)	92.53±0.69
F8 (3:2)	82.80±0.47
F9 (3:4)	75.37±0.88

*Mean±SD. SD: Standard deviation

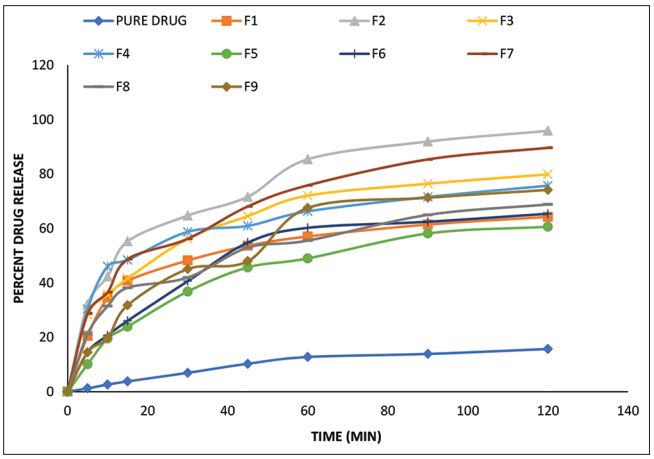

Fig. 1: Dissolution profiles of pure ellagic acid and the formulations prepared

Drug release kinetics of EA phytosomes

The promising formulation (F2) drug release kinetics were evaluated by plotting zero-order, first-order, Higuchi, and Korsmeyer–Peppas plot. The results of analysis as per various kinetic models are given in Table 4 and Fig. 2. A comparison of correlation coefficient (R^2) value of zero- and first-order indicated the drug is released by first-order kinetics as the R^2 value is highest in the first order (0.9833). The Higuchi plot (% cumulative drug release vs. √time) was found to be linear with correlation coefficient value of 0.979 indicating diffusion as the mechanism of drug release. The release exponent value (n) 0.6592 obtained from Korsmeyer–peppas plot (>0.45 and <2) indicated non-fickian (anomalous) diffusion as drug release mechanism. Correlation coefficient values of the plots are summarized in Table 5.

FTIR studies of EA

Fourier-transform infrared spectra (FTIR) of pure EA and promising formulation of EA phytosomes (F2) are shown in Figs. 3 and 4, respectively. FTIR spectra of EA showed its characteristic peak at 3556.9 cm^{-1} due to OH stretching, conjugated ketone (C=O) stretching at 1694.83 cm^{-1}, aromatic ring C-C stretching at 1510.37 cm^{-1}, aromatic

Table 3: Percentage drug dissolved versus time values of pure ellagic acid and ellagic acid phytosomes

Time (min)	Percentage drug release				
	Pure EA	**F1**	**F2**	**F3**	**F4**
5	01.23±0.88	20.53±1.20	32.13±0.74	28.40±0.61	30.38±0.55
10	02.62±0.37	34.62±1.86	42.35±0.36	35.60±0.61	46.07±0.13
15	03.80±0.39	40.80±1.55	55.25±0.21	41.58±0.38	48.40±0.55
30	06.94±0.35	48.22±0.98	64.71±0.47	56.24±0.23	58.74±0.45
45	10.27±0.44	53.62±1.62	71.63±0.34	64.51±0.71	60.94±0.78
60	12.75±0.24	57.03±2.33	85.40±0.34	72.05±0.06	66.26±0.26
90	13.88±0.67	61.43±1.64	91.91±0.22	76.43±0.43	71.55±0.49
120	15.70±0.42	64.25±1.14	95.86±0.71	79.85±0.62	75.72±1.04
Time (min)	**Percentage drug release**				
	F5	**F6**	**F7**	**F8**	**F9**
5	10.09±0.14	14.51±0.48	28.70±0.34	21.24±0.31	14.50±0.51
10	19.57±0.40	20.68±0.58	36.55±0.55	31.45±0.57	19.70±0.27
15	23.91±0.24	26.07±0.25	48.73±0.63	38.19±0.81	31.78±0.24
30	36.81±0.52	40.62±0.41	56.18±0.14	41.94±0.33	45.06±0.21
45	45.67±0.57	54.77±0.49	68.07±0.45	52.94±0.71	47.96±0.28
60	48.98±0.26	60.22±0.32	75.83±0.54	55.53±0.42	67.53±0.44
90	58.13±0.18	62.44±0.41	85.35±0.42	64.96±0.63	71.26±0.28
120	60.62±0.58	65.38±0.46	89.63±0.54	68.82±0.58	74.15±1.02

EA: Ellagic acid

Table 4: Data for drug release kinetics plots

Time (min)	Percentage cumulative drug release	Log percentage cumulative drug release	Percentage drug remained	Log percentage drug remained	Log time	√time
0	0	0.000	100	2.00	0.000	0
5	32.13	1.507	67.87	1.83	0.699	2.236
10	42.35	1.627	57.65	1.76	1.000	3.162
15	55.25	1.742	44.75	1.65	1.176	3.873
30	64.71	1.811	35.29	1.55	1.477	5.477
45	71.63	1.855	28.37	1.45	1.653	6.708
60	85.4	1.931	14.6	1.16	1.778	7.746
90	91.91	1.963	8.09	0.91	1.954	9.487
120	95.86	1.982	4.14	0.62	2.079	10.954

√: Square root

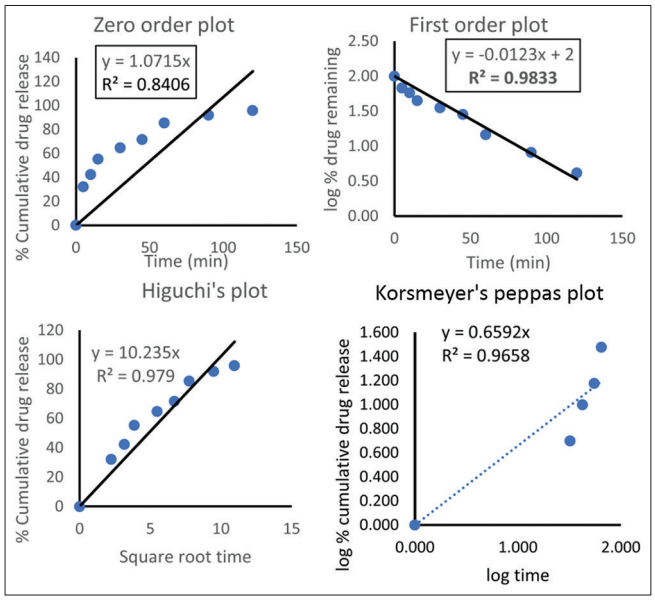

Fig. 2: Kinetic plots of drug release from ellagic acid phytosomes (F2)

C-H bending at 811.24 cm^{-1}, and ortho disubstituted aromatic SP^2 C-H bending at 755.10 cm^{-1}. Phytosomes of formulation F2 revealed OH stretching at 3556.65 cm^{-1}, conjugated ketone stretching at 1695.15 cm^{-1}, aromatic ring C-C stretching at 1510.18 cm^{-1}, aromatic C-H bending at 810.70 cm^{-1}, and ortho disubstituted aromatic SP^2 C-H bending at 754.19 cm^{-1}.

DISCUSSION

Phytosomes of EA were successfully prepared by anti-solvent precipitation method. A total of 9 formulations (F1–F9) were prepared by taking different ratios of EA and soya lecithin. The percentage entrapment efficiency was satisfactory in almost all formulations. The formulations F2 and F7 gave the better values compared to other formulations. The percentage entrapment efficiency was in the range 79.55–93.03%. The highest entrapment efficiency of 93.03% was found in formulation F2 prepared by taking EA and soya lecithin in the ratio (1:2). The dissolution was performed for the pure EA and all the nine formulations prepared. An enhanced dissolution rate of drug was observed in the phytosomes formulated. Formulation F2 showed a highest drug release of 85.40% in 60 min and 95.86% release in 120 min. Hence, the F2 formulation was further investigated for drug release kinetics. A comparison of correlation coefficient (R^2) value of zero- and first-order indicated that the drug is released by first order kinetics as the R^2 value is highest in the first order (0.9833) indicating that the release is dose dependent. The Higuchi plot (% cumulative drug release vs. √time) was found to be linear with correlation coefficient value of 0.979 indicating diffusion as the mechanism of drug release. The release exponent value (n) 0.6592 obtained from Korsmeyer–Peppas plot (>0.45 and <2) indicated non-fickian (anomalous) diffusion

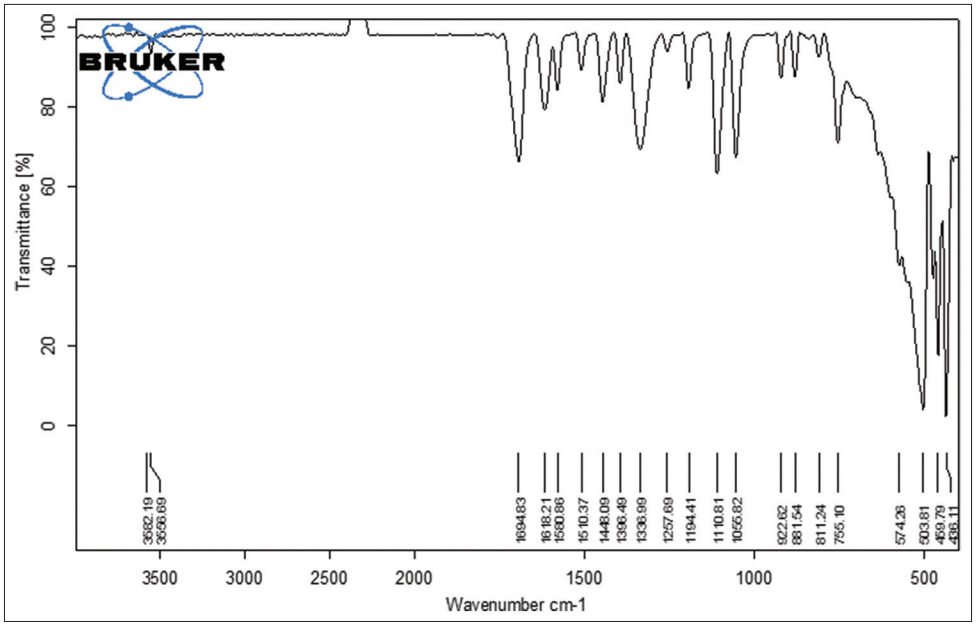

Fig. 3: Fourier-transform infrared spectroscopy spectrum of pure ellagic acid

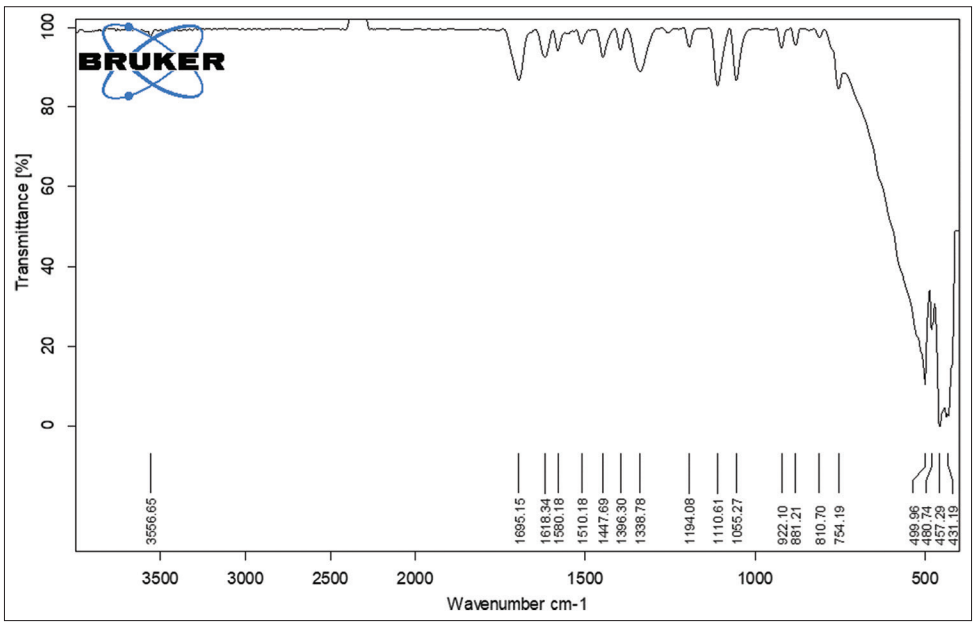

Fig. 4. Fourier-transform infrared spectroscopy spectrum of phytosomes formulation F2

Table 5: Correlation coefficient, R^2 values

Plot	R^2
Zero order	0.8406
First order	0.9833
Higuchi	0.9790
Korsmeyer's peppas	0.9658 (n=0.6592)

as drug release mechanism. In the FTIR study, there was a very minute shift in the peaks observed indicated that there is no interaction between the drug and polymers/excipients used.

CONCLUSION

Phytosomes of EA were successfully produced by anti-solvent precipitation method and the percentage drug entrapment efficiency was satisfactory in almost all formulations. From *in vitro* drug release study, formulation F2, prepared by taking 1000 mg of EA and 2000 mg of Soya lecithin exhibited highest percent of drug release as 85.40% in 60 min and 95.86% in 120 min to possess optimum bioavailability.

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AUTHORS' CONTRIBUTIONS

Present research work is carried out under the guidance of Jeevana Jyothi. B and is responsible for the outcome of this novel work as well

as preparation of manuscript. Ramya. K has done the experiments involved in the research.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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