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In-vitro studies of bioactive nanoemulgel from agro-waste and mathematical modeling of drug release

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A comparison of the bioactive components present in the 1:16 (% w/v) ethanol extracts of rinds of *Citrus limetta*, *Punica granatum* and leaves of *Musa acuminata* have been carried out. The leaf extract of *Musa acuminata* revealed the presence of about 88% of components with antimicrobial activities. The current work focuses on a comparative study of fitness of empirical (Zero, First, Higuchi) and semi empirical (Ritger-Peppas) mathematical models to describe the drug release mechanism of the formulated nanoemulgel. In-vitro studies are carried out, of which B1 posed 96% of cumulative drug release. The results revealed that Higuchi model fitted the best for the optimized formulation of B1, B2, and B3 with higher regression coefficient (R^2 values of 0.99). The formulation followed the Fickian type of release for the bioactive component, which can be used for treating the fungal infection.

Keywords: Agro-wastes, Bioactive component, Drug delivery, Release mechanism

Introduction

Skin, the largest organ of the human body, covers the entire body, serves as the primary interface between body and environment. It acts as a line of defence against microbial invasion and environmental stress[1,2]. A site-specific action of the drug is made possible through a topical delivery system. The duration of the narrow therapeutic drug action can be increased through the topical mode of delivery. Currently, the major challenge faced in modern drug delivery system is poor aqueous solubility of many new bioactive chemical molecules[3].

From the past literature studies, plants are the source of natural health products for human beings and are potent in producing new drug entities[4]. Citrus belongs to the family *Rutaceae*. Many studies revealed that the rinds of citrus fruits are rich in flavanoids, volatile oil and poly-methoxylated flavones[5]. Citrus rind extract analysis confirmed the presence of large number of phytochemical compounds with remarkable medicinal properties, making it as an emerging research area in pharmaceutical, cosmetics and nutraceutical[6,7,8]. The essential oils obtained from the rind extracts of citrus has properties such as anti microbial, anti-cancer, antioxidant, anti-inflammatory, anti ulcer and anti-thypoid[9]. Pomegranate belongs to the family *Punicaceae*. The rinds of which consist of polyphenols, tannins with more potent antimicrobial activities[10,11]. The rind extract of Pomegranate had reported for its antimicrobial, anti-cancer, anti-diarrheal and anti inflammatory activites[12,13]. Owing to its impending properties, the rind extract is acquiring an increased attention[14]. Banana belongs to the family *Musaceae*. Very few research works have been reported on the *Musa acuminata* leaves and hence study on the properties is vital in identifying a new chemical moiety with pharmacological activity[15].The present study focuses on extraction of bioactive components from rinds of *Citrus limetta*, *Punica granatum,* and the leaves of *Musa acuminata*.

The massive structure of the many biologically active compounds own reasons for poor solubility, absorption, and terribly precise targeted delivery, chiefs the effectiveness impairment. Therefore, the use of current drug delivery technique to deal with those troubles is probably a way out for those crucial issues[16]. Among diverse technological advances, nanotechnology is imparting a tremendous capability to develop an ideal drug with greater biocompatible, particularly targeted and pharmacokinetic ally controllable. A crucial parameter that dictates the bioavailability and hence biological impact of the drug is drug release from its system[17].

Nanoemulgel is an emerging technique for topical drug delivery. It is currently being examined to treat diseases caused by viruses, bacteria, and fungi[18,19]. Presently researchers are focusing on the development of hydrogel-based formulation for controlled release owing to its low cost of development, high adaptivity in delivering different active molecules with production simplicity[20, 21].

Mathematical modeling aids in forecasting the drug release process[22]. It is utmost important in designing the pharmaceutical formulation, as it predicts the rate and extent of drug release, which determines the biological efficacy of the developed drug systems. Experimental findings and empirical phenomena can be correlated through interpretation of mathematical models. Hence, effective and efficient drugs can be produced when the knowledge of pharmacists and engineers accompany with each other[23,24].

The aim of the current study is to identify bioactive rich extract pertaining to antimicrobial activity. The present research also focuses on the comparison of mathematical model for drug release behaviour and kinetic evaluation of the model for control release of the bioactive compound from extract mediated nanoemulgel. The drug release data obtained were quantitatively correlated and interpreted for empirical and semi empirical models to understand the mechanism of drug release.

Experimental Section

Materials

Agro wastes such as rinds of *Citrus limetta*, *Punica granatum,* and the leaves of *Musa acuminata,* were locally collected. Coconut oil, ethanol (99.9% purity) from Nice Chemicals (Kerala, India), Tween 80, Span 80 and Carbapol 940 from Loba Chemie (Mumbai, India), Triethanolamine from Sigma Aldrich (Bangalore, India) were purchased for the present research work.

Preparation of extracts

The agro wastes such as rinds of *Citrus limetta*, rinds of *Punica granatum* and leaves of *Musa acuminata* were collected separately, washed, shade dried, grounded and sieved. The powdered samples were subjected to extraction using Soxhlet extractor using ethanol as a solvent in 1:16 (%w/v) ratio for duration of 9 h. The samples were further analyzed using Clarus 680 GC (Perkin Elmer) Gas Chromatography-Mass Spectrometry (GC-MS) [25]. The presence of the bioactive components were analysed and compared for nanoemulgel formulation.

Preparation of nanoemulgel

Nanoemulgels were formulated by blending 2% carbapol 940 hydrogel with the extract mediated emulsion at room temperature using magnetic stirrer at 750 rpm for 15 min[26]. The pH of the nanoemulgel was adjusted with Triethanolamine. The S_{mix} of 1:1 was used for the emulsion in the present study[27]. The prepared formulations were named from B1 to B9 by varying the ratios of oiland extract[28].

pH determination

A high or very low value of pH can become destructive to the skin[29]. Hence, 1% aqueous solution of the nanoemulgel were taken, analyzed using a digital pH meter (Labman scientific Instruments model LMPH-9)

Swelling index

Swelling index of the nanoemulgel was determined by placing 1g of the gel in a dish containing 10 mL of 5.5 pH phosphate buffers. Following a time gap of 30 min, the sample was taken and reweighed after removing the excess moisture. The percentage of the swelling index was reported by considering the difference in weight of the gel[30,31].

In-vitro release study

In-vitro studies were performed by placing 1 g of nanoemulgel formulation on the egg membrane in the donor compartment of the Franz diffusion cell. 5.5 pH buffer solutions were filled in the receptor compartment, which was stirred using a magnetic stirrer. For every 1 h, about 1 mL of the sample was withdrawn and the equal amount was replaced with the fresh buffer. The withdrawn samples were analyzed using (Elico SL164 Double beam UV-Vis Spectrophotometer) at 295 nm. The cumulative percentages of drug release were plotted against time[32,33].

Kinetic analysis of data

The bioactive component release from the gels was studied using various mathematical models. These models were applied to the data from in-vitro studies. The models used for the study were categorized as empirical and semi-empirical models.

The mathematical models for empirical models are

Zero order: $Q_t = K_0 t + C_0$ (Linear form) (1)

First order: $Q_t = Q_0 \exp(-K_f t)$ (Exponential form) (2)

Higuchi: $Q_t = K_H \sqrt{t} + C_H$ (Square root of time form) (3)

The mathematical model for semi-empirical models are

Ritger-Peppas: $Q_t = K_{RP} t^n$ (Power law form) (4)

Where K_0, K_f, K_H, K_{RP} are the release rate constants corresponding to zero-order, first order, Higuchi, and Ritger-Peppas models respectively. 'Q_t' is the cumulative amount of drug released at any time't'. 'Q_0' is the initial amount of drug. 'C_0'& 'C_H' are the respective intercepts of the vertical axis. These models were used to predict the release mechanism and the fraction of drug released[34].

Results and Discussion

Based on the experimental investigations, *Citrus limetta* rinds consist of 8 components, out of which 6 components namely were reported to have biological properties such as anti-inflammatory, antioxidant, and anti allergent. For *Punica granatum* rinds, 10 components were recognized in which six components was found to exhibit antimicrobial activity. Similarly, 7 components of *Musa acuminata* leaf extracts recognized to have biological activity.

Fig. 1(a) shows the GC-MS chromatogram of *Citrus limetta* rinds. At the retention time of 10.69 min and 11.02 min, Cyclohexanol, 1-methyl-4-(1-methy lethenyl)-acetate, a fragrance compound, respectively,

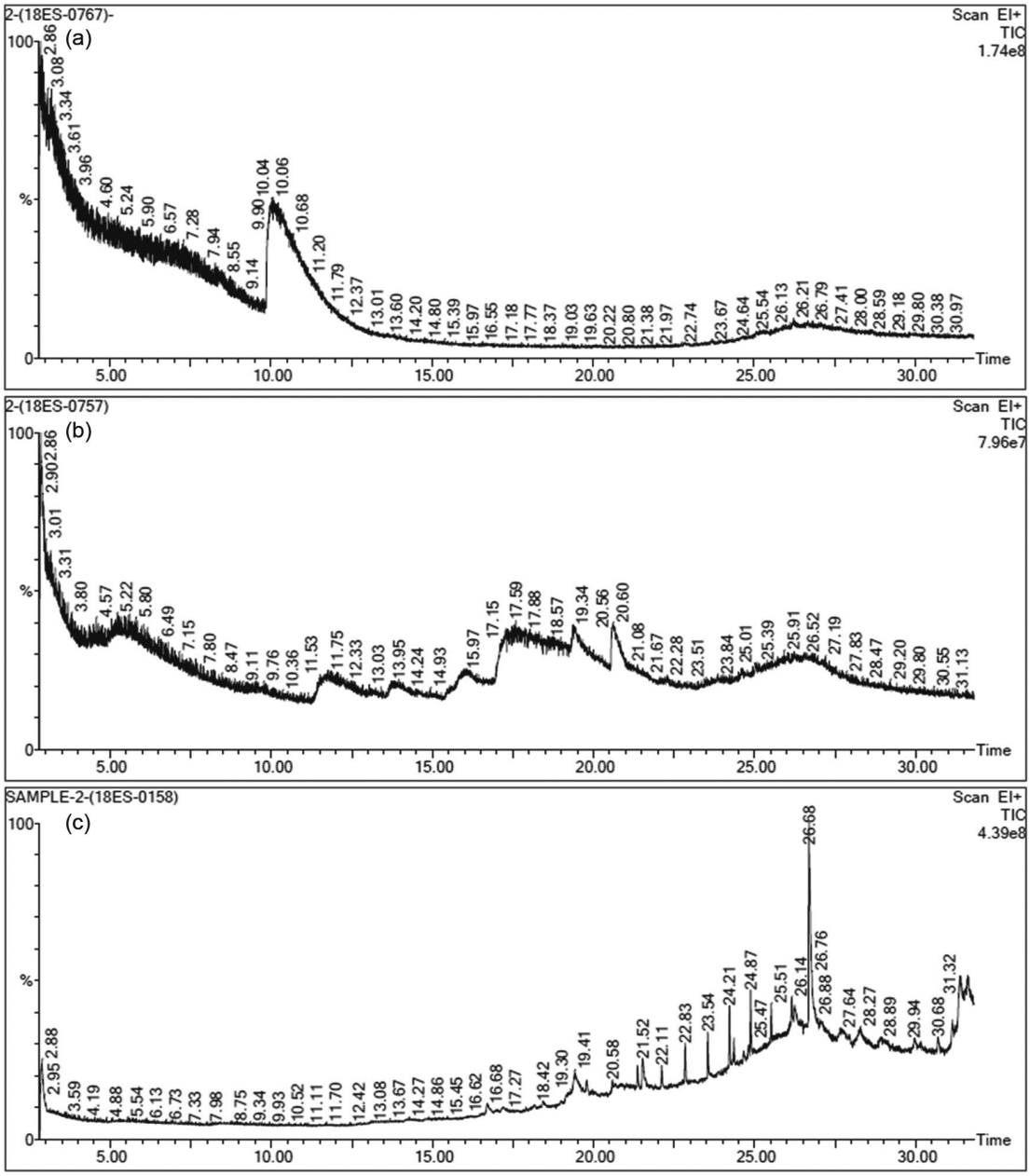

Fig. 1 — GC-MS chromatogram of (a) *Citrus limetta* rinds extract, (b) *Punica granatum* rinds extract and (c) *Musa acuminata* leaves extract

were reported. A retention time of 10.382 min corresponds to cyclohexene 4–isopropenyl-1-methoxymethoxymethyl. This compound has anti-inflammatory and anti allergent properties[35]. 2,3-anhydro-D-galactosan, a preservative was found at retention time of 19.050 min with a peak area of 5.475%[36]. Tetraacetyl–d-xylonic nitrile was identified and reported to show anti-tumour and anti oxidant properties with peak at the retention Time of 24.03 min[37]. 3,6-methano-8h-1,5,7-trioxacyclopenta[ij] cycloprop [a]azulene-4,8(3h)- was reported at retention time of 24.92 min, can act as an active pharmaceutical ingredient. Antibacterial activity was reported for 1,1' bicyclopropyl-2-octanoic acid- 2'- hexyl-, methyl ester with a retentiontime of 24.51 min[38].

Fig. 1(b) corresponds to GC-MS chromatogram of *Punica granatum* rinds extract, where 4-hexen-3-one, 4,5-dimethyl at 17.29 min are reported to be a fatty acyls in multiomics measurements with a peak area of 11.51%. At 17.58 min retention time, 2,3-anhydro-D-galactosan was identified. 2-Hexanol found at 19.08 min possesses fragrance and flavouring properties with peak area of 2.89 %. A flavouring compound, 1-hexanamine at retention time of 19.38 min was reported with peak value of 18.58 %. At retention time of 20.60 min, a cellular location identifying component, 9-octadecenoic acid, ethyl ester was recognized. A component of 3,7-diacetamido-7h-S-triazolo has been reported for its antibacterial and antifungal activity at retention time of 18.76 min[39].

Fig. 1(c) depicts the GC-MS chromatogram of *Musa acuminata* leaf extract. Phytol with a retention time of 19.41 min was reported to show the properties of antimicrobial; anti-inflammatory; anticancer; analgesic; hepatoprotective; anti-androgenic; anti-diuretic; immuno stimulatory and anti-diabetic activity[40,41,42]. At retention time of 21.52 min, antioxidant; antimicrobial; ant proliferative properties were reported for hexanedioic acid, Bis(2-ethylhexyl) ester[43]. Antimicrobial; Diuretic anti-inflammatory; antiasthma were reported for ethyl iso-allocholate with retention time of 24.20 min[44]. 1h-Benzocyclohepten-7-ol, 2,3,4,4a,5,6,7,8-octahydro-1,1,4a,7-tetramethyl-, cis and 1,6,10,14-hexadecatetraen-3-ol, 3,7,11,15-tetramethyl-, (E,E)- at retention time of 27.03 min was reported to exhibit antitumor; analgesic; antibacterial; anti inflammatory; sedative ; fungicide activity with a retention time of 26.238 min. Antimicrobial; antioxidant; anti-inflammatory were reported at retention time of 26.143 min for 2-piperidinone, N-[4-bromo-N-butyl]-[45]. Dl-alpha.-tocopherol has shown properties such as anticancer; antioxidant; antitumor;anti-inflammatory; hypocholesterolemic; cardioprotective; antidermatitic; antileukemic; antiulcerogenic; antispasmodic; antibronchitic; antitumor; apoptotic; antiaging and anti cataract[46,47].

The above GC-MS analysis has revealed the presence of many bioactive components in the extracts of *Citrus limetta* rinds and *Punica granatum* rinds and *Musa accuminata* leaves. For *Citrus limetta* extract, among the various bioanalytes, only 25% components has potential antimicrobial properties. With respect to *Punica granatum* rind extract, only 9% of the identified compound has antimicrobial activity, whereas for *Musa acuminata* , around 88% of the compounds has been reported. The extract with majority of antimicrobial activites was optimized for the further formulation of nanoemulgel.

The S_{mix} ratio of 1:1 ratio was utilized to formulate nanoemulgel with varying proportions of oil and extract. The compositions of the prepared nanoemulgel were tabulated in Table 1. Estimation of the physical properties is an important factor in the manufacture of the nanoemulgel. The prepared nanoemulgels were characterized for physical

Table 1 — Composition and characterization of Nanoemulgel

Formulation code	Nanoemulsion combinations			Hydrogel Carbapol-940 (g)	pH (no unit)	Swelling Index(%)
	Oil (%)	Extract (%)	Smix (%)			
B1	1	9	2	12	5.5	70.2
B2	2	8	2	12	5.2	68.0
B3	3	7	2	12	5.1	63.0
B4	4	6	2	12	5.3	59.3
B5	5	5	2	12	5.1	54.9
B6	6	4	2	12	5	49.7
B7	7	3	2	12	5.1	37.8
B8	8	2	2	12	5.2	31.6
B9	9	1	2	12	5.2	29.1

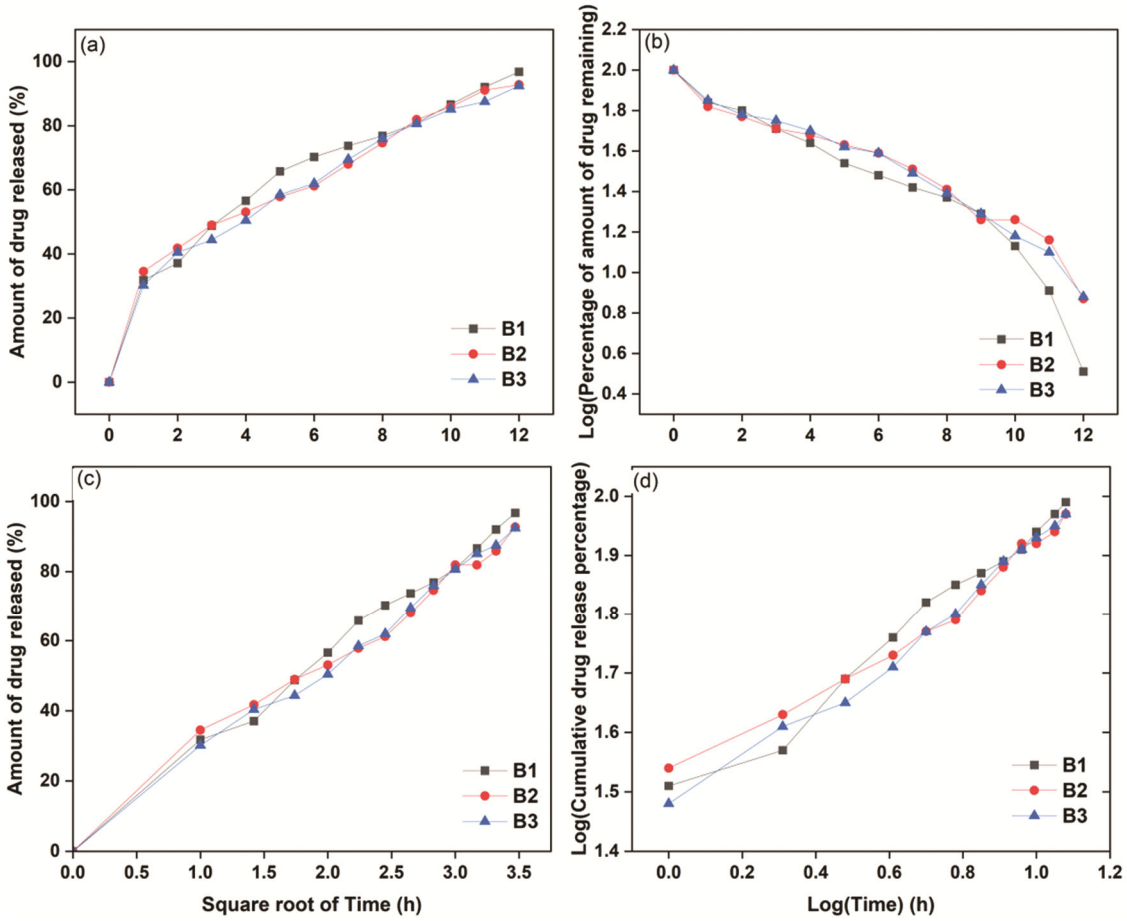

Fig. 2 — Kinetic data evaluation for B1, B2 and B3 formulation: (a) Zero order model, (b) First order model, (c) Higuchi model and (d) Ritger-Peppas model

properties such as pH and swelling index. The pH of the prepared nanoemulgels were in the range of 5-5.5,ensuring its suitability for topical application without any risk of irritation[48]. The swelling index of the nanoemulgel were also examined and reported. The better value of swelling index was observed for formulations for B1,B2,B3 comparatively. The hydrogel present in the nanoemulgel contains a large number of hydrophilic groups. On exposing the nanoemulgel to the surrounding fluid, the polymer chain gets extended resulting in a larger value[49]. Thus, based on the above characterisation studies, the formulations B1, B2 and B3 were considered for invitro studies.

The suitability of different models was examined using curve fitting. In Fig. 2, the independent axis is represented as time and the dependent axis as a function of cumulative drug release. In-vitro release data were analyzed for different empirical release kinetics and semi-empirical model. The obtained results revealed the mechanism of release and release diffusion. The cumulative drug release data were tabulated in Table 2 along with parameters estimated on fitting the kinetic data with drug release models.

Table 2 — Parameters estimation for release kinetic models with the correlation coefficient

Model	Parameters	Formulations		
		B1	B2	B3
Zero Order model	K_0 (µg/mL)	6.72	6.41	6.54
	C_0 (µg/mL h)	22.47	22.39	20.51
	R^2	0.90	0.91	0.92
First Order model	K_f $(h)^{-1}$	0.09	0.07	0.08
	R^2	0.91	0.94	0.96
Higuchi model	K_H	27.19	25.00	26.19
	C_H (µg/mL h)	1.56	3.83	0.76
	R^2	0.99	0.98	0.99
Ritger-Peppa model	K_{RP}	4.37	4.51	4.28
	n	0.46	0.40	0.46
	R^2	0.98	0.97	0.98
Cumulative release at 12 h in %		96.78	92.74	92.42

The zero-order model describes the process of uninterrupted drug release from the system and a concentration of drug remains steady throughout the delivery[50].The two-parameter such as K_0 and C_0 values of zero-order release were calculated and were in the decreasing order. The graphical depiction of cumulative percentage drug release against time as shown in Fig. 2(a) does not follow seamlessly the principle of zero order release kinetics, yet its slightly approaching the regression coefficient of 0.90, 0.91 and 0.92 for B1, B2 and B3 formulations, respectively. From the kinetic analysis, it can be inferred that there is no initial outburst of the drug from the hydrogel.

The first order process relates the concentration of the drug and the rate of drug release directly to each other as the rection progresses[51]. K_f value was calculated for the first-order model and was in the range of 0.09-0.08. A plot of logarthamic percentage of drug remaining against time was shown as in Fig. 2(b) for B1, B2 and B3 formulations. From these figures, it can be concluded that first order release kinetics principles was not approached, indicating that dissolution does not control the mecahanism of release of drug from the nanoemulgel, even with regression coefficient of 0.91, 0.94 and 0.96 for B1, B2 and B3 formulations, respectively.

Higuchi model describes the drug release and its dissolution. This model predicts the total depletion of the drug from the dosage form. The graphical depiction of percentage amount of drug release versus square root of time was plotted as shown in Fig. 2(c) , for B1, B2 and B3 formulations. For B1, B2, and B3 formulations, the best fit model was identified to be Higuchi with an R^2 value ranging as 0.99-0.98 and the K_H value ranges from 25 to 27.19 which infers that the formulations follow the diffusion-controlled release i.e., the drug molecule starts to dissolve into the solvent whereas the drug dispersion remains the hindered within the bulk liquid. A stable diffusion can be observed within the formulation. The graph depicts that there was a fast diffusion at the earlier stage for shorter duration and then the rate of release got slower with half power of time, thereby making a sense for most diffusion based release[52].

According to Ritger-Peppas model, when the value of n is less than 0.5, it indicates Fickian diffusion. The value of n for B1 and B3 formulations was reported as 0.46 and the B2 formulation as 0.40 as depicted in Fig. 2(d). The release rate constant (K_{RP}) was directly proportional to diffusion constant and as it depends on the physical and structural properties of the active component and the polymer matrix[53]. The K_{RP} value ranges between 4.28-4.51 which concludes the Fickian diffusion. Comparatively, the B3 formulation showed a higher value of R^2 than others for both empirical correlation and semi-empirical correlations.

Conclusion

The extracts of rinds of *Citrus limetta*, *Punica granatum*, and leaves of *Musa acuminata* were experimented for its bioactive components. GC-MS analysis was carried out to identify the bioactive components in the extract. Based on experimental investigations, it was found that Musa acuminata possessed more bioactive compounds than the rind extracts of *Citrus limetta, Punica granatum*. From the nanoemulgel formulations B1, B2, B3 were reported to have better values of pH and swelling index. After application of mathematical models, the drug release was found to be the best fitted by Higuchi square root model with higher regression values (R^2 = 0.99) which implies that the release of drug from matrix as a square root of time dependent process and diffusion controlled with Fickian type. On comparing and analysing the experimental data, it is concluded that B3 formulation of *Musa acuminata* was found to be the best amongst B1 and B2 in terms of physical characteristics and kinetic evaluation.

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