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Original Research

Unveiling Antiarthritic Potential of *Moringa oleifera* Lam. Extract: Evaluation and Hydroxypropylmethylcellulose-Based Formulation

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Abstract

Medicinal plants consist of complex chemical compounds that have been acknowledged for their important role in treating persistent human illnesses. This research concentrates on the phyto-physicochemical evaluation of *Moringa oleifera* Lam. extract and its *in vitro* anti-arthritic properties, as determined by inhibiting protein denaturation utilizing varying concentrations of the extract and a standard drug, diclofenac sodium. The study's results revealed a significant *in vitro* anti-arthritic impact of the extract, with an 85.8% inhibition compared to the standard drug's 99.9% inhibition. Furthermore, this research involved creating sustained-release tablets using hydroxypropyl methylcellulose (HPMC)-based formulations and various grades of hydrophilic polymers along with a fixed quantity of a hydrophobic polymer. The manufactured tablets demonstrated favourable sustained-release traits through direct compression, with the MF2 formulation showing a 73.8% cumulative release over 10 hours.

Keywords: *Moringa oleifera*; Plant extracts; Anti-arthritic; Hen's egg albumin; Diclofenac

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Introduction

Arthritis, as a chronic joint disorder, is a major cause of disability globally and occurs due to improper regulation of pro-inflammatory cytokines and enzymes which results in elevated levels of chemical mediators like leukotrienes, prostaglandins, matrix metalloproteinase enzyme, nitric oxide and adhesion molecules [1]. It is an inflammatory condition of various types which can be differentiated on the basis of tissue occurring in skin, muscle and joint respectively. These inflammatory conditions are treated with few synthetic medicines, available in the market; however, these medicines only exhibit symptomatic relief rather than cure of the disease. Synthetic medicines also possess various side effects; though inflammation reoccurs on discontinuation of treatment [2]. Herbal products are one of the most preferred choices due to the presence of certain active phytoconstituents which are reported for their significant potential against arthritis and other inflammatory disorders [3]. In the present study we selected a plant extract namely, *Moringa oleifera* Lam. (Sahijan) belonging to the Moringaceae family. It is a fast-growing small deciduous *tree native to tropical Asia* which is rich in vitamins and essential nutrients. It grows well in weakly acidic soil (pH 6.3 to 7). *M. oleifera* has been reported for various pharmacological activities. The moringa leaves contain polyphenols, β-carotene and quercetin. The seeds contain *p*-cymene, behenic acid and 25% α-phellandrene. These phytoconstituents contribute the treatment of bone fracture, piles, pain in joints, swelling, asthma, irregular menstruation, scurvy, bites of poisonous insects, worm infection, and labour pain [4,5]. The objective of the present study was to assess *in vitro* anti-arthritic activity of *M. oleifera* extract, as well as to develop a sustained-release tablet formulation.

Materials and Methods

Ethanolic extract powder of *Moringa oleifera* (100%) was procured from Vital Herbs, Delhi with authenticated certificate of analysis (COA). Various grades of hydroxypropyl methylcellulose (HPMC), ethyl cellulose (EC), magnesium stearate, talc and microcrystalline cellulose (MCC) were procured from S.D. Fine chemicals Ltd., Mumbai, India. All the chemicals and reagents used were of analytical grade. Phyto-physicochemical parameters and *in vitro* anti-arthritic activity of extract were evaluated prior to the design of sustained release formulation.

Phyto-physicochemical evaluation of extract

The dried ethanolic extract of *M. oleifera* was observed for organoleptic properties like color, odor, taste, and texture. Flame test, unsaturation test and other chemical tests were performed to confirm its characteristic properties. pH value (5% w/v solution) was determined using auto digital pH meter (Labtronics, LT-11 model). Qualitative tests were also carried out to confirm presence of phytoconstituents like flavonoids, tannins, glycosides, alkaloids, saponins, terpenoids, carbohydrates, and proteins [6,7].

Powder characteristics

Powder characteristics of the extract were determined by evaluating swelling index, total ash value, water soluble extractive value (% w/w), acid insoluble ash, bulk density, Hausner's ratio, angle of repose, loss on drying and Carr's index [12,13].

Thin Layer Chromatography analysis

Thin layer chromatography (TLC) of extract was carried out on a pre-coated silica gel G (0.25 mm Merck silica gel 60 F254) plate using toluene, ethyl acetate and methanol in the ratio of 7:2:1. R_f value was calculated after visualization of spots under UV light or by exposing them with iodine vapours [8,9].

Fourier Transform Infra-Red spectroscopic analysis

Moringa extract was dried in a hot air oven at 70-80°C for 3-4 hours and desiccated overnight. About 3-5 mg extract sample was triturated with dried potassium bromide of equal weight and KBr disc were prepared by compressing it. FT-IR spectrum was recorded at an absorbance mode from 4000 to 400 cm^{-1} using Shimadzu FT-IR spectrophotometer. Drug-excipient compatibility studies were performed by scanning of optimized formulation MF2 mixed with polymers by FT-IR spectrophotometer [10,11].

In vitro anti-arthritic activity

The antiarthritic activity is assessed based on inhibition of protein denaturation method [14,15]. The material used for the test was hen's egg albumin, Moringa extract, diclofenac sodium, phosphate buffer (pH 7.4). Test solutions (0.5 mL) were prepared by dissolving 0.45 mL, 5% solution of egg albumin into 0.05 mL extract solution of different concentrations (100, 250, 500, 1000 µg/mL) separately. Control solution (0.5 mL) was prepared by dissolving 0.45 mL egg albumin with 0.05 mL double-distilled water. Standard solutions (0.5 mL) were prepared by dissolving 0.45 mL egg albumin into 0.05 mL diclofenac sodium solution of similar concentrations as test solutions. All the solutions were incubated at 37°C for 20 minutes and then heated subsequently at 57°C for 10 minutes. Then, 2.5 mL of phosphate buffer saline was mixed with each reaction mixture. The absorbance of all the solutions were observed at wavelength maxima (281 nm) using UV-visible spectrophotometer. The percentage inhibition of protein denaturation was calculated for test solution and compared with standard

solution (diclofenac sodium) [16].

% Inhibition = 100- [(Absorbance $_{test\ /\ standard}$ - Absorbance $_{control}$) / Absorbance $_{control}$ x100]

Formulation of tablets

The non-aqueous dry granulation method was used for formulation of sustained release tablets of ethanolic extract of *M. oleifera*. Polymer mixture prepared by first triturating HPMC with EC followed by MCC. The powder extract was added part by part into polymer mixture along with continuous blending then blended mixture was passed through 60-mesh sieve to prepare granules. Preformulation parameters of granules i.e., angle of repose, Hausner's ratio, Carr's index, bulk and tapped densities were determined [17]. The granules were thoroughly mixed with magnesium stearate and talc for desired hardness and lubrication. Finally, granules compressed into round shape tablets using a rotary tablet compression machine (Grovers Enterprises, HICON, 12 mm punch size, single punch) with rpm of 18 and pressure of 4-5 kg for specified composition (Table 1) which was optimized by design expert software (version 13). The Formulations were named as MF1, MF2, MF3, MF4, MF5 and MF6. The tablets were evaluated for their characteristic parameters like weight variation, hardness, thickness, friability and dissolution test [18,19].

Evaluation of physical parameters

All the tablet parameters were evaluated for various quality control tests according to the pharmacopeial specifications [20]. Thickness (mm) of 5 tablets from each formulated batch (MF1- MF6) were measured by digital Vernier calliper and mean value was calculated. Randomly sampled 20 tablets were used for weight variation test on a precision balance. Hardness (kg/cm^2) was determined by HICON, Monsanto hardness tester using 10 tablets. Friability test was carried out in Roche friabilator using 10 tablets from each formulation batch at a speed of 25 rpm for 4 minutes.

In vitro dissolution study (Drug release studies)

The drug release studies of formulated tablets were carried out as per protocol given in United State Pharmacopoeia using type1 (basket type) apparatus [21,22]. In this method acidic solution completely replaced by buffer solution keeping other conditions constant like volume of dissolution medium (900 mL) and 37 ± 0.5 °C temperature at 50 rpm. For the first two hours, hydrochloric acid buffer (0.1N, pH 1.2) was used and it was replaced with phosphate buffer (pH 6.8) between 2-10 hours. The sampling time points were 0, 2, 3, 4, 5, 6, 7, 8, 9 and 10 hours. The percent drug release (% CR) of each formulation (containing 500 mg of herbal extract) was determined using a UV spectrophotometer.

Results and Discussion

Phyto-Physicochemical evaluation

Preliminary physicochemical properties and powder characteristics of extract are summarized in tables 2 and 3, respectively. The phytochemical evaluation revealed the presence of active phytoconstituents in the extract. The result showed that the extract is rich in alkaloids, flavonoids, tannins, glycosides, steroids and carbohydrates (Table 4). The presence of saponins offer additional advantages by combating infections and microbial intrusion as natural antibiotics. Flavonoids exhibit inhibitory properties, guarding against allergies, inflammation, platelet aggregation, and free radicals. Tannins, crucial in herbal medicine, effectively stop bleeding injuries.

TLC analysis

TLC analysis was performed to separate chemical constituents of extract. The optimized solvent system having good resolution was toluene: ethyl acetate: methanol (7:2:1) which showed two spots at R_f values 0.76 and 0.85.

HPTLC analysis

The extract was subjected to HPTLC for qualitative analysis of phytoconstituents. The chromatograms and fingerprints have been reported in figure 1. It revealed 6 spots at R_f values 0.04, 0.08, 0.15, 0.37, 0.56 and 0.85 in solvent system- toluene: butanol: ethyl acetate: formic acid (3: 3: 3.5: 0.5) at 254 nm and 366 nm.

Table 1. Composition of tablet formulation

Ingredients	Batch / Qty. Per Tablet (mg)					
	MF1	MF2	MF3	MF4	MF5	MF6
Drug (Extract)	500	500	500	500	500	500
HPMC K4M	35	35	70	70	140	-
HPMC K15M	70	35	35	70	-	140
EC	105	105	105	105	105	105
MCC	119	119	119	119	119	119
Mg Stearate	14	14	14	14	14	14
Talc	14	14	14	14	14	14
Total Powder	857	822	857	890	890	890

Abbreviation: HPMC: hydroxypropyl methylcellulose, EC: ethyl cellulose, MCC: microcrystalline cellulose

Table 2. Organoleptic properties of *M. oleifera* extract

Parameters	Observation
State	Solid coarse powder
Color	Brown
Odor	Pungent
Taste	Bitter

Table 3. Physicochemical parameters and powder characteristics of *M. oleifera* extract

Parameters	Observation
Flame test	Swelled up, no sooty flame
Unsaturation test	Decolorize 1% $KMNO_4$ solution
pH	4.6
Total Ash value (%)	5.5
Water sol. extractive (%)	63
Acid insoluble ash (%)	2.8
Swelling Index	11.4
Loss on drying (%)	12
Bulk density (g/ mL)	0.63
Hausner's ratio	1.18
Carr's Index (%)	14.3
Angle of repose (°)	41.7

FT-IR analysis

The FT-IR spectrum of Moringa pure extract and its compatibility spectra with selected polymers i.e. methyl cellulose and HPMC K4M is represented in figure 2. The spectra revealed that there is no appearance or disappearance of characteristic peaks of pure extract and in the physical mixture which depicts absence of chemical interaction between drug and polymer. The FT-IR spectra of pure ethanolic extract of *M. oleifera* showed the absorption peaks at 2927.94 cm^{-1} due to -NH stretching, 1627.92 cm^{-1} due to -C=O stretching and 3381.21 due to -O-H stretching whereas its polymer mixture containing formulation showed characteristic functional group peaks at 3053.34 cm^{-1} and 2836.73 cm^{-1} (-NH stretching), 1727.27 cm^{-1} and 1654.46 (-C=O stretching), 3412.10 cm^{-1} and 3504.20 cm^{-1} (-OH stretching) with minor shifts for ethyl cellulose and HPMC K4M respectively.

In-vitro anti-arthritic activity of Moringa extract

The result of *in vitro* anti-arthritic activity revealed that the ethanolic extracts of *M. oleifera* were capable of controlling the production of autoantigen and inhibition

Table 4. Qualitative phytochemical screening of Moringa extract

Procedure	Observations	Result
Sample +Conc. H_2SO_4	Yellow color	Flavonoids Present
Sample +NaOH	Yellow color	Flavonoids Present
Sample + shaken with water	Frothing	Saponins Present
Sample + $FeCl_3$	Green Black precipitate	Tannins Present
Sample + Molisch's reagent	Purple ring at junction	Carbohydrates Present
Sample + Fehling's reagent	Brick red color	Carbohydrates Present
Sample + Benedict's reagent	Red precipitate	Carbohydrates Present
Sample + Keller's reagent	Brown ring color	Glycosides Present
Sample + Salkowaski's reagent	Red brown color	Steroids Present
Sample + Ninhydrin reagent	Blue color	Proteins & amino acids Present
Sample + Biuret reagent	Purple color	Proteins & amino acids Present
Sample + Mayer's reagent	White precipitate	Alkaloids Present
Sample + Wagner's reagent	Red brown color	Alkaloids Present
Sample + Hager's reagent	Yellow precipitate	Alkaloids Present

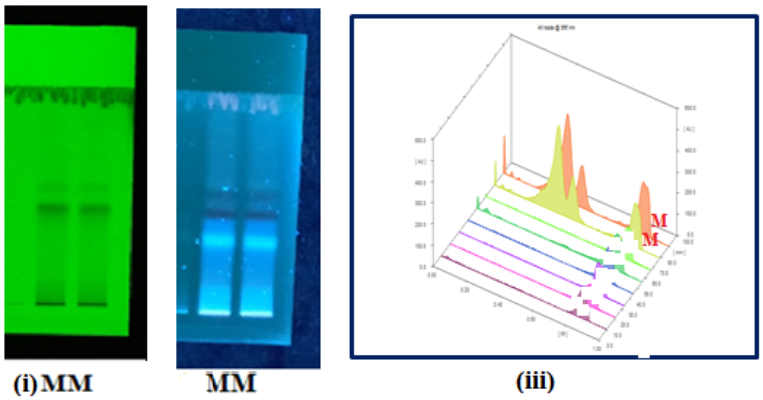

Figure 1. HPTLC Plates at (i) 254 nm (ii) 366 nm (iii) HPTLC fingerprints

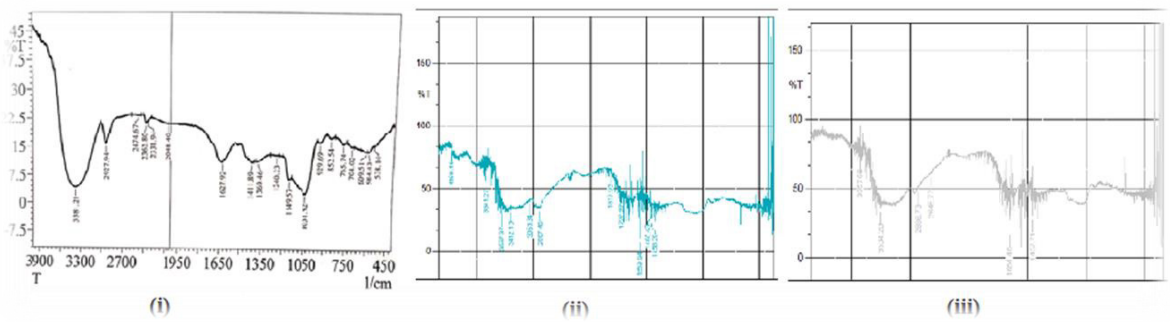

Figure 2. FT-IR spectra of (i) Pure Moringa extract (ii) Moringa with Ethyl cellulose (iii) Moringa with HPMC K4M

of protein denaturation that proved its remarkable anti-arthritic activity as compared to standard drug.

Evaluation of quality control parameters of Moringa tablets

The quality parameters of formulated tablets were evaluated according to the pharmacopeial specifications and results are summarized in Table 6. Thickness was controlled and found in the range of 4.4 - 4.6mm. The weight variation of the tablets was found to be ±3.6 %. Hardness for tablets of all batches was in the range of 9.7 to 12.5 kg/cm^2. Friability of tablets showed less than 1% loss.

In vitro dissolution study

Dissolution profiling or drug release kinetics is an important parameter of the sustained release tablet. On varying amount and ratio of different polymers, there will be significant change in drug release pattern. The Lab-India Dissolution Tester (Type 1-Basket) followed USP 2011 methodology, operating at 50 rpm and 37 ± 0.5 °C. A buffer change protocol was employed, with 900 mL of media. Initially, 0.1N hydrochloric acid buffer (pH 1.2) was used for two hours, followed by phosphate buffer (pH 6.8) for the subsequent eight hours. Sampling intervals were set at 0.5, 1, 2, 3, 4, 5, 6, 7, 8, and 10 hours. Absorbance at 246 nm, measured by a UV spectrophotometer, determined the release percentage from the formulation containing 500 mg herbal extract.

The release data was calculated and compared with the absorbance value obtained from UV-VIS spectrophotometer. In order to find the equation with best fit, the calculated release data were treated as per the equation given by zero-order, first order, Higuchi and Peppas-Korsemeyer models. The results of statistical analysis conclude that zero order and Higuchi models are showing highest linearity (r^2 = 0.96 to 0.99) and formulation MF2 showed remarkable drug release as compare to standard drug.

Table 5. Absorbance and % inhibition of protein denaturation

Absorbance of Control Solution	Conc. (µg/ml)	Standard Solution (Diclofenac sodium)		Test Solutions *(Moringa oleifera)*	
1.721		Absorbance	% Inhibition	Absorbance	% Inhibition
	100	1.861	91.9	2.409	60.0
	250	1.775	96.9	2.096	78.2
	500	1.742	98.8	2.084	78.9
	1000	1.713	99.9	1.966	85.8

Table 6. Evaluation parameters of Moringa tablets

Parameters	MF1	MF2	MF3	MF4	MF5	MF6
Thickness (mm) (n =20)	4.5	4.6	4.4	4.5	4.6	4.6
Hardness (kg/cm^2) (n =6)	10.0	9.7	10.0	12.5	11.0	11.5
Friability (%)(n =10)	0.91	0.61	0.27	0.97	0.73	0.70
Weight variation (%) (n =20)	0.48-3.2	0.34-2.9	1.5-3.8	0.28-2.4	0.8-2.1	1.2-3.9
Dissolution study(% CR) in 10 hours	69.79	73.84	63.20	50.18	69.02	65.03

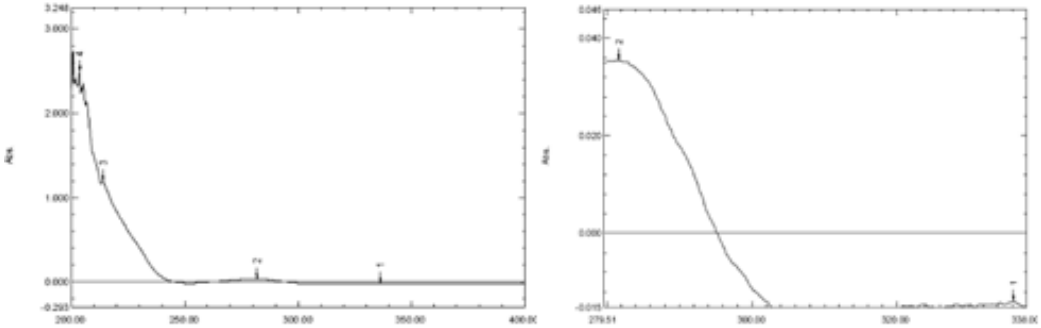

Figure 3. Left: UV Spectra of egg albumin (5%) at 281 nm (absorbance 0.035). Right: Extrapolation of left figure for more clarity to show absorbance at 0.035.

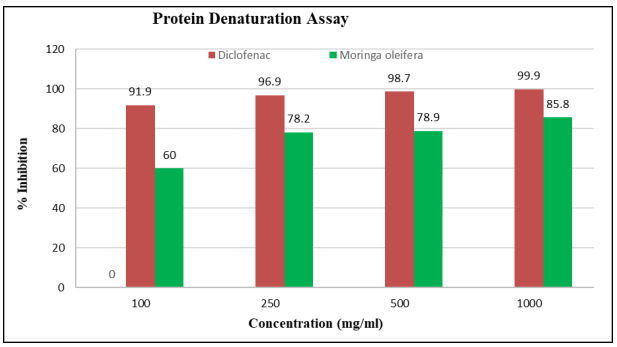

Figure 4. Effect of *Moringa oleifera* extract on hen's egg albumin denaturation

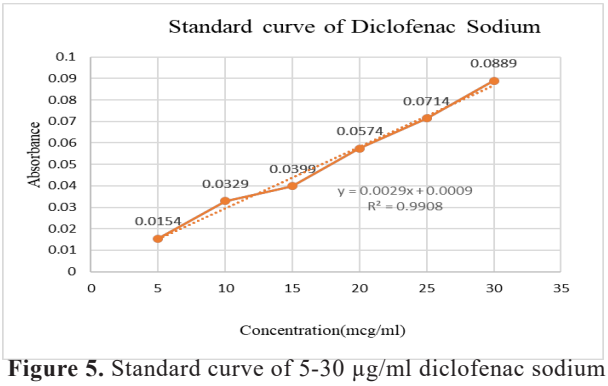

Figure 5. Standard curve of 5-30 µg/ml diclofenac sodium at 246 nm

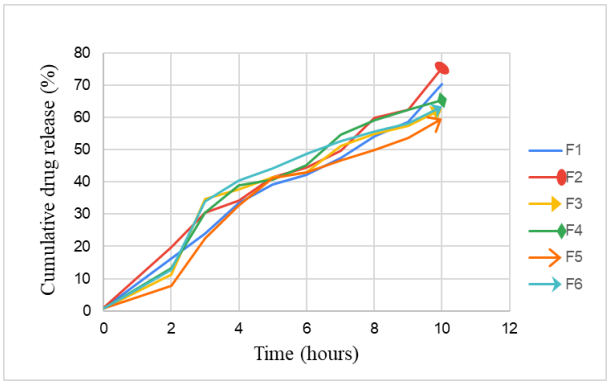

Figure 6. *In vitro* drug release profile of Moringa tablets (MF1-MF6)

Conclusions

The ethanolic extract of *M. oleifera* underwent physicochemical, phytochemical and preformulation investigation along with HPTLC and FT-IR analysis. The extract was assessed for *in vitro* antiarthritic activity by inhibition of protein denaturation method using hen's egg albumin. The percentage inhibition of extract was found to be 85.8 %, which reveals its potential against arthritis. The study also included formulation of sustained release tablets of extract using HPMC based polymer mixtures. There was no chemical interaction between extract and selected polymers used in formulation, which was represented in FT-IR

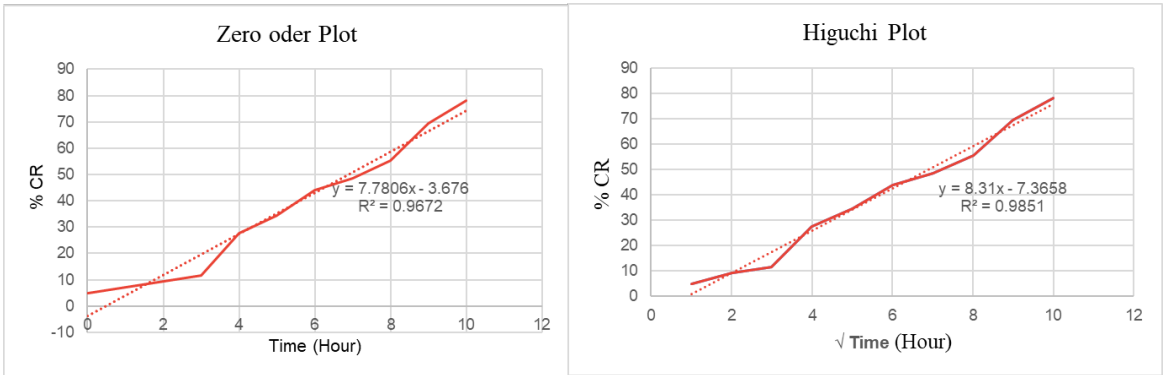

Figure 7. Plots for % drug release from **MF2** formulation of *Moringa oleifera*

Table 7. Cumulative drug release data (% CR) of MF2 up to 10 hours

Time (Hr.)	Absorbance (Y)	Conc. (X) µg/mL	Conc. µg/5 mL	Conc. µg/900 mL	Conc. mg/5 mL	Conc. mg/900 mL	CR (MF2)	% CR
0	0.01	3.13	15.68	2824.13	0.015	2.82	2.82	0.60
2	0.06	20.37	101.89	18341.37	0.101	18.34	18.35	3.93
3	0.21	72.10	360.51	64893.10	0.36	64.89	65.01	13.92
4	0.301	103.48	517.41	93134.48	0.51	93.13	93.61	20.045
5	0.42	144.51	722.58	130065.51	0.72	130.06	131.06	28.06
6	0.59	203.13	1015.68	182824.13	1.01	182.82	184.54	39.51
7	0.67	230.72	1153.62	207651.72	1.15	207.65	210.38	45.05
8	0.79	272.10	1360.51	244893.10	1.36	244.89	248.78	53.27
9	0.94	323.82	1619.13	291444.82	1.61	291.44	296.69	63.53
10	1.09	375.55	1877.75	337996.55	1.87	337.99	344.86	73.84

spectra. The percentage drug release for formulation MF2 was calculated as 73.84 % in 10 hours, which was shown in Figure 6. In view of above facts, we can conclude that the sustained release formulation of *Moringa oleifera* might be better alternative for management of arthritic disorders in future.

The findings regarding the *in vitro* anti-arthritic activity indicate the potential of the selected herbal extract as a viable treatment for arthritis and associated inflammatory conditions. Future investigations will include *in vivo* anti-arthritic assessments using established methods such as paw edema reduction in rat models, carrageenan and formaldehyde-induced inflammation in rat models. Moreover, the collected data from physicochemical, phytochemical, and pre-formulation analyses may facilitate the creation of a monograph and inform the development of novel drug delivery formulations utilizing these extracts in the future.

Conflict of Interests

The authors declare that there are no conflicts of interest.

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