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Evaluation of the Binding Properties of Pectin Isolated from *Citrullus lanatus* and *Cucumis sativus* Peels in Tetracycline Capsule Formulation

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: The advent of science and technology has greatly enhanced the conversion of agro-industrial wastes into various value-added products to meet the demands of increasing population. The study evaluated the binding properties of pectin isolated from *Citrullus lanatus* and *Cucumis sativus* peels in comparison with carboxymethyl cellulose (CMC) and pectin BP in oxytetracycline capsule formulations

Methods: Pectin was extracted from *Citrullus lanatus* and *Cucumis sativus* peels using standard procedures. Extracted pectin was subjected to phytochemical, organoleptic and spectral analysis and then used to prepare batches of oxytetracycline granules and capsules. Granule flow, capsules properties and drug compatibility studies using Fourier transform infrared (FTIR) analysis were determined.

Results: Phytochemical analysis of the powdered peels of *C. lanatus* and *C. sativus* showed the presence of alkaloid, saponin, terpenoid and carbohydrate. Percentage yield of pectin were 11.88 and 12.27% for *C. lanatus* and *C. sativus*, respectively. Oxytetracycline granules prepared exhibited the following micromeritic properties; Hausner's ratios - 1.12 to 1.33; Carr's indices - 11.11 to 32.61(%); angles of repose - 30.47 to 43.89° and flow rates - 1.17 to 5.67 g/sec. Disintegration times of formulated capsules were between 4.86 to 6.87 min and were within pharmacopoeial limits for capsules. FTIR studies showed no interaction between drug and excipients. Dissolution studies revealed decreased drug released with increase in binder concentration.

Conclusion: Pectin extracted from the peels of *C. lanatus* and *C. sativus* fruits compared favourably with pectin BP and CMC in their binding properties, hence a suitable substitute binder in the formulation of oxytetracycline granules and capsules.

Keywords: *Citrullus, Cucumis*, peels, pectin, excipients, oxytetracycline

INTRODUCTION

In recent years, natural polymers including those from plants, have evoked tremendous interest due to ease of conversion into pharmaceutical excipients employed in the formulation of pharmaceutical dosage forms (Somnache *et al*., 2016). Apart from various natural polymers, pectin occupies a prominent place due to its diverse pharmaceutical and therapeutic applications, hence various techniques are being adopted for obtaining high yield pectin from different agro-industrial wastes such as fruits and other plants parts (Martau *et al*., 2019; Mellinas *et al*., 2020). Pectin has excellent swelling and erosion properties, hence can be used as an adsorbent, emulsion stabilizer, bulk-forming agent and coating material for tablets, pellets, micro particles, and beads (Neckebroeck *et al.*, 2021; Obarisiagbon *et al*., 2022a). Watermelon (*C. lanatus*) is an important domestic and global fruit crop accounting for 7% of the worldwide area devoted to fruit and vegetable production (Guo *et al.*, 2013). Cucurbitaceae is a plant family, also known as gourd family, which includes crops like cucumbers, squashes, luffas and melons (Ajuru and Nmom, 2017). Watermelon contains about 6% sugar and 92% water by weight. As with many other fruits, it is a source of vitamin C (Guo *et al*., 2013). In China, they are stir-fried, stewed or more often pickled. When stir-fried, the de-skinned and de-fruited peel is cooked with olive oil, garlic, chili peppers, scallions, sugar and rum (Okafor *et al*., 2015). Pickled watermelon peel is also commonly consumed in the Southern US, and its juice can also be made into wine (Agricultural Marketing Service, 2020). Cucumber (*C. sativus*) is one of the monoecious annual crops in the Cucurbitaceae family that has been cultivated by man for over 3, 000 years. Economically, *C. sativus* ranks fourth after tomatoes, cabbage and onion in Asia (Eifediyi *et al*., 2010; Eleduma, 2023).

Tetracycline is one of the antibiotics groups used for veterinary, human therapy and for agricultural purposes. Amongst the different antibiotics used, more attention is paid to the tetracyclines as it exhibits serious environmental problems including ecological risks and human health damages. Their highly hydrophilic character and low volatility have resulted in significant persistence in the aquatic environment, and only few studies have described the fate and toxicity of oxytetracycline antibiotics in the environment (Fiaz *et al*., 2021)

Pectin has shown promising results as drug carriers for oral drug delivery and are widely used for various bio-medical applications; it therefore has great potential and opportunities for future developments. Pectin is an inert, biodegradable and biocompatible complex, widely used in various fields such as textiles, food industries as well as in pharmaceuticals (Freitas *et al*., 2021). This present study hopes to ascertain the validity of *Citrullus lanatus* (watermelon) and *Cucucmis sativus* (cucumber) peel pectin as an efficient binder in the formulation of oxytetracycline granules in a capsule as a pharmaceutical dosage form. The aim is to compare the binding property of watermelon and cucumber peel pectin with carboxymethyl cellulose (CMC) and pectin BP at different binder concentrations.

METHODOLOGY

Materials

Oxytetracycline hydrochloride powder (Venu Healthcare, India), carboxymethyl cellulose, pectin BP, lactose powder, starch BP powder, ethanol and concentrated hydrochloric acid (CDH, Vardaan, Daryangj. New Delhi, India), sodium hydroxide (Gunsgdong Chemical Ltd, China). *Citrullus lanatus* (watermelon) and *Cucumis sativus* (cucumber) fruits were collected from a farm at Okada Town, in Ovia North East, Edo State, Nigeria, identified and authenticated at the Department of Pharmacognosy, College of Pharmacy, Igbinedion University, Okada and voucher specimens deposited at the Department's Herbarium Unit (IUO/18/211 - *Citrullus lanatus* and IUO/18/232 - *Cucumis sativus*)

Methods

Preparation of watermelon and cucumber peels

Watermelon and cucumber peels were washed thrice with running tap water to remove any unwanted external materials before being chopped into pieces, sun-dried for 24 hours and then followed by another 72 hours of air drying until a constant weight of the peels was achieved. The dried peels were milled into powder with the aid of mini maize milling machine (HR 1500 Stainless, China) and stored in airtight containers for further analysis.

Phytochemical screening of watermelon and cucumber peel powder

The phytochemical screening was carried out to test for the presence of phytochemical components such as saponins, tannin, flavonoid, alkaloid, cardiac glycosides, steroids, terpenoids, anthraquinones and carbohydrates (Evans, 2009).

Extraction of pectin

Watermelon and cucumber peels pectin was extracted in line with an already reported method with some modifications (USP-NF, 2016; Thanaketpaisarn *et al*., 2017). The powdered peels (400 g) was weighed and carefully dissolved in deionized water (3000 ml, PH 1.65) adjusted with concentrated hydrochloric acid and sodium hydroxide. This was placed in a water bath at a temperature of 95°C, allowed to stand for 1.0 hour and filtered using double layers of cheese cloth to separate the residue. The filtrate was stored in a closed container while the residue was suspended in 400 ml of deionized water for 5 min, adjusted with concentrated HCl and sodium hydroxide. The resulting filtrate was mixed with 95% ethanol at a filtrate-to-ethanol ratio of 1:2 and left undisturbed in an enclosed container for 12 hrs. The precipitated pectin was harvested by filtration using cheese cloth, washed thrice with 70% ethanol and then with undiluted ethanol to remove impurities and dried in a vacuum oven. The dried pectin was milled, weighed and the percentage yield calculated before been packed and sealed in low density polythene bags until use.

Organoleptic properties of extracted pectin

The colour, taste and smell of the extracted pectin powders were assessed by five (5) persons and the consensus attribute accorded by a majority of three (3) persons for an organoleptic parameter was recorded.

Fourier transform infrared (FTIR) analysis of extracted pectin

The extracted pectin powders as well as pectin BP were subjected FTIR analysis. Using the potassium bromide (KBr) pellet method, 100 mg of KBr was weighed and mixed uniformly with 5 mg sample in fine particle size state. The mixed sample was placed in an evacuable KBr die of a hydraulic press and compressed into a pellet. Pelletized sample was placed in a cell holder and inserted into the FTIR machine (FTIR-4100 Spectrophotometer, Shimadzu Co. Japan) and scanned at a range of 750 - 4000 cm^{-1}.

Formulation of oxytetracycline granules

Using the wet granulation method, batches of oxytetracycline granules were prepared using the formula in Table 1.

Table 1: Formula of prepared paracetamol powder blends and tablets

Ingredients (mg)	Batches											
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**I**	**J**	**K**	**L**
Oxytetracycline	500	500	500	500	500	500	500	500	500	500	500	500
Lactose	60	60	60	60	60	60	60	60	60	60	60	60
Starch	15	15	15	15	15	15	15	15	15	15	15	15
WPP	5.8	17.4	29	-	-	-	-	-	-	-	-	-
CPP	-	-	-	5.8	17.4	29	-	-	-	-	-	-
Pectin BP	-	-	-	-	-	-	5.8	17.4	29	-	-	-
CMC	-	-	-	-	-	-	-	-	-	5.8	17.4	29

Key: WPP = Water melon peel pectin, CPP = Cucumber peel pectin, PBP = Pectin BP, CMC = Carboxymethyl cellulose.

Twelve (12) batches of the granules were prepared by using varying concentrations (1.0, 3.0 and 5.0 %w/v) of binder solutions (watermelon peel pectin (WPP) or cucumber peel pectin (CPP) or pectin BP (PBP) or carboxymethyl cellulose (CMC)) to wet mass the calculated quantities of oxytetracycline, lactose and starch powders already dry-mixed together and sufficient to produce 50 capsules per batch. The wet mass was dried in the oven at 50°C for 6 hours, milled and sieved through a stainless-steel sieve size 10 BSS. The granules were stored in sealed aluminum foils in an air tight container for further analysis.

Micromeritic properties of oxytetracycline granules

The following micromeritic parameters of the prepared granules were evaluated in accordance with standard procedures; bulk and tapped densities,

Hausner's ratio and Carr's index, angle of repose and flow rate (USP, 2005; Obarisiagbon *et al*., 2022b).

Bulk and tapped densities

Granules (50 g) were carefully placed in a measuring cylinder and the bulk volume occupied by each of the batch samples without tapping was noted. Bulk density was calculated according to the Equation 1. The bottom of the cylinder was tapped 100 times on a table top until no change in volume was observed. Tapped density was calculated as the ratio of weight of granules to the tapped volume using Equation 2.

$$\text{Bulk density} = \frac{\text{Weight of granules (g)}}{\text{Bulk volume (ml)}} \quad \text{- - - (1)}$$

$$\text{Tapped density} = \frac{\text{Weight of granules (g)}}{\text{Tapped volume (ml)}} \quad \text{- - - (2)}$$

Hausner's ratio and Carr's index

The Hausner's ratios and Carr's indices of the various batches of granules were calculated with the values obtained from the bulk and tapped densities measurements using Equations 3 and 4, respectively.

$$\text{Hausner's ratio} = \frac{\text{Tapped density}}{\text{Bulk density}} \quad \text{- - - (3)}$$

$$\text{Carr's index} = \frac{\text{Tapped density-Bulk density}}{\text{Tapped density}} \times 100 \quad \text{- - - (4)}$$

Angle of repose

This was determined by allowing the granules flow through a funnel and fall freely on table surface. Further addition of granules was stopped as soon as the cone has touched the tip of the funnel. The height and diameter of the resulting cone were measured, and the angle of repose was calculated from Equation 5.

$$\text{Angle of repose } (^{o}) = \tan\theta = \frac{h}{r} \quad \text{- - - (5)}$$

Where h = height of the cone, and r = radius of the cone base.

Flow rate

Flow rate was determined using the fixed funnel method. Fifty (50) grams of the granules were weighed into funnel and allowed to flow through the orifice of the equipment. The time taken for the granules to pass through was noted and the rate of flow per second was calculated from Equation 6.

$$\text{Flow rate} = \frac{\text{Weight of granules (g)}}{\text{Time of flow (sec)}} \quad \text{- - - (6)}$$

Drug-excipient interactions

Compatibility between oxytetracycline and the extracted pectin powders was investigated using FTIR analysis. Pure sample of the drug (oxytetracycline) and the batches of granules prepared with 5.0 %w/v pectin solution were subjected to the test.

Filling of granules into gelatin capsule shells

Granules weighing 580 mg were carefully hand-filled into empty gelatin capsule shells. The closed shells were cleaned of residual powder by dusting with a soft cloth and then packaged in airtight transparent bags containing silica gel until further analysis.

Evaluation of oxytetracycline capsules

Weight variation

The weight variation was determined using the British Pharmacopoeia (BP) recommended method. Twenty (20) capsules from each batch were individually weighed and their mean weight as well as their standard deviations calculated (BP, 2010).

Disintegration time

The disintegration time was determined using a Disintegration test apparatus (PX/DTA-1901, New Delhi, India). Six (6) capsules were used in carrying out the disintegration test. A capsule was placed in each tube of the disintegration apparatus and the basket rack of tubes was lowered into a 1.0 L beaker filled with 800 ml of water at 37 ± 05°C. The machine was switched on with the basket assembly oscillating at a frequency of about 30 cycles per minute until the capsules disintegrated into smaller particles that passed through the mesh at the bottom of the tubes. The time taken for each of the 6 capsules to completely disintegrate was read and the average time and standard deviation calculated.

Dissolution test

Dissolution test adopted was based on the USP 32-NF 27 method for dissolution test of solid dosage forms. Using the basket method, a capsule placed in the basket was immersed in 900 ml dissolution medium of 0.1 N HCl solution, maintained at 37 ± 0.5°C by a constant temperature bath and operating at a speed of 120 rpm. Aliquots of 5 ml of the dissolution medium were withdrawn at various time intervals for 90 min.

An equal volume of fresh dissolution medium at the same temperature was replaced each time withdrawal was made. The withdrawn samples were filtered into labelled test tubes, suitably diluted and the absorbance measured in a UV-spectrophotometer at 278 nm wavelength. Triplicate determinations were made and the mean and standard deviation recorded.

STATISTICAL ANALYSIS

Descriptive statistics was done for all data using Microsoft Excel (2007). Differences between mean was determined using one-way ANOVA while $p < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Phytochemical properties

Results from the phytochemical evaluation of the powdered peels of *C. lanatus* and *C. sativus* are outline in Table 2. Both powders showed presence of alkaloids, saponins, terpenoids and carbohydrate but absence of anthraquinone and cardiac glycosides. Only the *C. lanatus* powder showed evidence of flavonoids.

Table 2: Phytochemical properties of *C. lanatus* and *C. sativus* peels powder.

Parameter	*C. lanatus*	*C. sativus*
Alkaloid	+	+
Anthraquinone	-	-
Flavonoid	+	-
Saponin	+	+
Cardiac glycoside	-	-
Terpenoid	+	+
Polysaccharides	-	-
Tannin	-	-
Carbohydrate	+	+

Key: + (present), - (absent)

Percentage yield and organoleptic properties of extracted pectin

The result from the percentage yield calculation of the extraction process was 11.88% for *C. lanatus* and 12.27% for *C. sativus*. These values agree with results obtained by previous workers who extracted pectin from skin and cap of pumpkin as well as from fruits and berry juices (Yazdanpanah and Manochehr, 2021; Konrade *et al*., 2023). The pectin extract from *C. lanatus* was brown in colour while *C. sativus* extract was light brown but both extracts were bitter in taste with a characteristic smell.

FTIR analysis of pectin

Results from the FTIR analysis of pectin BP and the extracted pectin of *C. lanatus* and *C. sativus* peels powder is shown in Figure 1(a), (b) and (c), respectively. The spectrum of pectin BP demonstrated a broad trough in the –OH region (3230 - 3550 cm^{-1}), an indication of high saturation with hydroxyl moiety or the O-H stretching of hydrogen bonded hydroxyl groups. Following the broad trough are bands in the region of 2947 cm^{-1} (-CH2 stretching), 1760 - 1745 cm^{-1} (C-O vibrational stretching), 1640 - 1620 cm^{-1} (C-O-C asymmetric stretching) and 1650 - 1550 cm^{-1} (C-N stretching and N-H deformation). These bands are consistent in the spectra of the extracted pectin, confirming the extracted materials as pectin. Also, these bands are in conformity with bands obtained in previous works regarding infra-red scanning of extracted pectin (Simpson and Morris, 2014; Santos *et al*., 2020; Chen *et al*., 2021; Konrade *et al*., 2023).

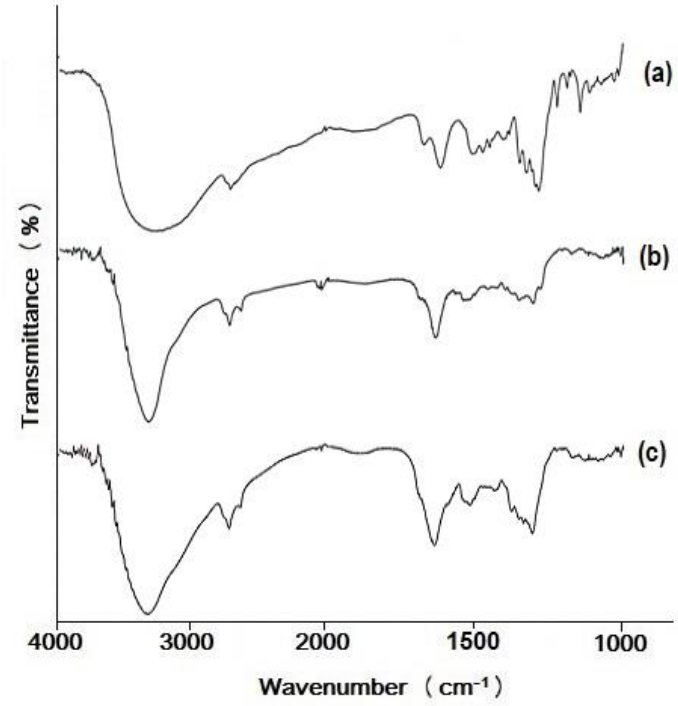

Figure 1: FTIR spectra of pectin BP (a), extracted water melon (b) and cucumber (c) peel pectin

Granule flow properties

Table 3 shows the bulk and flow properties of the various batches of oxytetracycline granules formulated with *C. lanatus* and *C. sativus* peels pectin, pectin BP and CMC as binders. Generally, the results of the bulk and the tapped densities of granules showed a significant reduction in volume. Granule particle shape and size distribution are largely responsible for these parameters and the resultant inter particulate interaction within the granule bed leading to granule densification with smaller particles filling the voids in-between the larger particles (Staniforth and Aulton, 2007).

The calculated values for Carr's indices and Hausner's ratios of oxytetracycline granules ranged from 1.12 ± 0.018 to 1.33 ± 0.012 and 11.11 ± 0.014 to 32.61 ± 0.010%, respectively. Also, the granules' angles of repose and flow rates ranged from 30.47 ± 0.022 to 43.89 ± 0.017° and 1.17 ± 0.018 to 5.18 ± 0.014 g/sec, respectively.

The micromeritic of the oxytetracycline granules formulated with the extracted plants pectin compared favourably with those of standard binders (CMC and Pectin BP), revealing good flowability and compressibility of the granules (BP, 2004).

Table 3: Micromeritic properties of oxytetracycline granules

Batch	Bulk density (g/ml)	Tapped density (g/ml)	Hausner's ratio	Carr's index (%)	Angle of repose (°)	Flow rate (g/sec)
A	0.45 ± 0.010	0.58 ± 0.020	1.29 ± 0.016	28.89 ± 0.011	30.47 ± 0.022	5.18 ± 0.014
B	0.46 ± 0.014	0.61 ± 0.011	1.33 ± 0.012	32.61 ± 0.010	34.01 ± 0.018	4.00 ± 0.014
C	0.58 ± 0.018	0.70 ± 0.013	1.21 ± 0.018	20.69 ± 0.012	43.89 ± 0.012	1.54 ± 0.018
D	0.50 ± 0.016	0.62 ± 0.019	1.24 ± 0.014	24.00 ± 0.021	32.47 ± 0.022	1.39 ± 0.016
E	0.54 ± 0.018	0.65 ± 0.018	1.21 ± 0.018	11.11 ± 0.014	34.87 ± 0.013	1.17 ± 0.018
F	0.57 ± 0.020	0.67 ± 0.018	1.18 ± 0.022	17.54 ± 0.018	36.66 ± 0.014	4.50 ± 0.021
G	0.47 ± 0.018	0.59 ± 0.018	1.26 ± 0.013	25.53 ± 0.016	33.29 ± 0.021	1.35 ± 0.016
H	0.50 ± 0.021	0.63 ± 0.022	1.26 ± 0.018	26.00 ± 0.016	35.85 ± 0.015	4.20 ± 0.018
I	0.57 ± 0.018	0.64 ± 0.017	1.12 ± 0.018	12.28 ± 0.020	43.89 ± 0.017	1.92 ± 0.020
J	0.46 ± 0.018	0.57 ± 0.018	1.24 ± 0.015	23.91 ± 0.019	31.43 ± 0.016	3.29 ± 0.011
K	0.48 ± 0.021	0.62 ± 0.016	1.29 ± 0.020	29.17 ± 0.013	33.06 ± 0.020	2.78 ± 0.013
L	0.52 ± 0.018	0.67 ± 0.014	1.29 ± 0.022	28.85 ± 0.018	37.01 ± 0.012	1.54 ± 0.028

Physicochemical properties of oxytetracycline capsules

The results of the physicochemical properties of oxytetracycline capsules formulated with different concentrations of the binders are shown in Table 4. The standard specification for uniformity of weight states that for capsules weighing above 324 mg of not more than 2 capsules should deviate from the average weight by more than 7.5% . Oxytetracycline capsules formulated passed the weight uniformity test for not having more than 2 capsules deviating from the average by not more than 7.5% (United State Pharmacopoeia, 2011). The disintegration times of the capsules ranged from 5.40 - 6.50 min (Batches A-C), 5.60 - 6.67 min (Batches D-F), 6.00 - 7.10 min (Batches G-I) and 6.09 - 7.40 min (Batches J-L) and they were within the pharmacopoeial limits for capsules (BP, 2010).

Table 4: Physicochemical properties of formulated oxytetracycline capsules

Batch	Weight (mg)	Disintegration time (min)
A	0.632 ± 0.023	5.40 ± 0.018
B	0.645 ± 0.014	6.10 ± 0.016
C	0.637 ± 0.025	6.50 ± 0.012
D	0.632 ± 0.018	5.60 ± 0.018
E	0.641 ± 0.036	6.30 ± 0.011
F	0.644 ± 0.028	6.67 ± 0.022
G	0.634 ± 0.022	6.00 ± 0.018
H	0.640 ± 0.031	6.40 ± 0.010
I	0.638 ± 0.029	7.10 ± 0.004
J	0.636 ± 0.027	6.09 ± 0.020
K	0.630 ± 0.027	6.40 ± 0.014
L	0.641 ± 0.021	7.40 ± 0.008

Dissolution profiles for the formulated oxytetracycline capsules

Dissolution profiles and some parameters of batches of the formulated oxytetracycline capsules are shown in Figures 2a, 2b and Table 5. Various batches exhibited variable maximum drug release at 60 min with the 1.0 %w/v of each binder (Batches A, D, G and J) showing the highest amount of drug release of 92.14, 89.23, 82.28 and 91.15%, respectively. These batches showed a burst released of their drug content with their low $T_{25\%}$ and apart from batches E and K capsules that also exhibited over 70% of drug release, their $T_{75\%}$ were much lower.

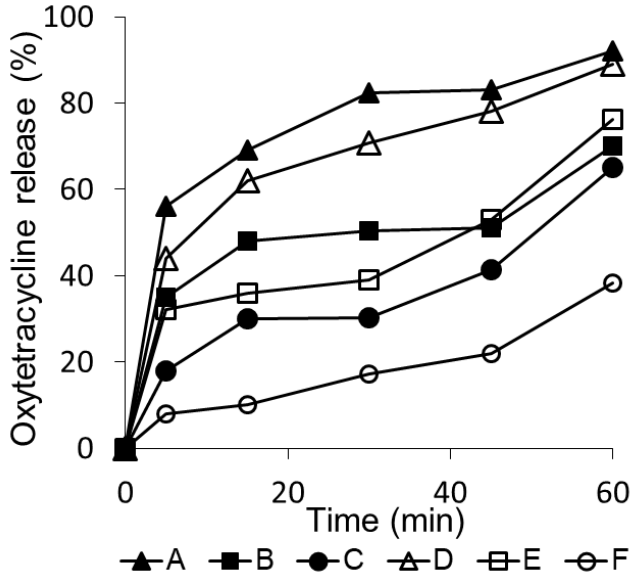

Figure 2a: Dissolution profiles of batches (A-F) of the oxytetracycline capsules

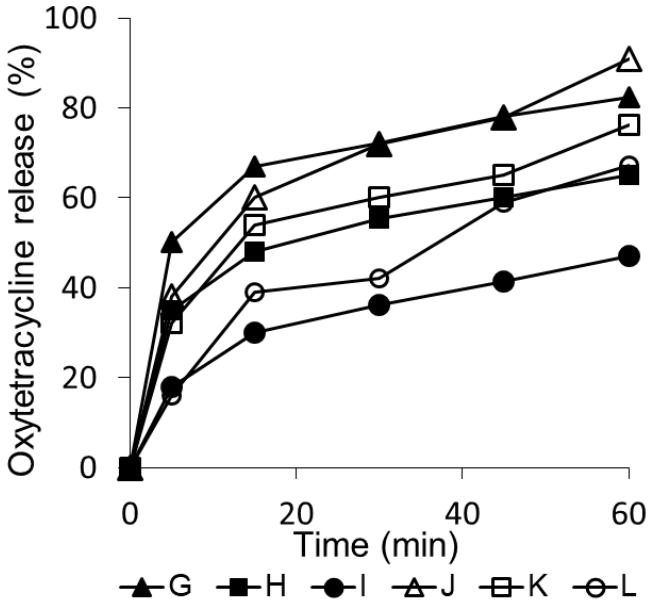

Figure 2b: Dissolution profiles of batches (G-L) of the oxytetracycline capsules

Table 5: Dissolution parameters of formulated oxytetracycline capsules

Batch	$T_{25\%}$ (min)	$T_{50\%}$ (min)	$T_{75\%}$ (min)
A	2.0	4.5	22.0
B	3.5	25.0	-
C	11.0	50.5	-
D	2.5	8.3	38.0
E	4.0	43.0	59.0
F	48.0	-	-
G	2.5	5.0	37.0
H	3.3	19.0	-
I	11.0	-	-
J	3.5	10.5	37.0
K	3.8	13.0	58.0
L	9.0	37.0	-

There were decreased amount of drug released with increase in pectin binder concentration for all batches. Comparatively, only the batches of capsules prepared with 1.0 %w/v of binder met the British Pharmacopoeia specification of 70% drug release in 45 min (BP, 2010). However, at binder concentrations of 5%w/w of the extracted pectin, the percentage drug released were relatively low hence, at this concentration and above, the extracted pectin may be employed for sustained released dosage formulation of oxytetracycline capsules (Benjabhorn *et al*., 2017).

Drug-excipient interactions

Figure 3 (a), (b) and (c) showed that the spectra of pure oxytetracycline (a) and oxytetracycline granules prepared with extracted water melon (b) and cucumber (c) peels pectin. The characteristic bands of pure oxytetracycline spectra are seen in the spectra of the formulated granules of oxytetracycline with watermelon and cucumber peels pectin. Also no new peaks were evident in the granule formulations, indicative of absence of chemical reactions in the dosage forms (Cocchi *et al*., 2004; Kopecka and Svobodova, 2014).

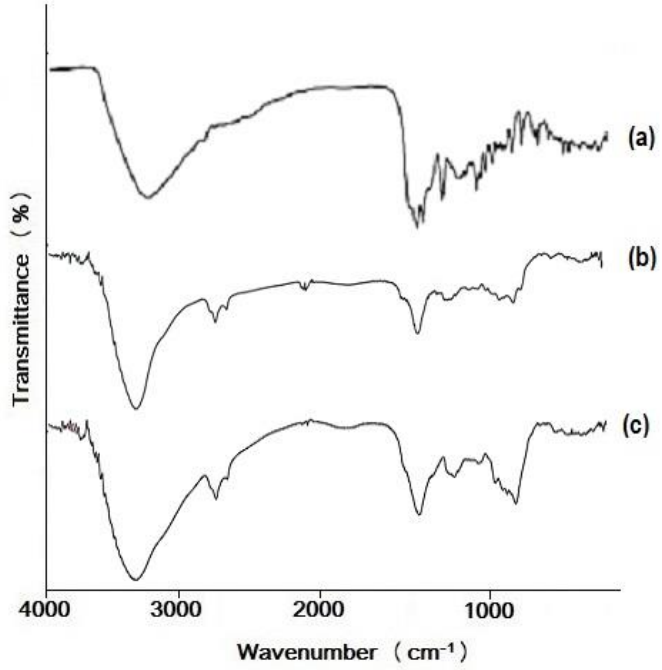

Figure 3: FTIR spectra for pure oxytetracycline (a) and oxytetracycline granules prepared with extracted water melon (b) and cucumber (c) peels pectin

CONCLUSION

Watermelon and Cucumber peels pectin were successfully extracted, identified, evaluated and used as binder in the formulation of oxytetracycline capsules. The percentage amount of drug released decreased as the concentration of the binder increased. Binding capacities of the extracted pectins were comparable to those of carboxymethyl cellulose and pectin BP. The FTIR analysis results showed no interactions between the drug and excipients, as there were no observable new peaks in the spectra of the formulations. Hence, pectin from the peels of water melon or cucumber may be a viable alternative to pectin BP and CMC as binders in capsule formulations.

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