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COMPARATIVE EVALUATION OF PHYSICOCHEMICAL PROPERTIES OF AMPICILLIN TRIHYDRATE CAPSULES FORMULATED WITH *CHRYSOPHYLLUM AFRICANUM* AND *DAUCUS CAROTA* PEELS PECTIN AS BINDERS

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ABSTRACT

Background and aim: Attempt has been made in this study to evaluate and compare the binding properties of two natural sources of pectin, *Chrysophyllum africanum* and *Daucus carota* peels with a standard binder, Carboxymethyl cellulose (CMC) in the formulation of Ampicillin trihydrate capsules.

Methods: Pectin was extracted from both fruit peels using ethanolic (95%)- HCL (1:20, pH 2.0) under reflux at 90ºC for 60 minutes. They were subjected to spectral analyses and determination of physicochemical properties for the formulation of Ampicillin trihydrate capsules using standard methods.

Results: Ampicillin trihydrate granules prepared with *C. africanum* peel pectin (CAPP) and *D. carota* peel pectin (DCPP) and Carboxymethyl cellulose (CMC) as binders had good flow properties. Their angle of repose ranged from 55.22º - 56.65º (CAPP); 44.70º - 57.99º (DCPP) and 53.13º - 58.26º (CMC); Hausner's ratio 1.488º - 1.60 (CAPP), 1.355 - 1.476 (DCPP) and 1.266º - 1.506º (CMC); Carr's index values from 32.78-37.78 (CAPP), 21.03-33.58 (CMC) and 26.70-32.26 (DCPP). Disintegration time (minutes) were 5.31 - 5.93 (CAPP), 5.92 - 6.27 (CMC) and 4.64 - 5.24 (DCPP) respectively, and are within Pharmacopoeial limits for tablets and capsules. Fourier Transform Infrared spectroscopy studies showed no evidence of incompatibility of extracted pectins with other excipients or Ampicillin trihydrate in the formulated dosage forms. Dissolution studies revealed decreases in the amount of drug released with increase in pectin binder concentration of 1-5% for all formulations - CAPP (70%, 60%, 40%) and DCPP (73%, 62%, 42%). However, increases were evident at 1%w/v binder concentration from 20-60 minutes as follows: CAPP (15%, 60%75%); and DCPP (45%, 62%, 73%). All these results compared with those of standard binder.

Conclusion: Peel pectins extracted from *C. africanum* and *D. carota* fruits exhibited good binding property when compared with standard binder, Carboxymethyl cellulose (CMC) and can thus serve as substitute binders in the formulation of Ampicillin trihydrate capsules, thereby conserving foreign exchange for the Nation and enhancing farmers' wealth.

Keywords: *Chrysophyllum africanum*, *Daucus carota*, peel pectin, Ampicillin trihydrate capsules

INTRODUCTION

Pectin is a polysaccharide consisting mostly of two moieties: homogalacturonan, (1-4) linked, a-D- galacturonic acid and its methyl ester; as well as rhamnogalacturonan 1, (1-2) repeating linked, a-L-rhamnose-(1-4) a-D-galacturonic acid disaccharide [1]. The various natural sources of pectin include citrus peels, carrot peels, cherry exocarp, sugar beets, and residues of mango, guava, dried apple pomace, papaya and cocoa processing [2, 3].

In recent years, plant-derived polymers have evoked interest due to their diverse industrial applications. Natural polymers like pectin are easy to isolate and purify being non-toxic and biocompatible. Pectins have been used in food industry, and recently explored for their other pharmaceutical applications as excipients (binding, thickening and suspending properties) [1, 4-8] and in cosmetics, textiles, paints, and paper industry [9]. The plant-based polymers have been studied for their applications in different pharmaceutical dosage forms like matrix controlled system, film coating agents [5, 6]. They have also been utilized as viscosity enhancers, stabilizers [10]. Pectin possesses several requisite characteristics to be used as polymer in drug development and release kinetics. It is employed in pharmaceutical industry as a carrier for drug delivery to the gastrointestinal tract, such as matrix tablets, gel beads and film-coated dosage form.

Many natural materials have advantage over synthetic materials since they are non-toxic, cost effective, readily available and biodegradable [11, 12]. Pectin is used extensively as a gelling agent in the food industry which remains the biggest markets for pectin. Pectin is a naturally occurring biopolymer that is finding increasing applications in the pharmaceutical and biotechnology industry [13, 14]. Cherry peel and carrot peel pectins have shown good property as natural binding agents. Pectin has the required stability under acidic conditions even at higher temperature, hence suitable as candidate to be used in drug delivery system. They have good gel forming ability in presence of divalent cations which makes them suitable carriers for delivering bioactive agents [8].

African star apple (*Chrysophyllum africanum,* Sapotaceae) is a dominant canopy tree of lowland and mixed rain forests, widely distributed in tropical West Africa countries such as Ghana, Nigeria, Kenya and Uganda. It attains 8–36 m in height, fruiting from December – April and is found abundantly in southern part of Nigeria [15]. Various parts of the plant have been studied for medicinal and nutritive properties [16, 17]. Carrot (*Daucus carota,* Apiaceae) is an important food crop utilized worldwide and its production is associated with by-products such as culled carrots and carrot waste (Carrot pomace). A variety of technologies aimed at adding value to the by-products or lessening the environmental impacts of current disposal strategies have been explored in the recent years. It is an important vegetable valued for its fleshy edible colourful roots. Plant and their products have always been a source of various drugs and excipients used in pharmaceutical formulations. Ampicillin is one of the drugs developed against bacteria which has gained broad use as an antibacterial agent. Ampicillin is on the World Health Organisation List of Essential Medicines. The WHO classifies ampicillin as critically important for human medicine. Ampicillin is used to treat infections by many Gram-positive and Gram-negative bacteria, including *Streptococcus pneumoniae, Streptococcus pyogenes*, some isolates of *Staphylococcus aureus*.

The aim of this study is to extract pectin from dried *C. africanum* fruit peel and dried carrot (*D. carota*) exocarp, and determine their binding properties in the formulation of ampicillin trihydrate capsules.

MATERIALS AND METHODS

Drugs, Chemicals and Solvents

Ampicillin trihydrate BP (Venu Health care, weight bridge, Kadi Road, India); pectin powder BP (Welfang Ensign Industries Co. Ltd, China); lactose powder BP (Danone®, Germany); carboxyl methyl cellulose powder BP (Central Drug House Ltd, Vardaan, New Delhi); ethanol 95% (CDH, Vardaan, Daryang, New Delhi); glacial acetic acid (CDH, Vardaan, Dariyang, New Delhi).

Plant collection and preparation of exocarp

The *C. africanum* (cherry) fruits purchased from a local market in Benin City were sorted and washed under running water. Mesocarp were carefully separated from the exocarp mechanically to obtain a thin skin (exocarp), and air-dried in the sun for 3 days to reduce the moisture content. The exocarp was then air dried on the laboratory floor for 5 days, milled and stored in an air-tight container for further use.

Daucus carota (carrot) roots were purchased from a local market in Benin City, Edo State, Nigeria. They were washed under running water and a scrapper was mechanically used to remove their exocarp layers. The exocarp was sun dried for 48 hours to reduce the water content and then air dried in the laboratory for 5 days. The dry peels were milled to powder and sieved using a stainless steel filter and then stored in an airtight container for further analysis.

Pectin extraction from *C. africanum* exocarp powder

Pectin extraction was carried out using the process described by McCready [17] as contained in Nsude, *et al* [18]. Milled exocarp powder (300 g) was converted into slurry by adding hot distilled water up to 100 ml while stirring, boiling, mixture and stirring for 15 minutes on a water bath for tissue disintegration. Aliquot of hot 95% ethanol (500 ml) was added to attain 1200 ml, mixture stirred continuously for 2 hours at 50^{o}C until no gelatinous particles were visible. Resultant slurry was rapidly filtered using 80 mm screen mesh (jute bag), residue washed back into beaker with hot water. Hot 95% ethanol was then added to the residue in small amounts, while stirring over a water bath for 15 minutes. Mixture was again filtered and added to the previous filtrate to give a final extract which was cooled rapidly to a temperature below 25^{o}C to minimize heat degradation of pectin. Fifty millimeters of 0.02 N NaOH was added and solution allowed standing for another 1 hour. Precipitated pectin was washed with acetic acid and carefully decanted before filtration. Finally, extracted pectin was oven-dried for 6 hours at 35^{o}C, milled to obtain uniformity and stored in an air-tight container for further analysis. Percentage yield was calculated on a dry weight basis and recorded.

Pectin extraction from carrot peels powder

The method of McCready [17] as contained in Nsude *et al*. [18] was adopted. Pectin was extracted from carrot peel powder (300 g) with water acidified with HCl (pH 2.0) under reflux condenser for 60 minutes (solute/solvent 1:20). Extracted pectin was filtered using a stainless steel filter and acid-pectin extract precipitated with acidic alcohol solution for 24 hours. Floating pectin precipitate was filtered, washed with alcohol several times, dried to constant weight at 40 –50^{o}C for 6 hours and cooled in a desiccator. The hard extracted pectin cake was milled,

sieved to obtain fine pectin powder and the yield determined.

Preparation of Ampicillin trihydrate granules

The wet granulation method [19] was employed to produce 12 batches of granules, each suitable for preparing 50 capsules of ampicillin 500 mg. Pure pectin, carboxymethyl cellulose, *C. africanum* and carrot peel pectin were separately used as binders at concentration of 1, 3, and 5% w/v. For each batch containing 500 mg of ampicillin trihydrate powder, finely powdered lactose and corn starch BP at their appropriate quantities per batch were dry mixed in a bowl mixer. The different binders at different concentrations were used to wet mass the powder mixture, dried in an oven at 60ºC for 30 minutes. Dried granules were passed through a sieve of aperture size BSS 22, and packed in an airtight glass container for further analysis.

Evaluation of Physicochemical properties of Ampicillin trihydrate granules

Angle of repose: Fifty grams of granules were allowed to flow freely under gravity through a funnel clamped on a retort stand with its tip 6 cm above a smooth aluminium foil placed on a flat horizontal surface. The height of the cone formed, h, and the radius of the base, r, were carefully measured. The tangent of the angle of repose was calculated using the equation:

$$Tan\,\theta = \frac{h}{r} \quad \ldots\ldots 1$$

Flow rate: The Erweka Granules Flow tester was employed in the determination. Fifty grams of the granules were allowed to flow through the orifice of the equipment time taken to pass through was noted and the rate of flow per second was calculated as:

$$Flowrate\ (g/sec) = \frac{Weight\ of granules\ (g)}{Time\ taken\ (\text{sec})} \quad 2$$

Bulk and tapped densities: Fifty grams granules (W_o) were placed in a 250 ml measuring cylinder and the volume (bulk), occupied by each of the samples without tapping was noted. Bulk density was calculated according to the equation 3. Bottom of cylinder was tapped for about 100 times on a tabletop until no change in volume was observed. Tapped volume occupied was noted and tapped density was calculated as the ratio of weight to the tapped volume as shown in equation 4.

$$Bulk\ density = \frac{Weight\ of\ granules\ (g)}{Bulk\ volume\ (cm^3)} \quad \ldots 3$$

$$Tapped\ density = \frac{Weight\ of\ granules\ (g)}{Tapped\ volume\ (cm^3)} \quad 4$$

***Hausner's Ratio*:** This was calculated as the ratio of tapped density to bulk density of the samples.

$$Hausner's\ ratio = \frac{Tapped\ density}{Bulk\ density} \quad \ldots\ldots\ldots 5$$

***Carr's (Compressibility) Index*:** This was calculated as follows:

$$\%\ Compresibility = \frac{Tapped\ density - Bulk\ density}{Tapped\ density} \times \frac{100}{1} \quad \text{---------- } 6$$

Evaluation of physicochemical properties of Ampicillin trihydrate capsules

The capsule properties evaluated include weight uniformity, disintegration time, and dissolution profile.

***Weight uniformity*:** The British Pharmacopoeia method [20] was used in the determination. Twenty capsules were weighed individually and collectively, and mean weight recorded. Percentage

coefficient of the capsule weight variation was calculated from the expression:

$$Percent\ (\%)\ variation = \frac{Standard\ deviation}{Mean\ weight} \times \frac{100}{1} \text{---------- 7}$$

***Disintegration test*:** The British Pharmacopoeia disintegration test method [20] was adopted. Six capsules were selected at random from each batch. The disintegration time for each batch was determined using the Erweka disintegration apparatus. Distilled water was used as the medium maintained at 37 ± 1^{o}C. One capsule each was placed in 3 test tubes of the machine. The time taken for the individual capsule to disintegrate and their particles to pass through the mesh of the tube was recorded. The average time for the 3 capsules was recorded as the disintegration time for each batch.

Dissolution test: The British Pharmacopoeia dissolution rate test method [20] was adopted. An Erweka dissolution test apparatus fitted with rotating paddle moving at 100 rpm was used. The dissolution medium was 800 ml, 0.1N hydrochloric acid (pH 1.2) maintained at 37±2^{o}C for 1 hour 30 minutes. Aliquot (5 ml) of the dissolution medium was withdrawn with a pipette at 0 min, 15, 30, 45, 60, 75 and 90 minutes' intervals, respectively and replaced with fresh dissolution medium at each instance. The absorbance was read at wavelength of 463nm with a Spectrophotometer Model HP211. The equivalent drug concentration was determined using the standard Beer-Lamberts plot.

***Preparation of calibration curve*:** Pure ampicillin trihydrate BP powder (100mg) was weighed and transferred into a 100ml volumetric flask. This was carefully dissolved with 0.1N HCl in the volumetric flask, solution made up to volume with 0.1N HCl, Serial dilutions were made and absorbance read spectrophotometrically (Model HP211) at 463 nm. The equivalent drug concentration was determined using the standard Beer-Lambert's plot.

***Fourier transform infra-red spectroscopy*.** Using the British Pharmacopoeial dissolution rate test method [20], potassium bromide (KBr) salt powder (100 mg) was weighed and mixed uniformly with 5 mg sample in fine particle size state. Finally, the sample was placed in an evacuable KBr dye and a 13 mm clear disk was pressed on it in a hydraulic press to form KBr pellet. Pelletized sample was placed in a cell holder and inserted into the machine (FTIR) and scanned at a range of 350 – 400 nm or more. After few seconds, the spectrum was displayed on both the computer screen and inspected compound page.

RESULTS AND DISCUSSION

The yields of the extracted pectins from *C. africanum* and *D. carota* were 2.44% and 2.9%, respectively. Organoleptic properties of peel powder of *C. africanum* were: light brown, odourless, bland taste; while *D. carota* recorded reddish brown colour, characteristic odour and bland taste (Table 1). These results are in agreement with previous work by Anokam [6].

Table 1. Organoleptic properties of *Chrysophyllum africanum* and *Daucus carota* peel powders

C. africanum		*D. carota*	
Parameter	**Result**	**Parameter**	**Result**
Percentage yield	2.44%	Percentage yield	2.90%
Organoleptic properties		Organoleptic properties	
Colour	Light brown	Colour	Reddish brown
Odour	Odourless	Odour	Characteristic
Nature	-	Nature	Amorphus
Taste	Bland	Taste	bland

Table 2. Phytochemical properties of *Chrysophyllum africanum* and *Daucus carota* peels powder

Phytochemical Constituent	*C. africanum* peel powder	*D. carota* peel powder
Alkaloid	+	+
Anthraquinone	-	+
Flavonoid	+	-
Saponin	-	-
Cardiac glycoside	-	-
Terpenoid	+	+
Tannin	+	+
Polysaccharides	+	-
Carbohydrate	-	+

Key: + (present), - (absent)

Micromeritis properties of ampicillin trihydrate granules

Table 3 shows micromeritis properties of ampicillin granules formulated with *C. africanum, D. carota* peel pectins and pure pectin powders. Results showed range value: Angle of repose (*C. africanum*: 55.22^o - 56.65^o), (*D carota:* 44.70^o - 57.99^o), (Pure pectin BP: 48.01^o -54.88^o); Hausner's ratio (*C. africanum*: 1.488 - 1.607), (*D. carota*: 1.355 - 1.476), (Pure pectin BP: 1.185-1.286); Compressibility index (*C. africanum*: 32.78% - 37.78%), (*D. carota*: 26.20% - 32.26%), (Pure pectin BP: 15.59% - 22.23%). The micromeritic of the extracted plant pectin compared favourably with those of standard binders (CMC and Pectin BP), indicating good flowability and compressibility of the granules [7, 8].

Table 3. Micromeritics properties of Ampicillin trihydrate granules

Formulation	Angle of repose (θ)	Bulk density (g/ml)	Tapped density (g/ml)	Hausner's ratio	Compressibility index (%)	Flow rate (g/sec)
Cherry pectin 1%	55.22	0.280	0.449	1. 607	37.78	0.033
Cherry pectin 3%	56.22	0.303	0.451	1.488	32.78	0.037
Cherry pectin 5%	56.65	0.335	0.500	1.491	32.94	0.035
Carrot pectin 1%	44.70	0.357	0.484	1.355	26.20	0.050
Carrot pectin 3%	51.63	0.357	0.503	1.410	29.50	0.089
Carrot pectin 5%	57.99	0.359	0.530	1.476	32.26	0.078
CMC 1%	53.13	0.340	0.475	1.398	28 .47	0.032
CMC 3%	57.60	0.303	0.456	1.506	33.58	0.024
CMC 5%	58.26	0.335	0.425	1.266	21.03	0.033
Pure pectin 1%	48.01	0.395	0.467	1.185	15.59	0.051
Pure pectin 3%	48.19	0.396	0.493	1.245	19.65	0.093
Pure pectin 5%	54.88	0.436	0.561	1.286	22.23	0.073

Physicochemical properties of ampicillin trihydrate capsules

Table 4 shows the results of the physicochemical analysis of the ampicillin capsules formulated with the different binders. Disintegration time ranged from 5.31 - 5.93 minutes (*C. africanum*), 5.92 - 6.27 (CMC), 4.64 - 5.24 (Carrot pectin), respectively and are within British Pharmacopoeial limits for tablets and capsules [20]. The compendia 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% [20]. Ampicllin trihydrate capsules formulated with 5% each of *C. africanum* pectin and *D. carota* passed the weight uniformity test for not having more than 2 capsules deviating from the average by more than 7.5% [21]. The variation could be as a result of human handling in the process of hand filling of the granules. All the formulations passed the disintegration time of not more than 15 minutes [20].

Table 4. Physicochemical properties of formulated ampicillin trihydrate capsules.

Formulation	Weight variation (mg)	Disintegration time (mins)
Cherry pectin 1%	0.637± 0.039	5.31
Cherry pectin 3%	0.670 ± 0.032	5.71
Cherry pectin 5%	0.659 ± 0.032	5.93
Carrot pectin 1%	0.638 ± 0.026	4.58
Carrot pectin 3%	0.620 ± 0.044	4.64
Carrot pectin 5%	0.654 ± 0.028	5.24
CMC 1%	0.652 ± 0.030	5.92
CMC 3%	0.654 ± 0.033	6.22
CMC 5%	0.667 ± 0.024	6.27
Pure pectin 1%	0.654 ± 0.023	6.09
Pure pectin 3%	0.651 ± 0.030	6.30
Pure pectin 5%	0.653 ± 0.031	7.15

FTIR spectra for ampicillin trihydrate capsule formulations

Fourier Transform Infrared (FTIR) spectroscopy is a time saving method that is used to detect a range of functional groups and is sensitive to changes in molecular structure. FTIR provides information on the basis of chemical composition and physical state, of the whole sample [20, 21]. From

Figure 6 A to Figure D and Figure 7A to Figure D, it showed that the spectra for cherry peel pectin and carrot peel pectin had the characteristic absorption bands of pure pectin BP. FTIR spectrum is also used to determine compatibility of active pharmaceutical ingredients with the excipients. From the results of spectra for Ampicillin trihydrate capsules formulated with cherry and carrot peel pectin, the characteristic bands for pure pectin were present. In addition, no new peaks were evident, indicating there were no chemical reactions in the dosage forms.

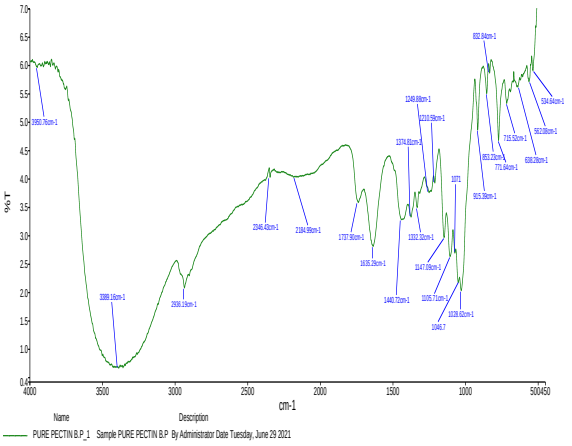

Figure 6A: FTIR spectrum of pure pectin B.P

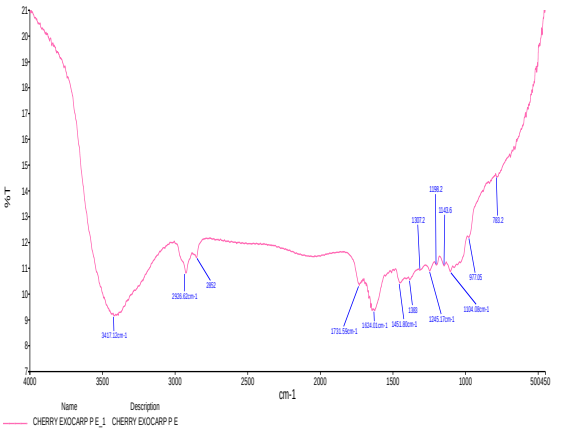

Figure 6B: FTIR spectrum of cherry exocarp pectin

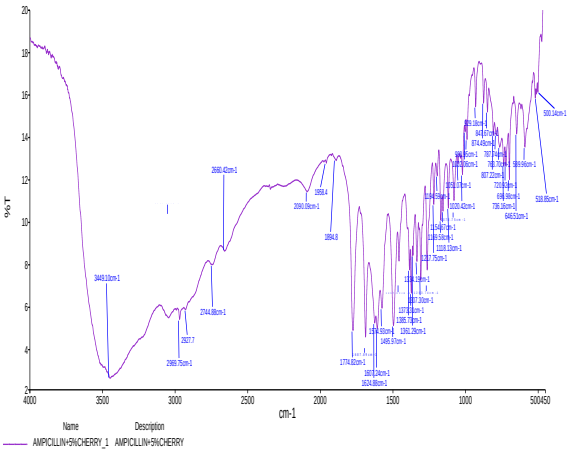

Figure 6C: FTIR spectrum of ampicillin trihydrate capsule formulated with 5% cherry pectin at wavelength of 4000-450 nm.

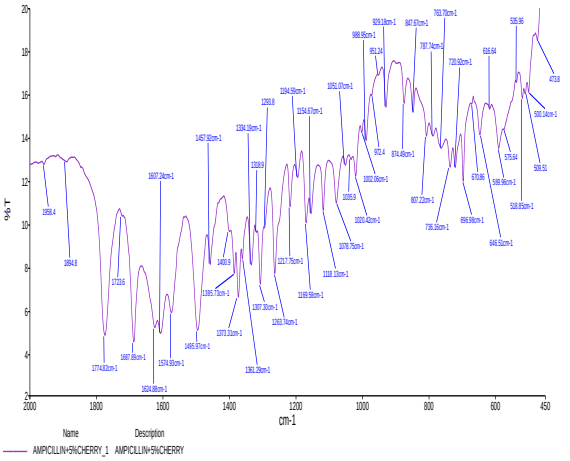

Figure 6D: FTIR spectrum of ampicillin formulated with 5% cherry pectin at wavelength 2000 - 450 nm

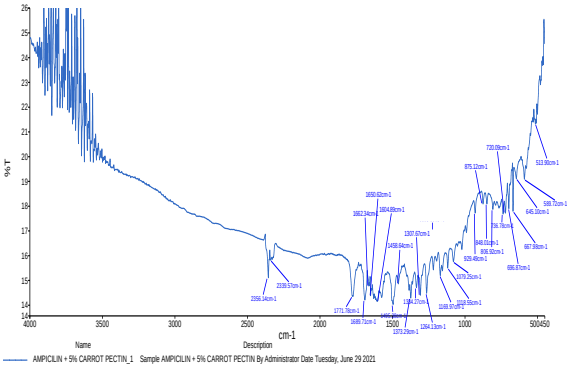

Figure 7A: FTIR spectrum of ampicillin trihydrate capsule formulated with 5% carrot pectin at wavelength 4000 - 450 nm

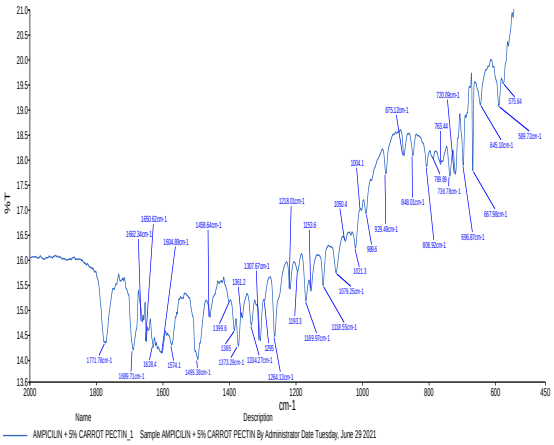

Figure 7B: FTIR spectrum of ampicillin trihydrate capsule formulated with 5% carrot pectin at wavelength 2000 - 450 nm

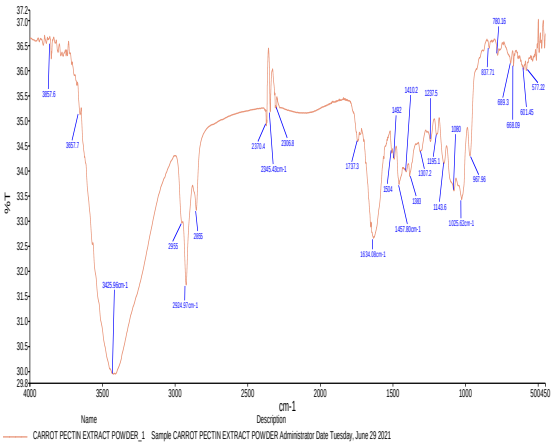

Figure 7C: FTIR spectrum of carrot pectin extract powder

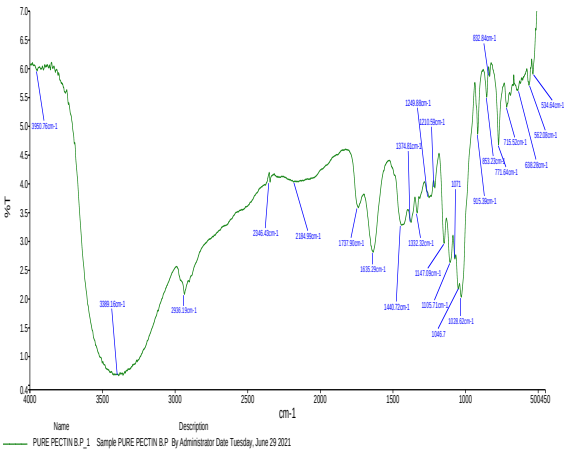

Figure 7D. FTIR spectrum of pure pectin B.P

Dissolution test results and profiles for formulated Ampicillin trihydrate capsules

The Standard calibration curve of pure Ampicillin trihydrate BP of known concentrations (µg/ml) is shown in Figure 8 with regression coefficient R^2 = 0.9916. Dissolution rate is the rate-limiting step in absorption of the drug *in vivo*.

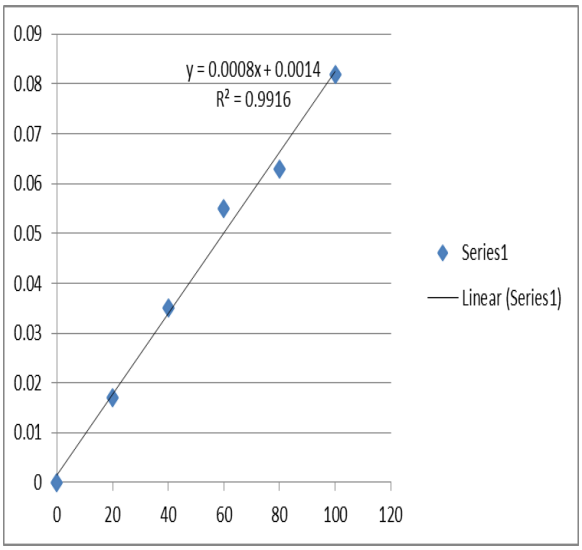

Figure 8. Calibration curve for Ampicillin trihydrate BP powder

Dissolution studies showed decrease in the amount of drug released with increase in pectin binder concentration for all batches as follows- *C. africanum* peel pectin (CAPP) [1%w/v (70%), 3%w/v (60%) and 5%w/v (40%)]; *D. carota* peel pectin (DCPP) – [1%w/v (73%), 3%w/v (62%) and 5%w/v (42%)] (Figures 9-11). Also, an increase in amount released with time at 1%w/v binder concentration was evident, CAPP [20 min (15%), 40 min (60%), 60 min (75%)]; while DCPP gave 45%, 62%, 73%, respectively (Figures 9-10). These results from Ampicillin trihydrate formulated with the extracted pectin showed same trend with results of the standard binder, CMC. Comparatively, the test binders (CAPP and DCPP) at binder concentration,1%w/v released 70-73% of their drug from the dosage forms. However,

at higher binder concentrations (3% and 5%), the amount of drugs released were relatively lower, hence at these concentrations, the test binders may be employed for delayed/sustained release dosage formulation of Ampicillin trihydrate capsules [7, 15].

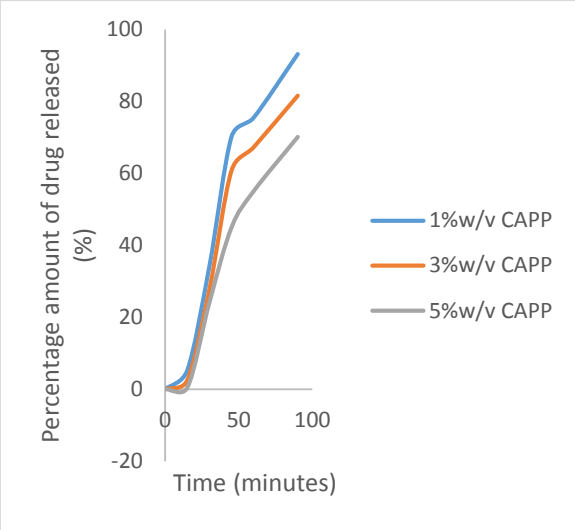

Figure 9. Dissolution profiles illustrating percentage drug released with time for Cherry peel pectin at various concentrations

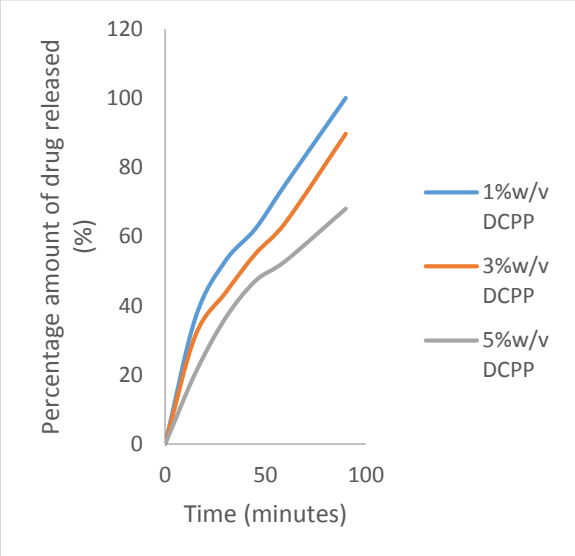

Figure 10. Dissolution profiles illustrating percentage drug released with time for Carrot peel pectin at various concentrations

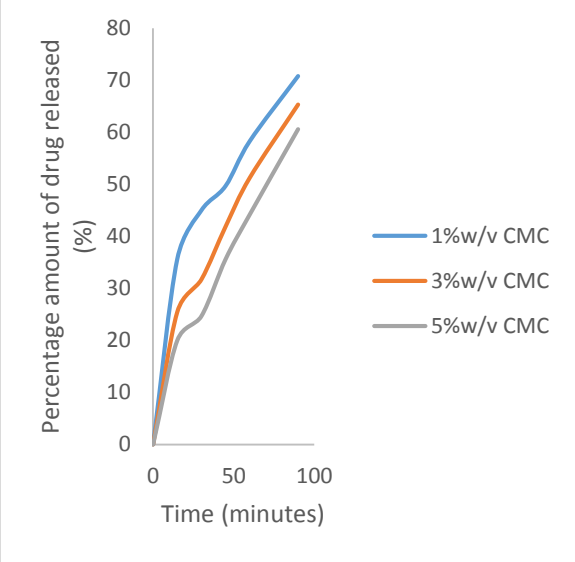

Figure 11. Dissolution profiles illustrating percentage drug released for CMC at different concentrations

CONCLUSION

This study evaluated C*hrysophyllum africanum* and *Daucus carota* peel pectin extracts as a binder in the formulation of Ampicillin trihydrate capsules. As concentration of the binders increased, percentage amount of drug released decreased. Binding capacities of extracted pectins were comparable to that of the standard binder, Carboxymethyl cellulose BP. The FTIR results also showed that extracted pectins were stable and non-reactive with neither the active pharmaceutical ingredient nor any excipients in the formulation, and therefore considered safe, and are recommended to be used as substitute to the standard binder (CMC). It is reasonable to infer from the results that the two extracted pectins can be used as substitute to Pectin powder BP and Carboxylmethyl cellulose BP which are imported binders, thereby conserving the

scarce national foreign exchange. These results point to the fact that the farmers' wealth can be increased, financial losses due to early post-harvest spoilage can minimize as these plant raw materials are processed into pharmaceutical excipients.

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