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Comparative Evaluation of The Physicochemical Properties and Dissolution Profiles of Amoxicillin Capsules Formulated with *Citrus Sinensis* and *Magifera Indica* Peels Pectin

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Abstract

Background and aim: The study evaluated the potential of extracted pectin from *Citrus sinensis* and *Magifera indica* fruit peels and compared with standard Carboxymethyl cellulose BP and Pectin BP respectively as binders in the formulation of Amoxicillin capsules.

Methods: Pectin was extracted from both fruits of *C. sinensis* and *M. indica* peels using ethanol (95%) – hydrochloric acid (HCL) (1:20, pH 2.0) under reflux at 90° C for 60 minutes. These extracts were dried, milled and subjected to spectral and phytochemical analysis. Dry granulation technique was used to prepare amoxicillin granules, milled, and evaluated for micromeritics properties. The granules were carefully hand- filled into hard gelatin shells to contain 500 mg of amoxicillin trihydrate per capsule. Weight uniformity, disintegration time and dissolution profiles of the various batches were determined.

Results: Both *C, sinensis* and *M. indica* peel powders contain alkaloid, flavonoid, saponin, cardiac glycoside, and tannin and carbohydrate. The average angle of repose for Amoxicilin granules containing orange and mango peel pectin, pure pectin and carboxymethyl cellulose were: 33.69° , 34.27° , 33.43° and 34.33° respectively. Bulk density – 0.06, 0.63, 0.61 and 0.69 g/cm³. Tapped density – 0.70, 0.71, 0.71 and 0.69 g/ml. Hausner's ratio – 1.17, 1.11, 1.17 and 1.14, respectively. The flow rates of the granules were generally satisfactory, hence can be readily compressed or filled into gelatin capsule shells. Weight variation of Amoxicillin capsules prepared with orange and mango peel pectin, pure pectin and carboxymethyl cellulose ranged from 0.68 to 0.069; 0.67 to 0.71; 0.67 to 0.71 and 0.69 to 0.70 mg respectively. Disintegration time with orange and mango peel pectin, pure pectin and carboxymethyl cellulose ranged from 4.29 to 7.07; 5.01 to 9.69; 5.17 to 6.17; 3.19 to 3.95 min, respectively. Dissolution profiles showed amount of drug released from Amoxicillin capsules formulated with orange peel pectin at 60 min to be between 50% (5% w/w) to 70% (1% w/w); mango peel pectin 58% (5% w/w) to 78% (1% w/w) and carboxymethyl cellulose 45% (5% w/w) to 63% (1% w/w) and pure pectin 52% (5% w/w) to 78% (1% w/w) respectively.

Conclusion: Pectins extracted from *Citrus sinensis* and *Magnifera indica* fruit peels exhibited good and comparative binding and release properties with those of amoxicillin capsules formulated with Carboxymethyl cellulose BP (CMC), and Pectin BP respectively; hence, can serve as substitute binders in the formulation of amoxicillin trihydrate capsules.

Keywords: Capsules, Pectin, Cellulose, Citrus, Magifera, Amoxicillin.

1. Introduction

Pectin are complex colloidal acid polysaccharide substances with high molecular weights and can be classified into four groups: protopectin, pectic acid, pectinic acid and pectins. Pectin is an important constituent in the middle lamella of cell walls of various plant parts such as citrus fruit, mango, papaya, quava fruits ^[1,2,3]. Pectin is defined as complex mixtures of polysaccharides that make up approximately one third of the cell-wall dry substance of most types of plants. Pectin is a heterogeneous polysaccharide having broad applications in the areas of food and beverage, pharmaceutical, cosmetics and biotechnology because of its thickening, gelling, and emulsifying properties ^[4,5].

Mango (*Magnifera indica* L. Anarcardiceae) is among the tropical fruits abundant in Nigeria. Mango peels are rich source of pectin, fiber and polyphenols. Mango peels represent about 7% to 24% (w/w) of the whole fruit ^[6]. It has been estimated that mango processing yields between 150,000 and 400,000 tonnes of wastes worldwide, which may cause environmental problems in the vicinity of the processing plants. The use of mango wastes in livestock feeding is a way of reducing environmental concerns. The recovery of valuable components of pharmaceutical origin/excipients from these waste by-products is considered to be a reasonable way of utilizing these wastes

and adding value to it while addressing both economic and environmental concerns $^{[7,8,9]}$.

The composition and structure of pectin are still not completely understood although; it has been isolated and described by Ningxian Yang *et al* ^[10] as containing mainly of galacturonic acid, a sugar acid derived from galactose. Pectin for use in food is defined as a polymer containing galacturonic acid units (at least 65%), and varies depending on different conditions of time, temperature and pH. The acid groups may either be free, combined as a methyl, ester, or as sodium, potassium, calcium or ammonium salts, and in some pectins, amide groups may also be present ^[11]

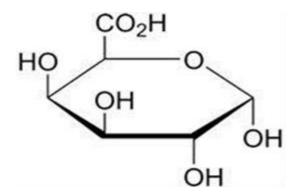


Fig. 1: Structure of Pectin

Citrus and apple pomace peels are acclaimed to be the most commercial and traditional sources of pectin. These are waste material from another industry such as apple pomace from a cider producer. Mostly Citrus peel has often been preferred material for pectin production due to its high pectin content and good colour properties. Generally lemon and lime peel are the preferred sources of citrus pectin.

Capsules are defined as unit solid dosage form of medicaments available as small containers (shells) made up of gelatin enclosing accurately measured drug substances. The term capsule is derived from the Latin word capsula, meaning a small container. Capsules occupy a significant position in the drug development. They are often believed as the primary oral dosage form because of their manufacturing process compared to other dosage forms. Gelatin has the property of disintegrating when it comes in contact with water, thereby releasing the medicament completely

Amoxicillin is one of the several semi-synthetic derivatives of 6-aminopenicillanic acid (6- APA) developed at Beecham, England in the 1960s. Amoxicillin is used to treat certain infections caused by gram-positive bacteria, such as pneumonia, bronchitis, and gonorrhea and infections of the ears, nose, throat, urinary tract, and skin, as well as some gram-negative bacteria. Amoxicillin belongs to a class of medications called penicillin-like (beta-lactam) antibiotics, effective broad-spectrum antibiotic, which is commonly prescribed to children for treatment of pneumonia and other illness ^[12]. Amoxicillin is mostly absorbed orally achieving peak concentrations in about 1.5 hours with 74-92% oral bioavailability and 20% bound to plasma proteins. The half-life of amoxicillin is 61.3 minutes and approximately 60% of an orally administered dose of amoxicillin is excreted in the urine within 6 to 8 h^[13].

2. Materials and Methods

2.1 Drugs, Chemicals and Solvents

Amoxicillin trihydrate BP (Venu Health care, Weigh bridge, Kadi Road, India), Pectin BP (Welfang Ensign Industries Co Ltd, China), Lactose powder BP (Danone ® Germany), Corn Starch BP. Others include Carboxylmethyl cellulose BP (Central Drug House Ltd, Vardaan, New Delhi), Ethanol 95% (CDH, Vardaan, Daryang, New Delhi), Glacial acetic acid (CDH, Vardaan, Daryang, New Delhi), Hydrochloric acid (HCL) (CDH, Vardaan, Daryang New Delhi, India)

2.2 Plant collection and preparation of peels

The peels of orange (*Citrus sinensis*) fruits were gathered from fruit vendors within Okada town, Ovia North East, Edo State, Nigeria. These were carefully sorted and washed under running water. The peels were sun dried outside the laboratory for 48 hours, milled, sieved and stored in an air tight container for further use.

Mango (*Magifera indica*) fruits were bought from New Benin market, Benin City, Nigeria; sorted and washed under running water and the skin/peels removed with the aid of a sharp knife. The skinned mango peels were further washed to remove some debris The orange and mango fruits were identified and certified by the Taxonomist in the Department of Pharmacognosy, College of Pharmacy, Igbinedion University, Okada.

2.3 Pectin extraction from orange (C. sinensis) peels

The procedure of extraction used by Obarisiagbon *et al* (2022) was adopted with slight modification ^[14]. Acidified water 0.5M HCl (2 l) with a pH 2.2 - 2.5 was measured and added to 250 g of the powdered orange peel in a round bottom flask. The round bottom flask was placed on a reflux condenser set to a temperature 70°c, and content boiled for about 3 hours. The mixture was filtered using a muslin cloth and the filtrate poured into 3 beakers and concentrated in an electric water bath at a temperature of about 100°c for 45 minutes. The concentrated filtrate was hydrolysed with 1:2 HCl, and washed with ethanol and

precipitate left undisturbed in the laboratory for 12 hours. The precipitated pectin was separated from the precipitating solution via filtration using a muslin cloth. Pectin was further dried in the oven at a temperature of about 60-70°c. This was milled and sieved to fine pectin powder, and stored in a dessicator for further use.

2.4 Pectin extraction from Mango (M. indica) peels

The extraction process of pectin from dried mango peel powder used by Kermani, Z J *et al* (2015) was adopted with slight modifications ^[15]. The powder was mixed with aqueous solution of HCl in a ratio 1:40 (w/v). The mixture was stirred continuously for 20 minutes and then filtered

Percentage yield (%) = $\frac{wt \, of \, extracted \, pectin}{wt \, of \, dried \, plant \, material \, (g)} \times 100$ - - - 1

2.6 Identification and phytochemical screening of pectin

Identification/confirmation and phytochemical screening of the pectin extract were carried out to identify the phytoconstituents using standard procedures.

2.7 Preparation of amoxicillin trihydrate granules

Amoxicillin trihydrate was used as model drug. Dry granulation method of Esratun Jannat et al (2016) was adopted with slight modification ^[16]. The first step involved weighing 30 g of amoxicillin trihydrate raw powder, appropriate amounts of internally added disintegrating agent, sodium lauryl sulphate, and magnesium stearate were sieved into a powder mixer. Appropriate quantities of Lactose and Starch BP, were weighed and sieved into the powder mixer. These ingredients were carefully mixed, until a uniform powder mix was achieved, slugged into tablet, crushed and sieved through 80-mesh sieve. The different binder types and concentrations respectively, were added to the crushed sieved power in granulating machine,

 $\theta = \tan^{-1}(h/r)$ -

Where, θ = angle of repose; h = height in cm; r = radius in cm.

Flow rate: Erweka Granules Flow tester was employed in the determination. Fifty grams of granules were allowed to

through a cheese cloth in order to separate the supernatant from the insoluble fraction. Pectin was precipitated by the addition of absolute ethanol (98% purity) with a ratio of 1:2 (w/w) into the supernatant and kept overnight at room temperature. The precipitated pectin washed with 98% (v/v) ethanol to remove the soluble impurities. The pellets were freeze dried until a constant weight was obtained.

2.5 Determination of percentage yield

The yields of the orange and mango peels pectin obtained from the extraction were calculated on a basis of dry weight as follows:

in and sieved through mesh 60 to form the granules with the required particle diameter. Same procedure was used to prepare granules with different concentrations of orange and mango peel pectin powder, pure pectin, carboxymethyl cellulose (1, 3, 5% w/w). The granules were evaluated for

2.8 Evaluation of the physicochemical properties of amoxicillin trihydrate granule

their physicochemical properties and later filled into hard

The method adopted by Abolfazl and Tahereh (2014) was used ^[17].

Angle of repose: Fifty grams of granules was weighed and 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:

gelatin shells.

flow through the orifice of the equipment and time taken to pass through was noted and the rate of flow was calculated as:

Flow rate
$$(g/sec) = \frac{Weight of granules (gm)}{Time (Sec)}$$
 - - - - 3

Bulk and tapped densities: Fifty grams' granules (W_o) were placed in a 250 ml measuring cylinder and the volume (V_b), occupied by each of the samples without tapping was noted. Bulk density was calculated according to equation

3. Buttom of cylinder was tapped for about 100 times on a tabletop until no change in volume was observed. Tapped volume (V_t) occupied was noted and tapped density calculated as shown in equation 4.

Bulk density $=\frac{Wo}{Vb}$	-	-	-	-	-	-	4
Tapped density $= \frac{Wo}{Vt}$	-	-	-	-	-		5
<i>Hausner's Ratio</i> : The ratio of tapped densit	y to bulk	density o	f the sam	ples was	calculated	1 as:	
Hausner's ratio = $\frac{Tapped Density}{Bulk Density}$	-	-	-	-	-		6
$Carr's Index (\%) = \frac{Tapped Density}{Tapped Density}$	x 100	-	-	-	-		7

2.9 Evaluation of the physiochemical properties of Amoxicillin trihydrate capsules

2.9.1 Disintegration test: This test was carried out to determine the disintegration time in accordance with the British Pharmacopoeia (2004) standard ^[18]. The apparatus

consists of a basket-rack assembly, a 900 ml, low-form beaker, 138 to 160 mm in height and having an inside diameter of 97 to 115 mm for the disintegration liquid medium. A thermostatic arrangement for heating the medium between 35 $^{\circ}$ C and 39 $^{\circ}$ C, and a device for raising

and lowering the basket in the liquid at a constant frequency rate between 29 and 32 cycles per minute. The volume of the fluid in the vessel is such that at the highest point of the upward stroke, the wire mesh remains at least 15 mm below the surface of the fluid, and descends to not less than 25 mm from the bottom of the vessel on the downward stroke. The apparatus contained distilled water as the disintegration medium with its temperature maintained at 37°C. One capsule is placed in each of the six tubes and the basket carefully placed in the flask, and apparatus started. When all the capsule has disintegrated and particles passed through the mesh, the time (min) was noted and recorded.

2.9.2 Dissolution test: The procedure adopted was based on the USP 32-NF 27^{19]}, method for dissolution test for solid dosage forms. Dissolution medium (distilled water), was placed in the vessel of a dissolution apparatus. Single capsule was placed in a small wire mesh basket fastened to the bottom of the shaft of a variable speed motor. The basket was immersed in the dissolution medium contained in a 900 ml flask, and maintained at 37 \pm 0.5 °C by a constant temperature bath. The motor was adjusted to turn at the specified speed, and aliquotes of 5 ml of the fluid are withdrawn at intervals to determine the absorbance at 425 micrometers wave length. Same volume of fresh dissolution fluid was replaced each time. The test was repeated on 3 additional capsules, and the mean determined.

2.10 Fourier transform infrared spectroscopy

Fourier transform infrared spectroscopy (FTIR), is an analytical technique used to identify organic, polymeric, and in some cases, inorganic materials. The FTIR analysis method uses infrared light to scan test samples and observe chemical properties.

The FTIR instrument sends infrared radiation of about 10,000 to 100cm⁻¹ through a sample, with some radiation absorbed and some passed through. The absorbed radiation is converted into rotational and/or vibrational energy by the sample molecules. The resulting signal at the detector presents as a spectrum, typically from 4000 cm⁻¹ to 400 cm⁻¹, representing a molecular fingerprint of the sample. Each molecule or chemical structure will produce a unique spectral fingerprint, making FTIR analysis a great tool for chemical identification. It is also used to ascertain possible interactions between excipients used in the formulation of dosage forms.

3. Results and Discussion

3.1 Phytochemical screening of *Citrus sinensis* and *Magifera indica* peel powder.

The qualitative tests of the phytochemical constituents of the powdered peels of *C. sinensis* and *M. indica* showed the presence of known chemical components with medical and pharmaceutical importance and are seen in the Table 1 below;

Phytochemical constituent	Tests	Sweet orange peel powder	Mango peel powder	
Alkaloid	Dragendorff	++	++	
Anthroquine	Borntrager's Test		_	
Flavonoid	HCL	++	++	
Saponin	Foaming Test	++	++	
Cardiac glycosides	Conc. H ₂ SO ₄	+	_	
Terpenoid	Conc. H ₂ SO ₄	_	_	
Tannin	Ferric Chloride	+	++	
Carbonhydrate	Molish test	+	+	

++ = Positive; - = Negative

Phytochemical analysis of orange peel powder showed positive to cardiac glycosides, saponin, alkaloid, flavonoid and tannin, and negative for terpenoid and anthraquinone; while those of mango peel powder revealed the presence of alkaloid, carbohydrates, flavonoid and tannin, and negative for terpenoid, cardialoidc glycosides and anthraquinone.

Table 2: Organoleptic properties of sweet orange (C. sinensis) and mango (M. indica) peels powder.

RESULTS				
Parameter	Orange peel powder	Mango peel powder		
Colour	Orange	Dark brown		
Odor	Characteristics	Characteristics		
Nature	Amorphous	Amorphous		
Solubility	Soluble in water	Soluble in water		
Ph	4.1	6.4		
Taste	Bitter	Bitter		

 Table 3: Mean and standard deviation of the physicochemical properties of Amoxicillin trihydrate granules formulated with different binders.

Parameters	Orange peel Pectin	Mango peel Pectin	Pure Pectin BP	Carboxymethyl cellulose BP
Angle of repose (°)	33.69 ± 4.41	34.27 ± 1.74	33.43 ± 4.85	34.33 ± 5.71
Compressibility index (%)	14.28 ± 3.14	10.14 ± 1.21	14.17 ± 0.71	11.49 ± 6.29
Hausner's Ratio	1.17 ± 0.04	1.11 ± 0.02	1.17 ± 0.01	1.14 ± 0.08
Bulk Density (g/ml)	0.60 ± 0.04	0.63 ± 0.01	0.61 ± 0.02	0.61 ± 0.04
Tapped Density (g/ml)	$0.70 \pm 0.75 s$	0.71 ± 0.01	0.71 ± 0.02	0.69 ± 0.01

4. Discussion

From the results, the angle of repose of the granules from the different formulations varies from $33.43^{\circ} \pm 4.85$ to $34.33^{\circ} \pm 5.71$. This shows that the granules had a good flow since they had angle of repose of less than 40° which shows that the granules are free flowing and acceptable for manufacturing purposes. The following batches namely; pure pectin (3%), orange pectin (5%), and carboxymethyl cellulose (5%) with the angle of repose 30.21°, 28.61°, 28.34° respectively had excellent flow characteristics. Mango peel pectin 3% and 5%, pure pectin 5%, and orange peel pectin 3% with the following angle of repose 34.03°, 32.66°, 31.07°, 35.94° respectively, had good flow characteristics. Pure pectin 1%, carboxymethyl cellulose 1% and 3%, mango peel pectin 1%, orange peel pectin 1% with the angle of repose 39.01° and 37.22° , 37.43° , 36.12° , 36.53° respectively, had a fair flow characteristic. The British Pharmacopoeia (2004) specifies that angle of repose of powders in the range of 40 to 50° are generally satisfactorily hence, all the granules from the above formulations had good flow properties.

The bulk density ranged from 0.60 ± 0.04 to 0.63 ± 0.01 . As the granule sizes increase, bulk density decreases. While the tapped density ranged from 0.69 ± 0.01 to 0.71 ± 0.01 . The flow rate was also in the range of 5.54 ± 9.59 to 13.40 ± 11.61 (gm/sec). The Hausner's ratio ranged from 1.11 ± 0.02 to 1.17 ± 0.01 for all the granules formulated. Generally, the physicochemical properties of Amoxicilin granules prepared with mango and orange peel pectin, carboxymethyl cellulose and pure pectin showed good flow characteristics hence indicating of good compressibility index.

The formulated capsules were evaluated for various physicochemical properties such as weight variation and disintegration time. The weight variation of capsules formulated with orange peel pectin ranges from 0.68 ± 0.064 to 0.69 ± 0.048 (mg) and complied with the uniformity of weight test as the % deviation was $\pm 4.8\%$ when compared with the standard limit of $\pm 5\%$ for capsules greater than 250 mg, while the capsules formulated with 3% orange pectin failed the test as the % deviation was $\pm 6.4\%$ because the value is greater than the accepted limit of $\pm 5\%$ for capsules greater than 250 mg.

The weight variation of capsules formulated with mango pectin ranged from 0.67 ± 0.039 to 0.71 ± 0.038 (mg). All the capsules formulated with mango peel pectin passed the uniformity weight test as the % deviation was within \pm 3.8% and $\pm 3.9\%$ for 3% and 5% mango pectin respectively. The weight variation of capsules formulated with 1% carboxymethyl cellulose was 0.69 ± 0.043 (mg). All capsules formulated with 1% carboxymethyl cellulose passed the uniformity of weight test as the % deviation was \pm 4.3%. The weight variation of capsules formulated with pure pectin ranged from 0.67 ± 0.039 to 0.71 ± 0.077 (mg). All capsules formulated with 3% and 5% pure pectin passed the uniformity of weight test as the % deviation was within $\pm 4.6\%$ and $\pm 3.9\%$ respectively. The variations observed within different capsule batches can be due to factors like particle size distribution, powder flow property, capsule size, specific volume and bulkiness of the powder. The disintegration time of capsules formulated with orange peel pectin ranged from 4.29 ± 0.20 to 7.09 ± 0.59 min, while that of mango peel pectin ranged from 5.01 ± 0.48 to 9.69 ± 1.52 min. The disintegration time for capsules formulated with pure pectin ranged from 5.17 \pm 0.17 to 6.17 ± 1.06 min. The disintegration time for capsules formulated with carboxymethyl cellulose ranged from 3.19 \pm 0.91 to 3.95 \pm 0.64. The accepted BP limit for soft gelatin capsules is 30 min. Hence, all the capsules formulated with orange and mango peel pectin, pure pectin and carboxymethyl cellulose passed the disintegration test since all the batches had disintegration time of less than 30 min. These results agree with earlier work by Obarisiagbon et al., 2022 s.

4.1 Calibration curve

Calibration curve, in bio-analytical method is a linear relationship between concentration (independent variable) and response (dependent variable) using a least squares method. This relationship is built to predict the unknown concentrations of the analyte in a complicated matrix. It is a general method for determining the concentration of a substance in an unknown sample by comparing the unknown to a set of standard samples of known concentration

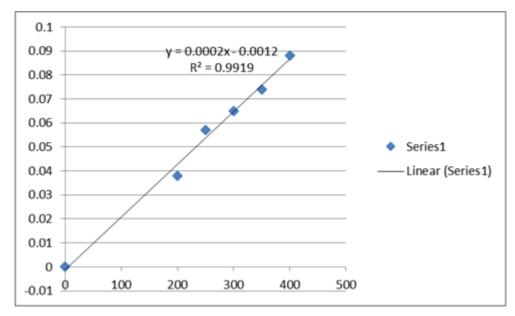


Fig. 2: Calibration curve for amoxicillin trihydrate BP

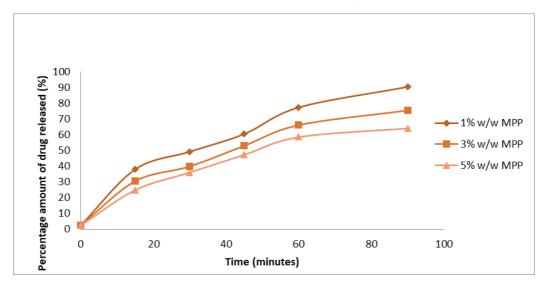


Fig. 4: Dissolution curves of amoxicillin trihydrate capsules with mango peel pectin as binder.

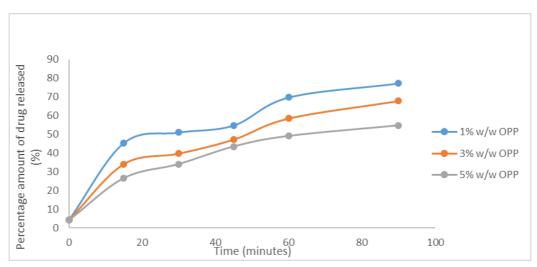


Fig. 5: Dissolution curves of amoxicillin trihydrate capsule with orange peel pectin as binder.

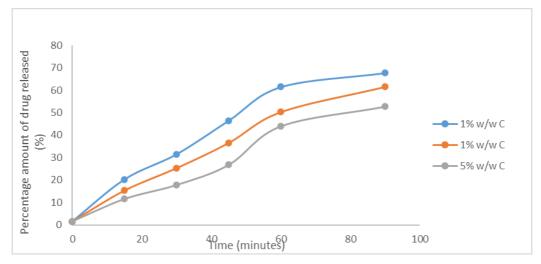


Fig. 6: Dissolution curves of amoxicillin trihydrate capsule with carboxymethyl cellulose as binder.

4.2 Fourier Transform Infrared Spectra (FTIR)

The various spectra of both mango and orange peel pectin (pure and in formulated dosage forms) show no interaction with either the active pharmaceutical ingredients (amoxicillin trihydrate) or the exipients used in the formulations as no new significant wave peaks were apparent. The spectra of the extracted pectin of orange and mango peels showed no significant difference from that of standard pure pectin BP. The spectra also showed that there was no form of decomposition both in the active drug or in the excipients used ^[20, 21]. These characteristics of the extracted pectins, showed that they can be effectively used in the formulation of amoxicillin trihydrate capsule oral dosage forms.

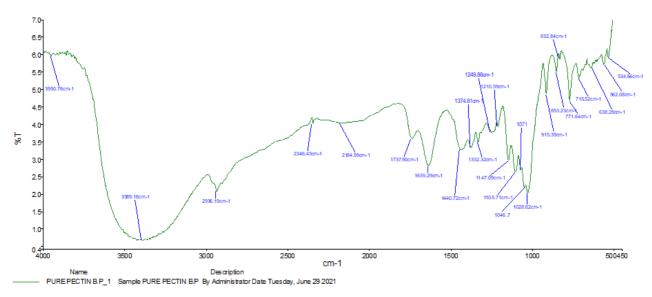


Fig. 7: FTIR of Pure Pectin BP powder

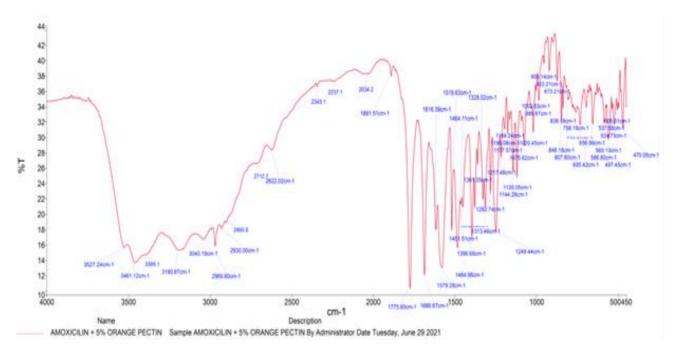
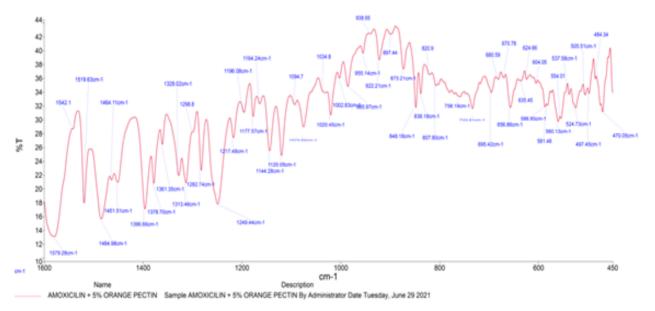


Fig. 8: FTIR of amoxicillin capsules formulated with (5% w/w) orange peel pectin within range $4000 - 450 \text{ cm}^{-1}$



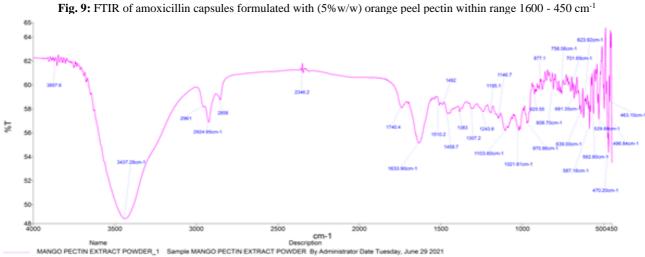


Fig. 10: FTIR of pure mango peel pectin within range $4000 - 450 \text{ cm}^{-1}$

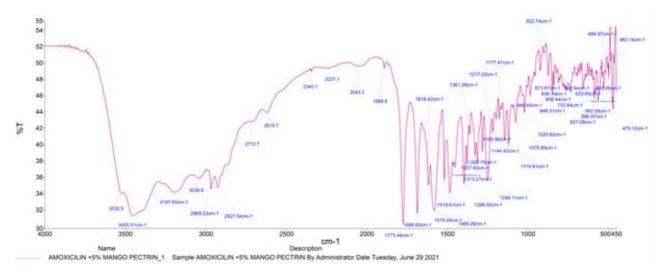
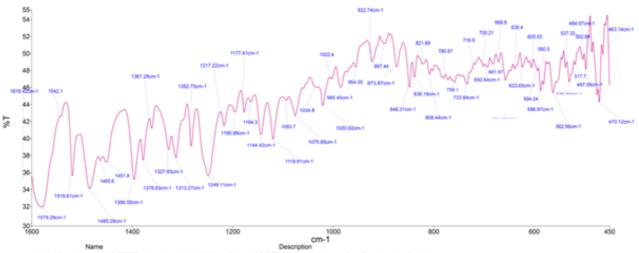


Fig. 11: FTIR of amoxicillin capsules formulated with (5% w/w) mango peel pectin within range 4000 - 450 cm⁻¹



AMOXICILIN +5% MANGO PECTRIN_1 Sample AMOXICILIN +5% MANGO PECTRIN By Administrator Date Tuesday, June 29 2021

Fig. 12: FTIR of amoxicillin capsules formulated with (5% w/w) mango peel pectin within range 1600 - 450 cm⁻¹

5. Conclusion

The physicochemical properties of the granules like, Hausner ratio, compressibility index, angle of repose were all in acceptable limits at all concentration of binders used which shows that the amoxicillin granules had good flow compressibility characteristics hence extracted pectin binders can be used for pharmaceutical formulation of solid dosage forms. Disintegration time increased with increased pectin binder concentrations, and capsules disintegrated within pharmacopoeial acceptable values of 15 minutes at different binder concentrations. The study revealed that sweet orange (*C. sinensis*) and mango (*M. indica*) peels are good sources of pectin and have the potential to become important raw material excipients in the formulation of pharmaceutical dosage forms.

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