**ORIGINAL PAPER**

https://dx.doi.org/10.4314/njpr.v21i2.7

**Nig. J. Pharm. Res. 2025, 21 (2) pp 115-122**
**ISSN 0189-8434 e-ISSN 2635-3555** Available online at http://www.nigjpharmres.com

# Effect of Alpha-Amylase Hydrolysis on the Physicochemical Properties of *Cissus Populnea* Gum

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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 the article.

## Abstract

**Background:** Intracellular gum extracted from the stem bark of Cissus populnea has potential applications in pharmaceutical, cosmetic, and food industries. However, in its native form, the gum is associated with starch from the storage sites in the stem. Alpha amylase hydrolysis of the associated starch may improve the physicochemical properties and functions of the resultant gum.
**Objective:** The objective of this study is to investigate the effect of purification of the native gum extracted from the stem bark of Cissus populnea by hydrolysis of the associated starch using alpha amylase.
**Methods:** Cissus populnea gum (CPG) was extracted by cold aqueous maceration and precipitated with 98% ethanol and dried in a hot air oven at 60°C for 1 h to obtain the native CPG. The polymer was further purified by introducing α-amylase enzyme with constant stirring at 70°C for 4 h to obtain the starch-free CPG. Both native and starch-free CPG were evaluated to elucidate the physicochemical properties.
**Results:** The extraction yield of the native and starch-free CPG was 33.5 and 29.3%, respectively. Enzyme hydrolysis significantly improved viscosity, moisture content, and moisture sorption capacity, which were 20.03, 5.63, and 19.14, respectively (compared to the native CPG). The purity and flow properties of the hydrolysed CPG were higher than those of the native.
**Conclusion:** The enzyme-hydrolysed Cissus populnea gum (CPG) exhibited improved physicochemical and flow properties, making it suitable for industrial and pharmaceutical applications in solid (tablets) and semi-solid (suspensions and emulsions) formulations.
**Keywords:** Cissus populnea, enzyme hydrolysis, physicochemical properties, paracetamol tablets.

## INTRODUCTION

Pharmaceutical excipients are components other than the active pharmaceutical ingredients that have been appropriately evaluated for safety and intentionally added to the formulation of a dosage form in order to achieve certain desired characteristics that make the dosage form suitable for formulation and administration to the patients (Patel et al., 2007). Excipients play a wide variety of functional roles that are crucial in the design of drug delivery systems, determining their quality and performance. Pharmaceutical excipients control the physicochemical properties as well as release profiles and availability of drugs from their formulated products (Oyi et al., 2007). They must be non-toxic, free of any unacceptable microbial load, and must be compatible with active pharmaceutical ingredients (Anekant et al., 2007).

Research into plant-based pharmaceutical excipients is on the increase since plant products have been found to serve as an alternative to synthetic products because of their biocompatibility, non-toxicity, biodegradability, environmentally friendly nature, and low prices compared to synthetic products (Patel et al., 2007; Meimei et al., 2007).

The plant, Cissus populnea Guill and Perr is a tropical plant belonging to the family Vitaceae. The plant is a tall woody climber of up to eight meters high; it is semicircular and grows mainly in the tropical regions of Africa, Asia, Australia, Central and South America, and North Mexico (Ojekale et al., 2006). It has a natural tendency to retain water; thus, it remains fresh almost throughout the season. It is gel-forming, and the gum is hydrocolloid and forms mucilage (Adeleye et al., 2015).

The plant is commonly known as 'okoho' by the Idomas, Igbo and Igala tribes of Northern Nigeria; 'Dafara' (Kano, Zaria); 'Latutuwa' (Katsina) by the Hausa language of the indicated towns of Northern Nigeria, and 'Orogboro' by the Yoruba tribes of Northern and Southern Nigeria. The plant Cissus populnea is also called food gum (Oladimeji & Okechukwu, 2016). The gum is used for soup and as a soup thickener.

The plant is associated with a myriad of medicinal uses in different parts of the world. Its extract has been credited with antibacterial properties (Kone et al.,2004), as an antitrypanosomal plant and a source of gum powder (Atawodi et al., 2002), and as a component of an herbal anti-sickling Nigerian formula (Moody et al., 2003). In Benin Republic, it is used for its diuretic properties (Adeleye et al., 2015), while in Ghana, it is used as a post-harvest ethnobotanical protectant (Belmain et al., 2000). In the Western part of Nigeria, it is used to improve genital erection in males and to improve spermatogenesis (Ojekale et al., 2006).

Some drug formulation studies have been done on this plant by some researchers in Nigeria. Ibrahim et al., (2002) investigated the mucilage obtained as a pharmaceutical excipient in tablet formulation, Abioye et al., (2000; 2001; 2000) studied the emulsifying properties of Cissus populnea gum, invitro release kinetics of salicylic acid from Cissus populnea gel and the stability effects of Cissus populnea gum in oil-in-water extemporaneous emulsions, Adeleye et al., (2011; 2015; 2018) evaluated the binding property of the gum in paracetamol tablets, physicochemical and rheological characterization of Cissus populnea gum extracted by different solvents, compaction and mechanical properties of Cissus populnea gum. However, there exists little or no information on the assessment of the starch-free gum extracted from Cissus populnea. In this study, native Cissus populnea gum (CPG) was enzymatically hydrolyzed using amylase (Nep et al., 2016), and the effect of this hydrolysis on the structural properties and functional indices of the enzyme-hydrolyzed Cissus populnea gum (emCPG) was evaluated.

## MATERIALS AND METHODS

### Collection, Identification, and Extraction of *Cissus populnea* Polymer

After identifying the aerial and underground parts in the herbarium of the Federal College of Forestry, Jos, Plateau State, Nigeria, the Cissus populnea plant was issued a voucher number FCF-H 00467. Stems of C. populnea were harvested in April in a town in Makurdi Local Government Area of Benue State, Nigeria. The stem bark was peeled, washed, and shredded, air-dried, and kept secured for extraction.

### Extraction of the Gum of *Cissus populnea*

The *Cissus populnea* polymer was extracted as reported by Ibrahim et al. (2000), with some slight modification. Instead of using a ratio of 2:1 of ethanol: acetone as the extraction solvent, 98% ethanol was used in this case. The fresh inner bark from the stem of *C. populnea* was washed thoroughly with distilled water and then shredded. The shredded material was macerated under ambient conditions in sodium metabisulphite for 48 h. The swollen gum obtained was separated from the residue by filtration through a muslin bag. The filtrate was precipitated from solution using absolute ethanol. The precipitated gum recovered was washed repeatedly with more ethanol to remove all water content until the gum became brittle. It was then dried in a hot air oven at $60^0$C for 48 h. The dried mass was milled to fine powder, passed through sieve number 250 (Fisher-brand test sieve, UK) and stored in an air tight amber coloured bottle, labeled CPG. The yield of the native gum was calculated by finding the percentage of the recovered gum (W2) divided by the dry weight of the shredded stem bark (W1), using equation 1.

$$(W2/W1 \times 100) \quad [1]$$

### Digestion of starch from CPG

The starch content in CPG, which initially tested positive to iodine, was digested according to the method of Nep et al. (2016). Briefly, 3000 mL of 1% w/v dispersion of CPG was treated with Termamyl 120® (1 % v/v) (Sigma Life Sciences) while stirring constantly at 70ºC for 4 h. The Termamyl was pre-treated by heating at 70ºC for 30 min to deactivate any pectinases and arabinoxylanases. Every 1 h an aliquot of the dispersion was removed and tested for the presence of starch using iodine. Starch digestion was complete in 3 h after which the sample did not test positive for starch. Subsequently, protein from the sample was precipitated by adjusting the pH to 4.5 with 2 M HCl and centrifuging at 4400 revolutions per minute (rpm) for 20 min. The recovered starch free CPG was further washed with absolute ethanol to get a brittle polymer precipitate. The precipitate was oven dried for 1 h at 60ºC. This sample was named enzyme modified Cissus populnea gum (emCPG), and the yield calculated by finding the percentage of final weight of starch-free gum recovered divided by the initial weight of gum used for starch digestion using equation 2:

$$W4/W3 \times 100 \qquad [2]$$

Where W4 is the weight of recovered emCPG and W3 is the initial weight of the nCPG used for starch digestion.

Evaluation of Physicochemical properties of the Cissus gum

**Loss on drying**

The moisture content of the CPG and emCPG were determined by weighing accurately 5 g each in a tarred evaporated dish on a Mettler AB54 Electronic balance (Mettler, A.G., Switzerland). This was then dried in an oven at 105ºC for 5 h and the final weight noted (Malviya, 2011). The percentage loss on drying was calculated using equation 3 until a constant weight was obtained.

Percentage Loss on drying = weight of moisture loss from sample /original weight of sample x 100 [3]

**Moisture sorption capacity of Cissus gum extract**

Two (2) g of CPG and emCPG were individually weighed and distributed evenly over the surface of a 70 mm tarred Petri dish and placed in a large desiccator containing distilled water in its reservoir. The desiccator was stored at room temperature at various time intervals over a period of five days. The weight gained by the exposed sample was recorded, and the amount of water absorbed was calculated from the weight difference (Ohwoavworhua et al., 2004).

**Swelling Capacity**

The swelling capacity was determined by weighing accurately 1 g each of CPG and emCPG into a 25 mL glass-stoppered graduated measuring cylinder and the volume occupied, Vo, was noted. About 20 mL of distilled water was added and the cylinder was closed. This was shaken vigorously every 10 min for 1 h and then allowed to stand for 6 h at room temperature (Odeniyi, et al., 2011). The volume, Vs, occupied by the sample including any adhering mucilage was noted and the swelling capacity was calculated using Equation (4).

$$\text{Swelling capacity} = V_s/V_0 \times 100 \qquad [4]$$

**Bulk and tapped densities of the powders**

The volume of known quantities of CPG and emCPG was obtained before and after tapping using a 100 mL flask. The volume before tapping was used to determine the bulk density while the volume after tapping was employed to determine the tap density mathematically. Furthermore, Hausner quotient and Carr's compressibility Index properties of the gum was obtained from equation 5 and 6

$$\text{Hausner quotient} = (\text{Tapped density})/(\text{Bulk}) \qquad [5]$$

$$\text{Carr's compressibility} = (\text{Tapped density-Bulk density})/(\text{Tapped density}) \qquad [6]$$

**Particle density**

The particle densities of the CPG and emCPG were determined by the pycnometer method using liquid immersion technique with xylene as the displacement liquid (Odeniyi, et al., 2011). A 50 mL pycnometer bottle was weighed, including the stopper, when empty (W). It was then filled with xylene to the brim till it overflowed, and excess was wiped off. The weight with the stopper was noted as (W1). The difference between this weight and the first was recorded as (W2). A 2 g quantity of the Cissus gum was weighed (W3) and quantitatively transferred into the pycnometer bottle and filled with the solvent to the brim. The excess solvent was wiped off, and the bottle was weighed again with the stopper (W4). The particle density, Pt, was calculated using equation 7.

$P_t=(w_{(2 .)} w_{(3 )})/(50(w_{(2 )}-w_4+w_2+w)$   [7]

**Angle of repose**

The angle of repose was determined by using the method adapted by Iwuagwu and Onyekweli (2002). The Cissus gum powders were allowed to fall freely through a funnel onto a plain white sheet of paper, placed on a flat surface until the apex of the cone formed by the powder just touched the tip of the funnel, clamped to a retort stand with its tip 2cm above the paper. The diameter of the base of the powder cone was obtained, and the angle of repose was calculated using equation 8

$\mathrm{Tan}\theta=h/r$   [8]

Where h is the height of the heap of powder, r is the radius of the cone, and Θ is the angle made by the heap with the base.

**Determination of pH**

A 1%w/v dispersion of the samples in water was shaken for 5 min, and the pH was determined using a pH meter (WA Saffron Walden, England).

**Solubility Test**

The emCPG and CPG were evaluated for solubility in water, acetone, chloroform, and ethanol in accordance with the British Pharmacopoeia specification (BP 2004).

**Moisture Content**

A 5 g each of the emCPG and CPG were weighed in a tarred evaporated dish on an electronic balance (Mettler, A.G., Switzerland). This was dried at 105°C for 5 h, and the final weight was noted. The percentage weight loss was calculated from equation 9:

% moisture content = wt. of moist/wt. of dried sample × 100%   [9]

**Total Ash and Acid Insoluble Ash Determination**

The ash content was estimated by the measurement of the residue left after combustion in a furnace at 450°C for 6 h (BP 2004). The ash obtained from the determination of ash is boiled with 25 mL of 2M hydrochloric acid for 5 min and the insoluble matter filtered and washed with hot water, ignited and the subsequent weight is determined. The percent acid-insoluble ash was calculated.

**RESULTS**

Extraction of the native gum Cissus populnea from the stem bark achieved a yield of 35.5%. Upon digestion of starch from the CPG the yield of enzyme-hydrolyzed CPG was 29.3%. Table 1 presents the results of the flow properties of CPG and enzyme-hydrolyzed CPG (emCPG), and Table 2 presents the results of some physicochemical properties.

**Table 1: Flow Parameters of CPG and emCPG**

| Parameter | Mean value (M±SD) | | P Value |
|---|---|---|---|
| **Description** | **CPG** | **emCPG** | |
| Angle of repose | 38.663 ±0.086 | 29.709 ±0.011 | .000 |
| Bulk density | 0.231 ±0.001 | 0.384 ±0.004 | .000 |
| Tapped density | 0.255 ±0.003 | 0.453 ±0.003 | .000 |
| True density | 2.095 ±0.003 | 1.913 ±0.007 | .000 |
| Hausner’s ratio | 1.282 ±0.001 | 1.181 ±0.001 | .000 |
| Carr.s index | 21.875 ±0.009 | 15.392 ±0.009 | .000 |
| Flow rate | 4.7 ±0.03 | 3.2±0.01 | .000 |

**Table 2: Some Physicochemical Properties of CPG and emCPG**

| Parameter | Mean value (M±SD) | | P Value |
|---|---|---|---|
| Description | CPG | emCPG | |
| Moisture content | 10.726 ±0.043 | 5.643 ±0.237 | .000 |
| Moisture sorption capacity | 37.470 ±0.142 | 15.140 ±0.108 | .000 |
| Viscosity @1.5%w/v | 8.16 ±0.007 | 20.03 ±0.006 | .000 |
| Swelling ratio in 0.1N HCl | 3.571 ±0.131 | 4.005 0.009 | .000 |
| Swelling ratio in $PO_4$ buffer 7.4 | 4.010 ±0.011 | 5.012 ±0.012 | .000 |
| Swelling ratio in water | 4.500 ±0.067 | 6.090 ±0.110 | .000 |
| Total ash (%) | 3.100 ±0.105 | 2.080 ±0.079 | .000 |
| Acid insoluble ash (%) | 1.080 ±0.103 | 1.080 ±0.092 | 1.000 |
| pH | 5.240 ±0.117 | 6.630 ±0.082 | .000 |

## DISCUSSION

The yield of both CPG and emCPG after extraction from the crude inner stem bark of the plant was 35.5% and 29.3%, respectively. Physicochemical and flow properties of the gum are summarized in Table 1. The gum is slightly soluble in water and practically insoluble in ethanol, acetone, and chloroform. The low solubility of the gum may be attributable to insoluble cell wall materials making up a larger proportion of the gum (Nep and Conway, 2010). The swelling characteristics of the extracts in different media - 0.1N hydrochloric acid (HCl), phosphate buffer (7.4) and water were evaluated, and results are shown in Table 2. The swelling was highest in water, followed by phosphate buffer (7.4) and least in 0.1N HCl. Generally, emCPG shows a higher swelling ratio (SR), suggesting that the gum may perform better as a binder/disintegrant/matrixing agent (Emeje *et al*., 2009). For example, hydrogels tend to swell more in water and phosphate buffer than in acidic solutions such as 0.1N HCl. *Cissus populnea* gum seems to have similar properties to other hydrophilic materials; therefore, we might expect its SR to be higher in water and phosphate buffer (7.4) than in 0.1N HCl.

In tablet formulation, SR and viscosity are crucial parameters that impact on the quality, stability, and performance of the final product (Emeje *et al*., 2012). SR is important because it indicates tablet ability to absorb fluid and swell; affects tablet disintegration and dissolution rates; influences drug release profiles; and impacts tablet's mechanical strength and stability (Isimi *et al*., 2019). Literature (Emeje *et al*., 2012) indicates that for immediate-release tablets, the SR range is 20 – 50%; for sustained-release tablets, it is 10- 30%; while for controlled-release tablets, it is 5 – 20%. For emCPG with SR of 6.09, it is clearly suggestive that the polymer can function efficiently as a controlled-release polymer. The gum is a pH-responsive polymer; it is therefore a "smart polymer" and may find application in controlled release dosage formulation (Emeje *et al*., 2009). Additionally, swelling is a primary mechanism in diffusion-controlled release dosage form (Jain *et al.,* 2004). Akhila and Emilia (2006), and Odeniyi *et al*. (2013) have shown that the capacity of material to capture water molecules influences parameters such as mechanical properties and surface mobility. The high swelling index of emCPG is an indication that it may be used as a sustained-release excipient in a matrix tablet system (Adeleye *et al*., 2015).

The emCPG exhibited a higher viscosity of 20.03 cp at 1.5% concentration of the gum against 8.16 cp for CPG at the same concentration. This shows clearly that enzyme hydrolysis greatly enhanced the viscosity of the gum. High viscosity is of particular interest in the formulation of suspensions and semi-solid dosage forms (Adeleye *et al*., 2015), where resistance to shear of agitation may impair easy pouring from the container (Sinko, 2011).

Viscosity plays a very significant role in the performance of excipients in pharmaceutical formulations: viscosity affects tablet's rheological properties; influences granulation and compression processes; impacts tablet's disintegration and dissolution; affects drug release profiles (Isimi *et al*., 2019). Factors affecting viscosity include excipient type and concentration (e.g., polymers, gums); solvent type and concentration; temperature and humidity; particle size and shape (Emeje *et al*., 2012).

The total ash (TA) and acid-insoluble ash (AIA) value of emCPG was found to be 2.080 and 1.080% w/w, respectively. Ash values reflect the level of adulteration or handling of the drug (Jain *et al*., 2004). Adulteration by sand or earth is normally composed of inorganic mixtures of carbonates, phosphates, silicates, and silica. In natural polymers, total ash and acid-insoluble ash are crucial for quality control, ensuring polymer purity and consistency. The USP (1995) standard limits for TA and AIA are 1 – 5% and 0.5 – 2%, respectively, implying that emCPG meets the USP requirement. The low values of TA and AIA obtained in this study indicate low levels of contamination during gathering and handling (BP, 2004). This result also indicates that emCPG, which gives lower values of TA and AIA, is purer than the CPG.

The angles of repose of CPG and emCPG extracts were 38.6630 and 29.7090, respectively. This is an indication that emCPG has excellent flow while CPG has fair flow (Olorunsola, 2021). The angle of repose is usually affected by particle shape, particle size, and size distribution among others. The angle of repose can indicate the cohesiveness of a powder material (Al-Hashemi *et al.,* 2018). The percentage compressibility (Carr's Index) is a quantitative descriptive assessment of the compressibility and flowability of a powder while Hausner's ratio is indicative of interparticle friction. As the values of these two parameters increase, the flow of the powder decreases. The emCPG, having Carr's Index of 15.3920% and Hausner's ratio of 1.1808, indicates good flow, whereas the CPG with an index above 21% and Hausner's ratio of 2.0954 indicates poor flow (Olorunsola, 2021).

The moisture content of gum shows 10.7333 and 5.6367 for CPG and emCPG, respectively. Moisture content in a powder may affect the frictional properties of the compact powder. The formation of a moisture film may reduce friction at the die wall by acting as a lubricant, thus decreasing tablet adhesion to the die wall. The emCPG when used in tablet formulation, will ease tablet ejection better than when CPG is used, because moisture is known to promote the sticking of powder to the die surface. The low moisture content of emCPG suggests its suitability in formulations containing moisture-sensitive drugs. Given suitable temperature, moisture will lead to the activation of enzymes and the proliferation of microorganisms, thereby affecting the shelf-life of most routine formulations. It is important to investigate the moisture content of a material because the economic importance of an excipient for industrial application lies not only on the cheap and ready availability of the biomaterial but also in the optimization of production processes such as drying, packaging, and storage (Emeje *et al.,* 2008).

The moisture sorption capacity of CPG and emCPG is 37.4733 and 19.1433, respectively. Moisture sorption capacity is a reflection of the relative physical stability of tablets made from the polymer when stored under humid conditions. The high value of the moisture sorption capacity of CPG and emCPG is an indication that they are sensitive to atmospheric moisture, which suggests that this may undermine the stability of hydrolysable constituents of a solid dosage form if used as an excipient in that formulation. Both CPG and emCPG should be stored in air-tight containers since they are susceptible to moisture sorption at atmospheric conditions. However, CPG is more susceptible to having a higher moisture sorption capacity.

A 1 % w/v suspension of emCPG in water gave a pH of 6.03; the near-neutral pH implies that when used in uncoated tablets, it may be less irritating to the gastrointestinal tract, for example, in tablet formulation. It may also find useful application in the formulation of acidic, basic, and neutral drugs. Knowledge of the pH of an excipient is an important parameter in determining its suitability in formulations since the stability and physiological activity of most preparations depend on pH (Nasipuri *et al.,* 1996).

**CONCLUSION**

The use of plant-based excipients such as *Cissus* p*opulnea* gum can offer several benefits because locally sourced natural gums are often more affordable than imported synthetic alternatives. The *Cissus populnea* gum is a known abundant, eco-friendly and renewable polysaccharide. Native *Cissus populnea* gum, irrespective of its biological origin, possesses inherent physicochemical properties that limit its pharmaceutical applicability. Modification of the native gum, however, confers new properties and improves its applicability. This study shows that enzyme hydrolysis of *Cissus populnea* gum improved its physicochemical properties, thereby making it suitable as a potential excipient for the pharmaceutical industry. Work on the use of modified gum in tablet formulation is ongoing.

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Conflict of Interest: None declared
Received: September 12, 2025
Accepted: March 23, 2026