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Characterization of Microwave-Assisted Extracts of *Citrus sinensis* Exocarp and Mesocarp Peels for their Pharmaceutical Excipient Potential

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ABSRTACT

Natural plants wastes are increasingly explored for their excipient potentials in alignment with the present global focus on economic and sustainable waste utilization. This study characterized microwave-assisted extracts of *Citrus sinensis* exocarp (Cs- Exop) and mesocarp peels (Cs-Mesop) using standard protocols.

The two extracts had distinctive organoleptic properties. Their respective mean particle sizes being (120.80 ± 0.12 and 99.70 ± 0.0.15 µm); pH (5.52 and 6.41); swelling index (2.08 ± 0.02 and 3.01 ± 0.07); water binding capacity (1.77 ± 0.03 and 3.32 ± 0.02 %); viscosity (12.6 ± 0.4 and 19.5 ± 0.4 mPa.s); moisture content (13.30 ± 0.17 and 18.78 ± 0.14 %); Hausner's ratio (1.35 ± 0.01 and 1.30 ± 0.01); compressibility index (22.2 ±0.01 and 20.3 ± 0.01) among others. The microbial evaluation revealed the absence of objectionable microorganisms; the total aerobic microbial counts were 8.00 x 10^2 and 19.50 x 10^2; yeast and mould counts were 2.00 x 10^2 and 6.00 x 10^2 respectively which were in conformity to the specifications of United States Pharmacopoeia. They both had safety profiles with the lethal dose (LD_{50}) values ≥ 5000 mg/kg. No occurrence of morbidity, mortality, abnormalities on the hematological and histopathology profiles at all the administered doses in the experimental animals. No statistical significance occurred between the Control and the CS-peels treated groups.

Citrus sinensis peel extracts have desirable pharmaceutical excipient attributes. Cs- Exop peel extract can be employed as a permeation enhancer, emulgent while Cs -Mesop can serve as a potential binder and disintegrant.

Keywords: *Citrus sinensis* peel *extracts*; characterizations; microbial and toxicological safety, excipient potential; microwave-assisted extraction.

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Introduction

Formulation design, development, and optimization require consideration of the physical, chemical and biological attributes of the active pharmaceutical ingredients and other formulation ingredients classified as excipients.[1,2] Excipients, though listed as inactive ingredients by FDA exhibit functional attributes that enhance the organoleptic, stability and therapeutic effectiveness of formulations hence patient compliance and adherence. [2, 3]

Excipients may be naturally sourced, chemically synthesized or biotechnologically derived. They can be classified on the basis of their origin, functionalities and utilization in dosage forms.[4]

Traditionally, in drug formulations, excipients are inert substances that have varied functions such as weight or volume diluents, binders, disintegrants, suspending agents, viscosity modifiers, lubricants, and glidants. However, in recent novel pharmaceutical designs and formulations, they often fulfill multi-functional roles such as release modifiers, stability and bioavailability enhancers, enhancement of patient acceptability and ease of manufacture.[5]

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The excipient choice and concentration must be established and justified with its intended function in the shelf–life performance of the finished/final product.[5, 6] The ideal excipient should possess acceptable organoleptic properties, stability, inertness (non- reactive), non-toxicity, compatibility with API and other formulation ingredients, safety, economical, cost-effectiveness and efficiency with regards to its intended use. [7]

There is an increasing global quest for the development of new and improved excipients that would meet the needs of advanced drug delivery systems.[8] Synthetic and natural polymers have been investigated extensively for development as potential novel drug delivery excipients but with greater global shift and preference for the natural polymers and their derivatives due to their comparative overriding advantages. The natural polymers used as excipients are macromolecule hydrocolloids which are abundant in nature existing as either gums, mucilages and glucans/ pectin possess a broad range of physicochemical properties. Additionally, they have comparative advantages of biodegradability, biocompatibility, inertness, low-toxicity, safety, cheapness, economical, availability, renewable, eco-friendly, modifiable (due to their large chains and functional groups) for diverse applications which make them appealing as pharmaceutical excipients over the synthetics and have unique properties that are tailorable based on their large chains and functional groups. [1,9] Consequently, the successful development of these polymers as excipients has yielded various applications including matrix controlled system, film coating agents, buccal films, microspheres, nanoparticles, release modifiers, tablet matrix formers, implants, viscosity enhancers, stabilizers, disintegrants, solubilizes, emulsifiers, suspending agents, gelling agents, and binders in drug formulations.[2,10]

Peels of fruits which consist of the epidermis / primary outer tissue (epicarp) and the peripheral inner tissue (mesocarp) function primarily to protect the parenchyma of fruits from pathogenic microorganisms,

do also have some constituents such as **(**chlorophyll, carotene, anthocyanin, etc.); nutrients (vitamins, minerals, water, sugar, fat, protein, etc.); bioactive (flavonoids, tannins, glycosides, saponins etc.) as well as inert hydrocolloid macromolecules (pectin, cellulose, etc.).[11]
Fruits (oranges, mangoes, pineapples, apples, bananas, etc.) peels have been in time past been often considered domestic and agricultural wastes that contribute to environmental pollution**.**
Following the increasing global focus on natural plants as pharmaceutical excipients and in the light of the aforementioned peels constituents, the necessity for sustainable environmental management and economic utilization of these aforementioned agricultural wastes, infers the quest to develop strategic processing technologies for their prospective optimization and utilization as natural excipients for the pharmaceutical industry. [12]
Plants phytoconstituents can be extracted conventionally using maceration, percolation and reflux techniques however, in recent times novel techniques such as microwave-assisted extraction (MAE), supercritical fluid extraction (SCFE) and pressurized solvent extraction (PSE) have found increasing use. MAE employs the microwave energy to disrupt the cell membrane/ intracellular lipids and release such into the organic solvent. During this process, polar compounds are caused to be aligned in the direction of the electric field and are rotated at a high speed that causes heat which leads to cell disruption. The MAE major advantages comparative to the conventional methods is the energy efficiency hence cost-effectiveness as it is faster, has high extractive index, suitable for thermolabile substances and utilization of smaller quantity of extraction solvent.[13-15]
Pectin a multifunctional constituent of cell wall is a high value functional food ingredient. It presents as a pale white or cream-brown colloid linear chain polysaccharide derivative. It is mainly extracted from fruits and exist as a multifunctional constituent of plants cell walls (primary and middle lamella) providing consistency and mechanical strength to it. It is a polydisperse and polymolecular biopolymer [16] and its composition varies with the plant source and the conditions applied during isolation Pectin is the methylated ester of polygalacturonic acid. The pectin backbone consists of a linear chain of α-(1-4)-linked*α-d*--galacturonic acid residues [17] with methyl esters of uronic acid.
The pectin structure contains the homogalacturonan (HG- i.e. the smooth) and type I rhamnogalacturonan (RG-I i.e. the hairy).[18] The galacturonic acid polysaccharides consist of various sugars such as, galactose, rhamnose, arabinose, xylose and glucose and thus variable number of methyl ester groups.[19, 20] It is produced commercially as a white to light brown powder being mainly extracted from fruits through esterification of the galacturonic acid with methanol or acetic acid.
The percentage esterified groups are expressed as degree of methoxylation (DM) and degree of acetylation (DA) respectively. Pectin with high degree of esterification form thermo-irreversible gels and implies that the degree of methoxylation /aceytylation is > 50% (HM) and conversely for low degree esterification pectin forms thermo-reversible gels and implies that the methoxylation/aceytylation is < 50% (LM). The lower the methoxyl content, the slower the set [21]. Thus, pectin has diverse potential excipient applications in formulation systems as gelling agents, thickeners, stabilizers,[22, 23] binders,[24, 25] film-formers, fast dispersants,[26] matrix formers and controlled release carriers (colon-specific, oral, ophthalmic and transdermal).[27]
Citrus sinensis Linn. (sweet orange, family: Ruta*ceae*) is a small evergreen which originated in Southern China, where it has been cultivated for millennia but grown worldwide in tropical, semi-tropical and some warm temperate regions. It is the most widely planted fruit tree in the world. The orange tree is a small, spiny tree, typically growing into heights of between 7.5 m to 15 m with a compact crown. The leaves are leathery and evergreen and range from elliptical to oblong to oval, 6.5-15 cm long and 2.5-9.5 cm wide with narrow wings on the petioles (leaf stems). The fragrant white flowers produced singly or in a cluster of up to 6 are about 5 cm wide with petals and 20-25 yellow stamens. The fruit which may be globose to oval is typically 6.5 to 9.5 cm wide and ripens to orange or yellow. The fruit skin (rind or peel) contains numerous small oil glands. The flesh or pulp of the fruit is typically juicy and sweet, divided into 10 or 14 segments (though there are seedless varieties) and ranges in colour from yellow to orange to red.
The orange tree is the most widely grown and commercialized citrus specie, its different parts possess various medicinal properties and nutritive values. Oranges are the world's most popular fruit as they are eaten fresh and used for juice.
The orange fruit is composed of an external layer (peel) formed by flavedo the outermost covering (epicarp or exocarp) and albedo (mesocarp), and an inner white material called endocarp that contains vesicles with juice. The juice, flavedo and albedo account for about 50, 10 and 25% (w/w) respectively of the whole fruit.[28]
Citrus is an important fruit that is not only rich in vitamins, organic acids, polysaccharides and other nutrients, but also contains a variety of important bioactive substances such as essential oils, flavonoids, carotenoids and pectin that have potential pharmaceutical and therapeutic applications [28-30].
The orange peels which present as domestic and agro wastes as well as a source of environmental pollution can be harnessed for the achievement of sustainable economic development through strategic and effective conversion and eco-friendly waste management technologies of this aforementioned biomaterial (pectin) and bioactives (essential oils, flavonoids, tannins, terpenes, carotenoids, etc.) for utilization in the pharmaceutical sector as formulation excipients and as alternative food products or food additives.
MAE technology has been reportedly used for the extraction of essential oils from *Citrus sinensis* peels by several workers in recent times.[31- 34] This may be attributable to its technological simplicity, economical affordability, cost-effectiveness and infers its suitability for the extraction of the exocarp and mesocarp peel constituents in resource limited settings.
In line with the ongoing quest for excipient development from natural plants and the need to optimize the economic value of agro-wastes as well as address environmental waste pollution, this study sought to characterize microwave-assisted extracts of *Citrus sinensis* exocarp and mesocarp peels for their pharmaceutical excipient potential.

Materials and Methods

Materials
Ethanol, hydrochloric acid, acetone, alpha naphthol, sulphuric acid, ferric chloride, Draggendoff's reagent, glacial acetic acid, ethyl acetate, methanol, lead acetate, methyl red solution bromocresol green (0.1%), Fehling's solution, Resorcinol, petroleum ether and chloroform, all of BDH Laboratory Chemicals Limited, Poole, England and all other reagents were of analytical grades and were used as received from suppliers.
The ripe fresh fruits of *Citrus sinensis* were obtained in November 2016 from a local market in Mushin area of Mushin Local Government, Lagos State, Nigeria during its fruiting season**.** The plant was authenticated at the Department of Botany, Faculty of Science, University of Lagos, Nigeria by Dr. Nodza George with an authentication code of LUH 7021. These fresh fruits were stored in a cool dry aerated place until needed for use.

Orange peel extraction and purification
The fresh fruits of *C. sinensis* were sorted out and thoroughly washed with clean water followed by adequate rinsing with distilled water. The greenish-yellow outer skin peels (exocarp) of the oranges were manually and carefully removed with a clean knife which exposed the mesocarp (inner peel). The peeled fruits were then cut in the middle into two equal halves to expose the pulp and seeds which were carefully removed manually from the mesocarp. The wet exocarp and mesocarp peels were then separately spread out neatly on two different aluminum trays and sundried for six (6) weeks. These sundried exocarps and mesocarp peels were subsequently subjected to further oven drying for five days at 40°C. The dried exocarp and mesocarp peels were then comminuted into fine powders using a traditional kitchen handmill (Corona^{R}). Then powdered exocarp and mesocarp peels were separately passed through sieve #20. These powdered peels were separately stored in air-tight containers and appropriately labeled until required for further use (micro-wave extraction).
The extraction technique was undertaken as described by Nilesh *et al*., [25] with slight modification, briefly, the dried powdered *C. sinensis* fruit

peels (50 g) each were separately soaked and blended in 300 mL distilled water acidified with 40% citric acid and pH was maintained at 1.2 in a Pyrex beaker. The acidified mixture of the blended peel powders was then separately heated at 60° C for around 10 min using a domestic microwave oven with adjustable microwave power and irradiation time (Akai, Tokyo; Model: MW033A-26, 23L, 50Hz, 230V at working frequency 2450 MHz and maximum power input (1250 W) and output (800 W). After the microwave treatment, the mixtures in the two Pyrex beakers were allowed to cool down to room temperature. The mixtures were separately passed through a two-fold muslin cloth and were cooled to room temperature. The sample was then centrifuged in a centrifuge (Clifton Laboratory Centrifuge, Nickel Electro Ltd., England) at 4 ºC, 8000 rpm for10 min.[35] The insoluble residues were recovered and the supernatants were precipitated with the use of three times the volume of 95% (v/v) ethanol mixture with continuous stirring for 15 min. The precipitated extracts' mass was separately washed with 95% (v/v) ethanol three times to remove the mono and disaccharides as indicated by Maran *et al.*[36] The precipitated mixtures were kept aside for 2 h without stirring, after which the precipitated peel extracts were separately filtered through a four-layered muslin cloth.
The precipitates were washed 2 to 3 times using 95 % ethyl alcohol, to further remove any remaining impurities. Finally, precipitates were dried for five days at 40ºC using (Gallenhamp Oven 300 plus series, England). They were weighed to determine the percentage yield and then finely powdered with a traditional hand mill. They were then packed separately in air-tight containers (with desiccators), labeled appropriately and stored until further use.[25, 37, 38]

Characterization of C. sinensis (Cs-Exop and Cs-Mesop Extract) Powders
Physiochemical parameters
The organoleptic properties (colour, odour, taste, texture), bulk and tapped densities, angle of repose, hausner's ratio, compressibility index, flow rate, solubility profiles, pH, moisture content, swelling capacity, and hydration/swelling index of the dried powdered *C.sinensis* peels (exocarp: Cs-Exop and mesocarp: Cs-Mesop) extracts were determined using standard procedures as described in an earlier publication by Ologunagba *et al*., [39] and briefly indicated below:

Bulk and Tapped Densities (BD and TB)
The Bulk and Tapped densities (BD/ TD) of the peel extracts (Cs-Exop and Cs-Mesop) were determined by accurately noting the respective weights (Wt) and the occupied volumes in a 25 mL measuring cylinder either in the loose packing condition (Vb) or when tapped 100 times on a plane hard wooden surface (Vt). Triplicate determinations for these two parameters were undertaken for each peel extracts.
The bulk density (BD) and tapped density (TD) were calculated by the following formula:

$$BD = \frac{Wt}{Vb} \quad (1)$$

Where: Wt = Weight of granules
Vb = Loose Volume of packing

$$TD = \frac{Wt}{Vt} \quad (2)$$

Where Wt = Weight of granules

Vt= Tapped Volume of packing

Angle of repose (AºR: θ)
This parameter which measures the maximum angle possible between the surface of a pile of powder and the horizontal plane indicates the interparticulate frictional forces in a loose powder system. Thus, the powders were separately allowed to flow through the funnel fixed to a stand at definite height (h). The angle of repose was then calculated by measuring the height and radius of the heap of powders formed.

$$\text{Tan } \theta = \frac{h}{r}$$
or (3)

$$\theta = \tan^{-1}\frac{h}{r}$$

Where, θ = Angle of repose, h = height, r = radius
Values of angle of repose ≤ 30° indicate free flowing granules and ≥ 40° suggest poorly flowing material.

Hausner's ratio (HR)
Hausner's ratio was calculated as the ratio of tapped density (TD) to the bulk density (BD) as given by the equation below:

$$HR = \frac{(TD)}{BD} \quad (4)$$

Compressibility / Carr's index (CI %)
Percent compressibility of the powdered peel extracts as determined by Carr's compressibility index was calculated by the following formula**:**

$$\textbf{CI \%} = \frac{(TD-BD)}{TD} x100 \quad (5)$$

Where, TD = Tapped density
BD= Bulk density

Solubility profile
The solubility profiles of both extracts were separately determined in aqueous and organic solvents (acetone, ethanol, methanol, diethyl ether) by shaking one part of each of the dried peel powder extracts with different aforementioned solvents.

Swelling Capacity/ Index (Hydration Profile)
The swelling capacity/Index (hydration profile) of each of the extract types were determined as described in a previous publication by Ologunagba *et al.*[39] Swelling index is the volume (mL) taken up through absorption by a weighed powdered test material when a specified volume of water at room temperature is poured into it. Thus, the swelling indices of the peel extracts (Cs-Exop and Cs-Mesop) were determined by accurately weighing 2 g of each sample into two separate 50 mL glass-stoppered measuring cylinders which were tapped on a tabletop for 200 times to ensure good packing arrangements of the particles of the powdered extracts. The volumes occupied by the extracts in the cylinders were recorded. Water was added to the 40 mL mark of the cylinders which were then left to stand for 24 h. The new volumes of the extract types were recorded. The swelling capacity of each extract type was calculated as the ratio of the final volume to the initial volume. The procedure was undertaken in triplicate for each extract type and the mean of the readings taken.

The swelling index (SI) is given by the equation

$$SI = \frac{VF-VI}{VI} \quad (6)$$

Where VI= Initial volume of powdered extract
VF= Final volume of powdered extract (after water absorption)

Water binding capacity
This was determined in accordance to the described procedure by Muazu *et al.*[40] One gram (1 g) of the powder was placed in a pre-weighed centrifuge tube and covered with 10 mL distilled water. The tube was shaken for 2 min and left to stand or 10 min before centrifugation at 3000 rpm for 10 min. The supernatant was decanted and the weight of the powder and the tube after water uptake and centrifugation was determined.
Water binding capacity was calculated using the equation:

$$WBC = \frac{WtC}{WtI} \quad (7)$$

Where, WBC=Water binding capacity

WtC = Weight of powder after centrifugation

WtI = Weight of powder at the initial

The pH determination of the peel extracts

The pectin was weighed and dissolved in water separately to get a 1 % w/v solution. The pH of solution was determined using digital pH meter (Mettler Toledo; Serial No: B236292275) as described in a previous publication by Ologunagba *et al.*,[39] This determination was undertaken in triplicate for each extract type.

Moisture content determination
The moisture content was determined by heating five grams of the sample to a constant weight in a crucible placed in an oven (Gallenhamp , England ; 300 plus series) maintained at 105° C.
The percentage moisture content was determined using the formula:

$$\frac{W2-W3}{W2-W1}x100 \quad (8)$$

Where, W1=Initial weight of crucible
W2= Weight of crucible + sample before drying
W3= Weight of crucible + sample after drying

Rheological parameters
The viscosities of the 5 % w/v dispersions of the Cs-Exop and Cs-Mesop extracts of *C. sinensis* were determined at 25°C with a Brookfield viscometer (Model DV-1Prime) at 100 r.p.m. with spindle number 2.

Phytochemical screening
Phytochemical screenings for the peel extracts of *C.sinensis* were carried using well-established standard methods described by Evans.[41] All these qualitative determinations rely on the visual color change reaction as a basic response to the presence of a specific phytochemical compound.

Proximate /Probate analysis
Moisture content determination
Standard methods of the Association of Official Analytical Chemists (AOAC)[42] and as described by Ologunagba *et al.*,[39]

Ash values determination
Ash values such as total ash, acid insoluble ash and water-soluble ash were determined in accordance with the procedure detailed by Ologunagba *et al.*, [39]

Determination of Crude Protein
Crude proteins of the two samples were separately determined by the Kjeldahl method as described by Alim-un-Nisa, *et al.*, [43] with placement of five (5) grams of each of the samples in the Kjeldhal's flask for digestion with sulphuric (H_2SO_4) acid in the presence of (selenium, mercury, or copper salts as the catalyst) to form ammonium sulfate at 380 ºC which is then dissolved in water, followed by distillation and titration.
The crude protein is calculated and obtained by the equation:

$$\% \text{ Total Nitrogen} = \frac{100x\,(Va-Vb)xNAx0.001401}{Wx10}x100 \quad (9)$$

Where, Va = Vol of standard acid used in titration
Vb= Vol of standard acid used in blank
NA= Normality of acid
Wt = Wt in grams of sample

Determination of crude fiber
The crude peel extract samples (Cs-Exop and Cs-Mesop) were de-fatted using petroleum ether. The determination of crude fiber in the peel extracts was in accordance with the method described by Ilodibia *et al*., [44] briefly, the weighed (5fat-freefree residues of the peel extracts were each separately subjected to digestion by treating successively with 150 mL of 1.25 % boiling solution of sulphuric acid for 30 min under reflux. The boiled samples were then washed in several portions of hot water using a two-fold cloth to trap the particles. It was returned to the flask and boiled again in 150 mL of 1.25% sodium hydroxide (NaOH) for another 30 min under the same condition. After washing in several portions of hot water the sample was allowed to drain dry before being transferred quantitatively to a weighed crucible where it was dried in the oven at 105°C to a constant weight (W_2). This was then incinerated /ashed in an oven maintained at 550 °C for 5 h. The weight of the residue was noted as (W_3). The loss of weight that results from ashing would correspond to the crude fiber present in the sample is as calculated by the equation:

$$\frac{W3-W2}{W1}x100 \quad (10)$$

W1= Weight of sample taken
W2= Weight of oven dried sample
W3= Weight of incinerated sample

Determination of crude fat
Two grams (2 g) of *C. sinensis* peel extract types were separately weighed (W_1) and transferred into a conical flask. 10 mL of petroleum spirit was added to the flask and left to stand. After 24 hours, the supernatant was decanted into an empty pre-weighed/tarred beaker (W_0). A further, 10 mL of petroleum ether was added to the residue in the conical flask which was allowed to stand for 12 h and decanted into the same beaker. The procedure was repeated a third time and the new weight of the beaker was noted as (W_2). Following the evaporation of the petroleum ether, the weight of the beaker was noted as (W_3). The weight of fat present in the sample was obtained by the difference in the beaker weights before and after extraction with petroleum ether. This determination was undertaken in triplicate. The weight of fat was obtained from equation 11.

$$\% \text{ Fat} = \frac{W3-W2}{W1}x100 \quad (11)$$

Where:
W_1 = weight of each peel extract type (Cs-Exop/Cs-Mesop)
W_2 = weight of beaker + Fat + Petroleum ether
W_3= weight of beaker + Fat
$W_3 - W_2$ = Fat

Determination of carbohydrate content
Total crude carbohydrate content was obtained by difference using the fresh weight derived data as shown in the equation below:

% Carbohydrate =100 - (% Protein + % Fat + % Ash + % Moisture + % Fiber) (12).

Each analysis was carried out in triplicate and ANOVA statistical method was used for the statistical analysis

Microbial load
Microbial load determinations of both the powdered peel extracts of *C. sinensis* were carried out in accordance with the US Pharmacopoeia procedures for the microbial limit test, [45] using 1 in 10 and 1 in 100 sterile dilutions of the extracts in 4 % Tween 20 and inoculated into the different microbial media as described in an earlier study by Ologunagba *et al.*,[39] The plates containing Sabouraud Dextrose Agar (fungal medium) were incubated at the Laboratory ambient temperature and observed daily for one week. Observations and colony count from other plates were done after 72 hours.

Toxicological evaluation
Study protocol
Male mice weighing between 18-36 g were used for the study. They were housed in a standard environmental condition and fed with rodent standard diets and water ad libitum. Animal care and handling conformed to OECD guidelines. [46,47] Ethical approval for the study was sought and obtained from the health research and ethics unit of the college of medicine of the University of Lagos .The Lethal Dose (LD_{50}) of the orange peel extract (Cs-Mesop) and its acute toxicity profile on experimental animals was determined.

Lethal Dose (LD_{50}) and Acute Toxicity Determinations

The limit test dose of 5000 mg/kg (LD_{50}) and acute toxicity evaluations on mice (18-36 g) were in accordance with the officially stipulated protocols and guidelines by Organization for Economic Cooperation Development (OECD, 2001; 2002) were undertaken[46,47]. The animals were observed individually for acute toxicity signs and behavioural changes 1 h post-dosing, and at least once daily for 7 days. Haematological parameters (red blood cell, white blood cell) of the extract (Cs-Mesop) treated and control mice were evaluated as described by Ibrahim *et al*., 2016 [48]

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) with Tukey test to evaluate significant differences between groups. Significant differences between control and experimental groups were assessed with student's t-test. Values of $p < 0.05$ were considered significant. All statistical analyses were carried out using the SPSS for Window XP Software Program (Version 13.0)

Results and Discussion

The results on the physicochemical properties of the two extract types are shown in Table 1. The extract yields from the exocarp and mesocarp peels of *C. sinensis* using the micro-wave assisted technique were below 20% w/w, respectively being 15.5% w/w and 5.1 % w/w, with an indication of higher extract yield from the exocarp extract. The totality of these extract values were in accordance with the 20 % w/w extract yield reported for the orange peel (a composite of the two aforementioned peels) by Tyagi *et al*.[49] A higher methanolic peel extract yield 60 % was however reported by Arora and Kaur[50] using Soxhlet extraction technique. It would be required therefore, that an optimized cost-effective micro-wave extraction technique and protocols be developed for higher orange peel extracts yield.

The organoleptic properties of the exocarp (Cs-Exop) and mesocarp (Cs-Mesop) peels were found to be acceptable. The Cs-Exop and Cs-Mesop powders were with characteristic tastes being respectively bitter and sour. They both had brownish-white colors, with Cs-Exop presenting with a darker shade, and a more pungent taste and odour which could be due to its higher content of tannins and flavonoids. This implies that Cs-Mesop would be preferentially used in formulations that are not coloured and gives consideration to the use of Cs-Exop in coloured formulations. Cs-Mesop comparatively had finer particle size and texture which infer the existence of higher inter- particulate cohesive forces that could affect the powder flow property (angle of repose) and pattern.

Table 1: The Comparative Physicochemical Properties of *Citrus sinensis* Peel Extract Types

Evaluation Parameter	**Extract of *Citrus sinensis* Peel Type**	
	Cs-Exop	***Cs-Mesop***
Yield (% w/w)	15.5 ± 0.01	5.1 ± 0.01
Organoleptic Odour	Sharp	Sharp
Taste	Bitter	Soar
Average particle Size (µm)	120.8 ± 0.02	99.7 ± 0.01
Solubility		
Water	Sparingly soluble-(Dispersible)	Sparingly soluble (Dispersible)
Organic solvents (Ethanol, Acetone)	Insoluble	Insoluble
Viscosity (mPa.s)	12.6 ± 0.10	19.5 ± 0.15
pH	5.52 ± 0.15	6.41 ± 0.11
Swelling Index (%)	2.08 ± 0.02	3.01 ± 0.03
Water binding Capacity	1.77 ± 0.15	3.32 ± 0.05
Bulk Density (g/mL)	0.43 ± 0.02	0.56 ± 0.02
Tapped Density (g/mL)	0.54 ± 0.01	0.72 ± 0.01
Hausner's ratio	13 ± 0.03	13 ± 0.05
Carr's Compressibility Index	20.3 ± 0.04	22.2 ± 0.08
Flow Rate (g/s)	1.67 ± 0.05	7.32 ± 0.01
A^{o}R	27.91 ± 1.65	29.4 ±1.15

Key: Results expressed as average of triplicate determinations; *Cs-Exop =Citrus sinensis* exocarp peel; *Cs-Mesop =Citrus sinensis* mesocarp peel; A^{o}R= Angle of Repose

This probably explains why its angle of repose was higher than that of Cs-Exop, thus a formulation powder system that contains Cs-Exop as an excipient would be expected to comparatively have a better flow pattern than that which contains Cs-Mesop; however, in this study, Cs-Mesop extract had an impressively comparative better flow pattern (7.32 ± 0.01) to Cs-Exop which was 1.67 ± 0.05. This indicates the contribution of other intrinsic material attributes of Cs-Mesop extract to its flow pattern, perhaps this extract type has an inherent glidant property which acts by the reduction of the material inter-particulate cohesive forces. In the light of this finding, Cs-Mesop portend as a glidant and lubricating excipient.

They were found to be sparingly soluble in water, insoluble in organic solvents, are acidic, with the mesocarp being less acidic, more viscous with comparatively smaller particle size.

The solubility behavior of any excipient is an important parameter to determine its suitability in different formulations. Extracted Cs-Exop and Cs-Mesop swelled on hydration in aqueous media (cold or hot) to form gels and would therefore be useful as pharmaceutical excipients as binders, thickening agents, suspending agents, gelling agents, matrix formers, release retardants and carriers. The fact that they both were completely insoluble in organic solvent such as benzene, ether, chloroform, n-butanol methanol and ethanol was in agreement with the characteristics of organic materials.

The pH values of 1% solution of the extracts (Cs-Exop and Cs-Mesop) being slightly acidic respectively, (pH 5.52 and pH 6.41) infers its low index of mucosal irritation and suggests their potential excipient suitability/ usefulness in oral formulations as preparations.

The water binding capacity for Cs-Mesop was comparatively higher (3.32 ± 0.05) than that of Cs-Exop (1.77± 0.15) which implies that the latter would exhibit comparatively higher hydration capacity and swelling index which will confer better material attribute for the aforementioned potential excipient utilities

The micromeritics properties of both the Cs-Exop and Cs-Mesop were acceptable and were in conformity with the findings of Tyagi *et al*.[49]

When the angle of repose is less than 30^0, then it indicates that powder is free flowing and values greater than 40^0 suggest a poorly flowing powder. Carr's index values up to 15 % generally show good to excellent flow properties of a powder which indicate desirable packing characteristics and values above 25% are often sources of poor tableting qualities. So, the values that are between these two indices may result in less than the optimum performance and modification of the particle size distribution could be advisable

The mesocarp also had a comparatively higher swelling index, water binding capacity, bulk and tapped densities and better flow properties which infers its better excipient potential as a matrix former, release retardant, micro/nanoparticulate carrier in novel drug delivery systems.

The result of proximate composition (Table 2) shows that the moisture contents of the two peel extract samples were low (≤ 10.00 % w/w). This is expected since the sample has been subjected to drying for five days to reduce the moisture content. High moisture content is an index of spoilage. It also indicates the hygroscopic nature of a powdered substance that would entail the necessity of storage in air-tight containers. These obtained values were in slight agreement with the findings of Gade *et al*., [51] who indicated 9.2 % w/w in the moisture contents of the orange peels obtained from a local market in Mumbai, India. The observed comparatively lower moisture content of *Cs*-Exop extract to Cs-Mesop extract is expected and could be explained that, being the outermost layer, is more exposed to environmental conditions such as sunlight.

The low ash values (≤ 10.00 % w/w) of the two peel extract types are in agreement with the findings of Gade *et al.,*[51] who indicated value of about 7.8 ± 0.01 in their study of oranges from Mumbai. This infer the high purity levels of these extracts, though the comparatively higher ash content of *Cs*-Mesop extract could be due to its comparatively higher content of crude fiber and carbohydrate. Ash values are an important parameter to characterize natural excipients. The higher contents of carbohydrate (about 50% w/w) in the two extract types compared to other constituents infers and suggestion of good potential for utility as a diluent in pharmaceutical formulations which aligns with the opinion of Olabinjo *et al*.,[52]

The presence of phytoconstituents in both peel extracts has been elucidated as displayed in Table 3. The crude fat contents of both peel extracts were (≤ 12.7 % w/w), the comparatively higher crude fat of *Cs*-Exop extract could be due to the presence of essential oils (monoterpenes especially d-limonene, myrcene, α-pinene, linalool, octanal and decanal)[53,54] This infers the potential excipient utility of these extracts as flavoring agents in pharmaceutical formulations which was also similarly reported by Edris.[55]

The presence of essential oils in these extracts also suggests their permeation and antimicrobial potentials. This submission is in line and drawn from the earlier reports by several workers Liu *et al*., and Guo *et al*.[54,56] that the essential oils from *Citrus sinensis* had a good inhibitory effect on microbial growth with inference to its potential use as a novel bacteriostatic agent. These findings were also in conformity with the report of its anti-oxidative, anti-bacterial, anti-inflammatory effects by Dosoky *et al*.[57] Furthermore, its anti-cancer activity has been reported Yang *et al*.[58]

The protein contents of both extracts which was (< 20 % w/w) indicate the presence of bioactive such as tannins, alkaloids, flavonoids and saponins etc. which may be higher in the Cs-Exop extract due to its higher protein content. The comparatively higher contents (>40 % w/w) of crude carbohydrates to other crude constituents (protein, fat and fiber) in both peel extracts infer their high polysaccharide constituents and suggests their potential excipient usefulness in pharmaceutical formulations as diluents, binders, disintegrants matrix formers and carriers.

Table 2: The Comparative Proximate Properties of *Citrus sinensis* Peel Extract Types

Proximate Content (%)	***Citrus sinensis* Peel Extract Types**	
	Cs-Exop	***Cs-Mesop***
Crude Fat	12.7 ± 0.16	11.75 ± 0.72
Moisture	8.78 ± 0.14	10.3 ± 0.17
Ash	7.57 ± 0.04	9.36 ± 0.25
Crude Protein	17.8 ± 0.83	12.3 ± 0.65
Crude Fiber	7.53 ± 0.41	9.5 ± 0.6
Crude Carbohydrates	45.62 ± 0.05	46.79 ± 0.59

Key: Results expressed as average of triplicate determinations; *Cs-Exop =Citrus sinensis* exocarp peel;
Cs-Mesop =Citrus sinensis mesocarp peel

Table 3: Comparative Pharmacognostic Properties of *Citrus sinensis* Peel Extract Types

Phytonutrient type	***Citrus sinensis* Peel Extract Types**	
	Cs-Exop	***Cs-Mesop***
Alkaloids	+	-
Reducing sugars	+	+
Tannins	+	-
Glycosides	+	+
Fats and Oils	+	+
Anthraquinones	-	-
Carbohydrates	+	+
Ketones	-	-
Flavonoids	+	+
Phenolics	+	+
Saponins	+	_

Key: Results expressed as average of triplicate determinations; (+) = indicates presence; (-) = indicates absence*; Cs-Exop =Citrus sinensis* exocarp peel; Cs-*Mesop =Citrus sinensis* mesocarp peel

The assertion of their binding potentials is in conformity with the reported work in a paracetamol tablet system by Srivastava *et al*.[24]

The findings of the proximate analysis are in slight conformity with those reported by Adewole *et al*.[59] with regards to the crude fat, moisture content for the Cs-Mesop extract, whereas, for the Cs-Exop extract, the aforementioned parameters were higher. The difference could be as a result of geographical location, environmental conditions like climatic and soil conditions, specie types, age of plant and harvesting period/conditions, etc.

Further characterization confirmed the presence of these phytonutrients: reducing sugars, glycosides, alkaloids, tannins, flavonoids, carbohydrates, fats and oils and proteins in both the exocarp and mesocarp peels. The phytochemical constituents of the two extracts have high contents of secondary metabolites which are in conformity with the findings of Bruneton [60] and Kumar *et al*.[61]

The exocarp peel extract comparatively contained more phytoconstituents as it additionally had alkaloids and tannins ,which is a contrast findings to the absence of same as reported by,[49] in the phytochemical constituents of their reported orange peel , but are in conformity with the reports of Manthey[62] and Kanaze *et al.*[63] who indicated presence of flavonoids in the orange peel. These two extracts were found not to contain ketones and anthraquinones.

These observed differences could be due to differences in environmental conditions such as soil type, climatic conditions and other plant variability factors such as harvest period/season, specie type,

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age of plant, extraction techniques etc. The presence of secondary metabolites will confer additional antimicrobial, antioxidant, anti-inflammatory, tissue repair/modifier property/attribute of the extracts.

The presence of secondary metabolites in the orange peels are good indication that if the peel is subjected to intensive research/study, bioactive compounds with good pharmacological activities may be isolated, characterized using various spectroscopic techniques and novel compounds may also be discovered. These two extract types thus have the potential utility as anti-oxidants, ant-inflammatory and antimicrobial agents in semi-solid oral and topical pharmaceutical formulations.

The result of the microbial load assessment is displayed in Table 4. The two extract types conformed to the microbial load specifications of the Pharmacopoeial [45] as the microbial evaluation revealed the absence of objectionable microorganisms and the total aerobic microbial counts were 8.00 x 10^2 and 19.50 x 10^2; yeast and mould counts were 2.00 x 10^2 and 6.00 x 10^2 respectively. Thus, are both microbiologically safe. They were also both effective against some microbes **(***Salmonella spp, E. coli, Staphylococcus spp, Pseudomonas aeruginosa, Shigella spp and Proteus spp***.)** using the agar well diffusion method, which infers their usefulness as antimicrobial. This finding is in agreement with the findings of Shetty *et al*.[64] who indicated that the peel extracts of *Citrus sinensis* had effective antimicrobial properties against dental caries bacteria (*Streptococcus mutans* and *Lactobacillus acidophilus*). The Cs-Exop extract was found to have more comparative antimicrobial inhibitory activity to the Cs-Mesop extract (result not shown); this could be due to its higher bioactive constituents such as flavonoids as reported by Manthey[62] and Kanaze *et al.*[63]

The toxicological evaluation outcome on Cs-mesop extract is displayed in Tables 5 and 6. It is toxicologically safe, as the lethal dose (LD_{50}) value was greater than 5000 mg/kg. Furthermore, at all the administered doses, there was no occurrence of morbidity, mortality and abnormalities. Tables 5 and 6 show that in all the administered concentrations of the extract at both acute and sub-chronic assessments, there was no abnormalities in the haematological profiles of the treated groups and no statistical significance was obtained between the control and the treated groups.

Furthermore, no histopathological abnormality in both the liver and kidney tissues of the treated groups comparative to the control group were observed at all the listed concentrations in Tables 5 and 6, though (histopathology micrograph not shown).

These outcomes suggest the toxicological safety of Cs-mesop extract with inference of its usefulness as a potential pharmaceutical excipient.

Table 4: The Comparative Microbial Load of *Citrus sinensis* Peel Extract Types

Microbial Group	**Colony Count (cfu/g)**		
	Cs-Exop	**Cs-Mesop**	**USP Limit (cfu/g)**
Total Aerobic Microbial Count	800	1950	Not more than 10^3
Total Combined Yeast and Mould Count	200	600	Not more than 10^3
Bile Tolerant gram –ve organisms	1950	1540	Not more than 10^3
Salmonella spp	Absent	Absent	Absent
Shigella spp	Absent	Absent	Absent
Escherichia coli	Absent	Absent	Absent
Klebsiellaspp	Absent	Absent	Absent
Pseudomonas aeroginosa	Absent	Absent	Absent
Proteus spp	Absent	Absent	Absent
Staphylococcus spp	Absent	Absent	Absent

Key: Results expressed as average of triplicate determinations

The peel extracts (Cs-Exop and Cs-Mesop) exhibited *in vitro* antimicrobial activities against *Salmonella spp, E. coli, Staphylococcus spp, Pseudomonas aeruginosa, Shigellaspp and Proteus spp*. The Cs-Exop extract exhibited comparatively higher antimicrobial activity to the Cs-Mesop extract (result of comparative antimicrobial susceptibility not shown).

Table 5: The Effect of post-24-hour administration of different concentrations of aqueous dispersions of Cs-Mesop peel extract on the haematological Parameter of mice

Haematological Parameter	**Doses of Extract (mg/kg)**					
	200	**400**	**600**	**800**	**1000**	**Control Group**
RBC	5.5 ± 0.37	5.1 ± 0.26	5.4 ± 0.27	5 ± 0.58	4.9 ± 1.08	5.1 ± 0.39
HCT	32.9 ± 3.75	28 ± 0.37	27 ± 4.95	25.8 ± 5.39	23.5 ± 8.63	29.9 ± 1.78
PLT	381 ± 55.2	410 ± 3.54	425 ± 15.6	392 ± 50.2	524 ± 41.7	610 ± 86.6
WBC	2.1 ± 0.42	2 ± 0.28	2.3 ± 1.06	1.1 ± 0.21	2.3 ± 0.92	2.2 ± 0.44
HGB	8.8 ± 0.21	8.1 ± 0.42	9.6 ± 1.49	10 ± 0.42	7.4 ± 0.71	9.8 ± 0.15
LYM	76.3 ± 5.8	77.4 ± 3.8	80.7 ± 4.4	81.0 ± 12.9	71.9 ± 7.5	78.0 ± 11.60

Key: Key: RBC= Red Blood Cell; HCT=Haematocrit; PLT=Platelet; WBC=White Blood Cell; HGB= Haemoglobin; LYM=Lymphocytes; Results expressed as average of triplicate determinations

Table 6: The Effect of post-one week (7 days) administration of different concentrations of aqueous dispersions of Cs-Mesop peel extract on the haematological parameter of mice

Haematological Parameter	Doses of Extract (mg/kg)					
	200	400	600	800	1000	Control Group
RBC	5.1 ± 0.80	5.24 ± 0.27	5.3 ± 0.47	4.5 ± 1.2	4.2 ± 1.87	5.0 ± 0.4
HCT	28.5 ± 2.75	31.8 ± 3.68	29.8 ± 3.96	26.3 ± 8.13	25.2 ± 10.68	30.03 ± 1.59
PLT	436 ± 77.80	472 ± 55.2	442 ± 1.56	420 ± 68	355 ± 33.1	634 ± 11.8
WBC	1.3 ± 0.14	1.9 ± 0.85	1.8 ± 1.41	1.4 ± 0.6	1.3 ± 0.85	1.7 ± 0.4
HGB	8.6 ± 0.85	9.2 ± 0.92	8.7 ± 1.41	7.6 ± 2.2	7.5 ± 3.2	8.5 ± 0.42
LYM	85.0 ± 7.0	82.5 ± 7.4	83.4 ± 6.7	86.4 ± 9.3	73.5 ± 6.5	81.0 ± 14.1

Key: RBC= Red Blood Cell; HCT=Haematocrit; PLT=Platelet; WBC=White Blood Cell; HGB=Haemoglobin; LYM=Lymphocytes; Results expressed as average of triplicate determinations

Conclusion

Cs-Exop and Cs-Mesop extracts have been successfully obtained from *C. sinensis* peels using micro-wave extraction, a cost-effective technique. This study has established the good and desirable physicochemical, microbiological and toxicological properties of these two extracts which infer their potential pharmaceutical excipient utilities as diluents, binders, disintegrants, emulgents, lubricants/glidants, thickening, suspending, gelling agents and release modifiers (fast dispersants, release retardants, matrix formers, micro/nanoparticulate carriers). In the light of their constituent bioactive, they are furthermore suggested as permeation enhancers, antioxidants, anti-inflammatory and antimicrobial agents in oral and topical formulations.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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