Research Article

PRELIMINARY EVALUATION OF THE PROPERTIES OF BIOMATERIAL OF *TYMPANOTONUS FUSCATA* SHELL AS PHARMACEUTICAL EXCIPIENT

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ABSTRACT

Tympanotonus fuscata var *radula* (L) or periwinkle is a mollusc and abounds in the Niger Delta, Nigeria. It is highly consumed as source of protein. The empty shells, hitherto poorly utilized add to environmental menace. This pilot study seeks an industrially based economic value for the shell. The physico-technical properties of the powder processed from it was evaluated as excipient for the pharmaceutical sector. A 10 % w/v aqueous dispersion of the biomaterial has pH of 11.20. It was insoluble in water and several organic solvents, effervesces in dilute or concentrated mineral acid. High level of calcium and other trace elements below the recommended pharmacopeia limits were analysed. Since its $LD_{50} > 15,000$ mg/kg body weight in albino mice, it is considered non-toxic and may serve as pharmaceutical aid in formulating orally administered medicines. Its low moisture content and moisture absorption capacity below 3 % in 96, 84, 75 and 52 % (30°C) relative humidity shows that it may offer a stable formulation with hydro-degradable drugs. The compacted pellets showed high friability, though powder demonstrated poor flowability, implying that it may serve as tablet diluent in wet granulation technique and not as directly compressible filler. Its trace metal and high calcium content indicates that it could be processed commercially as calcium supplement for livestock. These findings if harnessed will cause a shift of economic responsiveness to periwinkle shell, protect our environment, extend agricultural activities, offer job prospects and generate raw-material for the indigenous drug and food industries.

Keywords: Evaluation, Properties, Biomaterial, Shell, *Tympanotonus fuscata*, Pharmaceutical, Excipient.

INTRODUCTION

The *Tympanotonus fuscata* var *radula* (L) often called periwinkle belongs to the phylum, mollusca. They are commonly found in lagoons, estuaries, mangroves, shallow waters and occasionally in inter-tidal zones where they burrow into the mud in beds of the river. They are endemic to West Africa¹⁻⁹. It is common as sea food and cheap source of animal protein in the Niger Delta region of Nigeria where its collection and marketing constitute an important industry¹⁰. The empty shells are discarded as waste and it adds to the environmental menace in this region. The shell is the hard, rigid outer calcium carbonate covering of certain animals. Though many other animals, particularly those that live in the sea produce exoskeleton, usually, only those of molluscs are considered to be shells. Chitin contained in shells adds to its hardness. They are also made of nacre, an organic mixture of outer layer of horny conchiolin which is a scleroprotein, followed by an intermediate layer of calcite or aragonite and then a layer of calcium carbonate in the form of platy crystals. Nacre is secreted by the ectodermic cells of the mantle tissue of certain species of mollusc. The blood of mollusc is rich in liquid form of calcium which concentrates out and can crystallize as calcium carbonate. The layers of calcium carbonate may incorporate conchiolin which binds the calcium carbonate crystals together. Large amounts of shells may form sediment and become compressed into limestone¹. In the Niger Delta areas of Nigeria, some people use the shells of *Tympanotonus fuscata* as cheap substitute for chippings in structural work. Some local livestock farmers add pulverised periwinkle shells to livestock feed as source of calcium supplement. Other uses of periwinkle shells have been reported. Periwinkle shell ash (PSA) has been investigated as a pozzolanic material, a step taken to improve on the

strength and durability performance of concrete. PSA has also been investigated for its suitability as partial replacement for ordinary Portland cement in concrete^{6,11-14}. The objective of this pilot study is to process and evaluate the properties of the powder of the periwinkle shell to ascertain its possible usefulness in the food or pharmaceutical industry as excipient. It is expected that findings from this work will cause a shift of economic responsiveness to periwinkle shell, protect our environment, extend agricultural activities, offer job prospects and generate raw-material for the indigenous drug and food industries.

MATERIALS AND METHODS

The materials were used as procured and include: nitric acid, acetone, acetic acid, methanol, chloroform, ether, glycerine, propylene glycol, hydrochloric acid and sodium hydroxide (M and B, England), *n*-hexane (Riedel de Haen, Germany), sulphuric acid, ethanol (BDH, England), sodium hypochlorite (3.85 % w/v), *Tympanotonus fuscata* (periwinkle) shells were collected from Borokiri, Port Harcourt, Nigeria.

Processing of periwinkle shell powder (PSP)

A 1 kg quantity of periwinkle shell was washed with portable water and soaked in 5 L of 3.85 % w/v sodium hypochlorite solution for 3 h to soften and aid the removal of the dark outer coat. The shells were washed severally by agitating in a sink under continuously flowing tap water until they were freed from the dark outer coats. The shells were washed further until the pH of the water content became neutral. The shells were dried in hot air oven (Uniscope, England) at 60°C for 72 h. Dried shells were pulverized in a

Hammer Mill (Thomas Willey, Model 4, USA). The powder obtained was classified through 250 µm stainless steel sieve (Endecott, England). In this work, the pulverized biomaterial was referred to as Periwinkle Shell Powder (PSP). It was stored in amber coloured air-tight jar for further investigations.

Characterization of periwinkle shell powder Organoleptic test

The powder was assessed for colour, odour, taste and texture.

Elemental analysis

A 1 g quantity of PSP was digested with concentrated nitric acid in Kjeldahl flask and filtered. The resulting solution was transferred into a 100 ml volumetric flask and made up to 100 ml with distilled water. Elemental analysis of the resultant solution was carried out using Atomic Absorption Spectrophotometer (Unicam-929 AAS, UK). Sodium and potassium were analysed by flame photometry. Barium was qualitatively analysed using the method of USP¹⁵.

Acute toxicity test

Aqueous dispersion of PSP was administered orally to male albino mice having an average body weight of 19 g. The animals were fasted for 24 h prior to the administration of the sample. This test was carried out in two stages. The first stage consisted of three groups of eight animals each. Each mouse per group was fed with 10 mg/kg, 100 mg/kg and 1000 mg/kg of body weight of PSP respectively. In the absence of lethality after 24 h, the animals were regrouped into four groups of six animals per group. Each mouse in a group was administered with a dose of 2900, 5000, 8000 and 15000 mg/kg of body weight respectively. They were also observed for a period of 24 h for any sign of toxicity. This test was performed in accordance with the ethical guidelines for the acquisition, care and disposal of animals.

Solubility

The solubility of 0.1 g of PSP was verified in water, ethanol (96 %), methanol, chloroform, ether, glycerine, propylene glycol, dilute or concentrated mineral acid respectively at room temperature. Similarly, these were also investigated at elevated temperatures using a thermostated water bath (Bio-Technics, India) maintained at 40°C.

pH of 10 % (w/v) dispersion

The pH of 10 % (w/v) aqueous dispersion of PSP was determined with a pH meter (Omega, USA).

Determination of ash values

Total ash, acid insoluble and water soluble ash was determined using the methods outlined in the British Pharmacopoeia 2012¹⁶.

Moisture content

The moisture content of PSP was determined using digital moisture balance (Citizen, MB-50, China). A 2 g quantity of sample was weighed on the equipment adjusted to 105°C. The equipment automatically switches off when optimum moisture contained in the sample has been removed. The value of moisture content was recorded as digitally displayed on the equipment.

Moisture absorption studies

A 0.5 g quantity of PSP each was stored in different air tight desiccators containing saturated aqueous solution of potassium sulphate, potassium chloride, sodium chloride and magnesium nitrate in contact with their excess solute at about 30°C to maintain

relative humidity of 96, 84, 75 and 52 % respectively¹⁷. Studies lasted for 30 days. The increase in weight of sample was calculated as percentage moisture gain:

$$\% \text{ Moisture gain} = \left(\frac{\text{Moisture gain}}{\text{Original weight}} \right) \times 100 \quad \dots (1).$$

Determination of densities of PSP

A 50 g of the sample was delivered into a glass measuring cylinder through a glass funnel and the bulk volume was noted. The measuring cylinder was later tapped until no further change in volume was observed. Five determinations were made. The bulk and tapped densities were calculated from the following relationships:

$$\text{Bulk Density} = \text{Mass/Bulk Volume} \dots\dots (2)$$

$$\text{Tapped Density} = \frac{\text{Mass of powder}}{\text{Tapped volume of powder}} \dots (3)$$

The true density of PSP was determined using the displacement method as reported by Odeku *et al*¹⁸. A 0.5 g was used and n-hexane was adopted as non-solvent.

Evaluation of flow properties of PSP

A 50 g of sample was used to determine the flow rate of PSP using the funnel method reported by Carstensen and Chan¹⁹. Its angle of repose was measured by pouring 50 g of powder into a cylindrical paper roll fixed on to a flat base whose diameter is known and the same as the internal diameter of the cylinder. The cylinder was slowly pulled out vertically so as to form a cone of powder on the base. The height of the cone was measured. This is a modification of the method of Jones and Pilpel²⁰. Calculations were made as follows:

$$\text{Flow rate} = \text{Mass of powder/Time} \dots\dots(4)$$

$$\text{Angle of repose; } \theta = \tan^{-1} \frac{h}{r} \dots\dots\dots (5)$$

The compressibility index, CI was calculated from the values of bulk and tapped volumes of the material obtained during the determination of bulk and tapped densities respectively. This was calculated from the formula:

$$CI = \left\{ 1 - \left[\frac{V}{V_0} \right] \right\} \times 100 \dots\dots\dots (6)$$

Where: V = tapped volume and V₀ = bulk volume

Hausner's quotient was calculated from the values of tapped and bulk densities of PSP using the formula:

$$\text{Hausner's quotient} = \text{Tapped density/Bulk Density} \dots\dots\dots (7)$$

The porosity of sample was calculated using the values of bulk and true densities as follows:

$$\text{Porosity} = \{ 1 - [\text{Bulk density/True density}] \} \times 100 \dots\dots\dots (8)$$

Compactability of periwinkle shell powder

A 400 mg of PSP was compressed at varying pressure loads of 2.45, 4.90, 7.35, 9.80 and 12.25 kN respectively using a 10.5 mm die and flat- faced punches on a hydraulic hand press (Model C, Carver Laboratory Press, Menomonee Falls, WI. USA).

Table 1: Physico-technical properties of PSP

Physico-technical parameter	Value
LD ₅₀	> 15,000 mg / kg
Processing yield (%)	93.50 ± 1.24
pH	11.20
Moisture content (%)	0.40 ± 0.01
Moisture absorption (%)	2.10 ± 0.04, 0.48 ± 0.02, 0.37 ± 0.01
96, 84, 75 and 52 % Relative Humidity	and 0.2 ± 0.01
Bulk density (g/ml)	0.99 ± 0.03
Tapped density (g/ml)	1.96 ± 0.01
True density (g/ml)	1.96 ± 0.03
Porosity (%)	49.34 ± 0.03
Compressibility index (%)	49.23 ± 1.25
Hausner's quotient	1.97 ± 0.02
Flow rate (g/s)	No free flow
Angle of repose (degree)	38.53
Total ash (%)	9.50 ± 0.12
Acid insoluble ash (%)	1.00 ± 0.01
Water soluble ash (%)	0.50 ± 0.03
Compact hardness (N)	Very friable

RESULTS AND DISCUSSION

The results of the physico-technical tests for PSP are presented in Table 1. The powder was moderately coarse, tasteless, off white in colour with characteristics pleasant, non-irritating and non-pungent odour. A high yield of 93.50 ± 1.24 was obtained after processing. The pellets compressed from the powder were very friable. The powder was practically insoluble in water, ethanol (96 %) and other organic solvents. A prolonged effervescence occurred when the powder was brought in contact with dilute or concentrated mineral acid releasing gas which turned lime water milky. This shows that the shell of *Tympanotonus fuscata* may contain carbonate suspected to be calcium carbonate. Calcium carbonate has been reported to be practically insoluble in water and ethanol (96 %) ²¹. A pH of 11.20 was obtained for a 10 % w/v aqueous dispersion of the powder. A pH of 9.0 was reported for a 10 % w/v aqueous dispersion of calcium carbonate ²¹. The higher pH value obtained for periwinkle shell powder may be due the presence of several other alkaline metals in its natural state. Elemental analysis show the following metals: iron, 4.0×10^{-7} , copper, 2.8×10^{-6} , zinc, 1.3×10^{-6} , manganese, $< 1.0 \times 10^{-7}$, lead, 3.0×10^{-7} , cadmium $< 1.0 \times 10^{-7}$, nickel, 3.48×10^{-4} , vanadium, 3.94×10^{-4} , chromium, 7.35×10^{-4} , sodium 3.53×10^{-2} , potassium, $< 1.0 \times 10^{-7}$, calcium 38.50, magnesium, $< 1.0 \times 10^{-7}$ and arsenic $< 1.0 \times 10^{-7}$ %. Barium, selenium and mercury were below detectable levels. Trace metals such as zinc, lead, cadmium, copper and nickel have been identified in the shell of *T. fuscata* by other investigators ²². Mollusc blood is rich in liquid form of calcium which concentrates out from the blood and crystallizes as calcium carbonate. Mollusc shells consist mainly of Calcium carbonate ²³. Large amounts of shells may form sediment and become compressed into limestone. Calcium occurs abundantly and is widely distributed as calcium carbonate which is found as chalk, lime stone, marble, calcite or aragonite ¹. Some pharmaceutical excipients employed in the formulation of orally administered medicinal products contain some of the metals detected in PSP. Acacia for instance contains calcium, potassium and magnesium ^{21,24}. The British Pharmacopoeia ¹⁶ indicates limits for heavy metals, 4.0×10^{-4} ; arsenic, 1.0×10^{-3} and iron, 4.0×10^{-1} %. The monograph for calcium carbonate shows the following elements and their maximum limits ^{15,24}: lead $\leq 3.0 \times 10^{-4}$; iron, ≤ 0.1 ; heavy metals, $\leq 2.0 \times 10^{-3}$; arsenic, $\leq 3.0 \times 10^{-4}$; magnesium and alkali metals, ≤ 1.00 and mercury, $\leq 5.0 \times 10^{-5}$ %. The values of metals such as arsenic, iron, magnesium and alkali earth metals other than calcium obtained for PSP in this work are low compared to the limits documented in the pharmacopoeias ^{15,16,22}. These findings coupled with its LD₅₀ above 15,000 mg/kg body weight in albino mice shows that it may not be toxic if used in livestock feed, food or other orally administered pharmaceutical dosage formulations. A total ash value of 9.5 ± 0.12 % obtained for PSP is high and may

indicate that the material consists mainly of compound that cannot be broken down further by heat. It may also be as a result of other crystalline materials formed or deposited in the shell of the *T. fuscata* such as calcite and aragonite ¹. Periwinkle shell powder contains 0.40 ± 0.01 % moisture. Moisture absorption studies showed low values of 2.10 ± 0.04 %, 0.48 ± 0.02, 0.37 ± 0.01 and 0.2 ± 0.01 in 96, 84, 75 and 52 % relative humidity respectively at 30°C after 30 days. Though, moisture absorption gradually increased with increase in relative humidity, the values are not very high. This shows that the powder may be suitable for the formulation of hydro degradable drugs. The results of the tests on flow rate, angle of repose, compressibility index and Hausner's quotient (Table 1) show that PSP has poor flow properties. A high flowability of powder is desirable for content uniformity and less weight variation in tablet formulation especially for directly compressible fillers. It has been reported that Carr's compressibility index < 20 %, angle of repose < 32° and Hausner's quotient < 1.2 are indications of good flowability for any pharmaceutical powder ^{15,21,25}. A true density of 1.96 ± 0.03 g/ml for periwinkle shell powder shows that it is not very light material. Bulk and tapped densities of 0.99 ± 0.03 and 1.96 ± 0.01 g/ml respectively were obtained for PSP. These parameters assist in the prediction of the ability of the material to undergo compression and final volume of the tablet ²⁶. The fact that the values of bulk and tapped densities were almost close coupled with low value of porosity indicate why the compacts of PSP were very friable ²⁷⁻³¹.

CONCLUSION

The non-toxicity of PSP makes it useful as excipient in the formulation of medicinal products. With high calcium content, it could be processed and packaged commercially as calcium supplement for livestock. Since direct compressions of the powder failed to yield cohesive compacts, it could be utilized as diluent in tablet preparation using the wet granulation method especially for those drugs that are moisture and thermo-stable. Further particle engineering of PSP is necessary to possibly generate a powder that will be effective as directly compressible diluent for solid pharmaceutical dosage forms. This will be very useful for low dose drugs as well as other active pharmaceutical ingredients that are neither hydro nor thermo-stable. These findings if effectively harnessed could cause a shift of economic responsiveness to *T. fuscata* shell. This will save our environment from menace of these shells, extend agricultural activities, provide more job prospects and generate raw-material for the indigenous drug and food industries.

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