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Chemical content of the periwinkle shell and its suitability in thin layer chromatography

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Abstract

The calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) contents of the periwinkle (*Tympanotonus fuscatus* and *Littorina littorea*) shell found in Uyo, Akwa Ibom State were determined by complexometric titration. Results indicated that *Tympanotonus fuscatus* and *Littorina littorea* had high level of $CaCO_3$ (88.22 ± 0.75% and 85.38 ± 0.80% respectively) and a relatively low but substantial level of $MgCO_3$ (10.25 ± 0.42% and 9.43 ± 0.60% respectively). The result is discussed with particular reference to the suitability of the periwinkle shell being used as slurry in thin layer chromatography.

Keywords: $CaCO_3$, $MgCO_3$, periwinkle shell, slurry, thin layer chromatography

1. Introduction

The periwinkle is a sea snail. Several species exist which include *Littorina littorea*, *Littorina scutulata, Littorina irrorata and Tympanotonus fuscatus*, having characteristic shapes and marks with which they can be identified. They are invertebrates of the phylum mollusca, class gastropoda and sub-class prosobranchia. The first three are of the order mesogastropoda, family littorinidae and genus littorina [1] while *Tympanotonus fuscatus* is of the order sorbeoconcha, family potamididae and genus tympanotonos [2]. The periwinkles are found in Nigeria, mainly in riverine areas within Rivers, Akwa Ibom and Cross Rivers States. The periwinkles move by crawling, though very slowly with a maximum speed of 2.5mms^{-1}. They live near low tidal level and feed on seaweeds and algae [3]. The periwinkles have robust shells big enough to withdraw into, hence they are also known as shellfish. Their shells are usually coiled and often beautifully coloured and marked [4]. The mantle covers the body of the periwinkle with a thin sheet of tissue and it is the part of the body that builds the shell [5]. The mantle contains cells which produce an external organic matrix that is rapidly mineralized with calcium carbonate. It is the presence of calcium carbonate that makes the shell hard [6]. The periwinkle shell has found great application in the glass industry, water treatment, construction industry [7], paper and paint industries, and in diet formulation especially as a source of calcium in poultry feed [8, 9].

Thin-layer chromatography (TLC) is a chromatographic technique used to separate non-volatile mixtures [10]. It is performed on a sheet of glass, plastic or aluminium foil, which is coated with a thin layer of adsorbent material, usually silica gel, aluminium oxide (alumina), or cellulose [10]. This layer of adsorbent is known as the stationary phase. TLC can be used to determine the number of components in a mixture, the identity of compounds, and the purity of a compound. By observing the appearance of a product or the disappearance of a reactant, it can also be used to monitor the progress of a reaction. TLC consists of three steps - spotting, development, and visualization.

The aim of this study is to determine the calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$) contents of the shell of *Littorina littorea and Tympanotonus fuscatus* and their possible use as slurry material in thin layer chromatography.

2.1 Materials and Methods

2.1.1 Sample Collection and Preparation for Analysis

Life samples of the periwinkle (*Tympanotonus fuscatus* and *Littorina littorea*) available in Uyo, Akwa Ibom State of Nigeria were purchased from Uyo main market, Uyo, Akwa Ibom State. These were first steeped into hot water for about 20 minutes to enhance the separation of the edible portion from the shell. The edible portion was separated from the shell using a sharp needle. The shells hence obtained were well washed with water and oven dried at a temperature of 80ºC for 20minutes. After drying, the shells were ground into fine powder using a grinding machine and sieved to homogenous particle size. The residue was discarded and the powdered samples stored in well labeled containers for analysis.

2.1.2 Methods

2.1.2.1 Biochemical Analysis

$CaCO_3$ and $MgCO_3$ were determined by complexometric titration [11].

2.1.2.1.1 Determination of $CaCO_3$

2.1.2.1.1.1 Principle

$CaCO_3$ is very insoluble in pure water but will readily dissolve in acid according to the reaction:

$$2HCL_{(aq)} + CaCO_{3(s)} \longrightarrow Ca^{2+}_{(aq)} + CO_{2(g)} + H_2O + 2Cl^-_{(aq)}$$

This reaction cannot be used directly to titrate the $CaCO_3$ because it is very slow when the reaction is close to the endpoint. Instead, the determination is achieved by adding an excess acid to dissolve all of the $CaCO_3$ and then titrate the remaining excess H_3O^+ with NaOH solution to determine the amount of acid that did not react with the $CaCO_3$. The difference between the amount of acid initially added and the amount left over after the reaction is equal to the amount used by the $CaCO_3$. The reaction used to determine the leftover acid is:

$$HCL_{(aq)} + NaOH_{(aq)} \longrightarrow H_2O + Na^+_{(aq)} + Cl^-_{(aq)}$$

2.1.2.1.1.2 Procedure

0.50g of powdered periwinkle shell was poured into a conical flask and few drops of ethanol added. The addition of ethanol acts as a wetting agent and helps HCL to dissolve the $CaCO_3$. 10ml of 1.0M HCL was added slowly to the conical flask with swirling of the flask to wet the sample. The solution was heated until it boiled and then allowed to cool; it was not boiled to dryness. 3 drops of phenolphthalein were added to the conical flask. A burette was filled with 0.1M NaOH solution. Titration was done to the first persistent pink colour which faded when close to the end point. The remaining NaOH was added dropwise until the colour change remained for at least 30 seconds.

2.1.2.1.1.3 Calculations

1. Number of moles of HCL added to the sample:
   Moles added= (0.01liter HCL) x (1.0 mole HCL/liter) = 1.0 x 10^{-2} moles HCL.
2. Number of moles of HCL left in each sample after reaction with $CaCO_3$:
   Moles left= (liter NaOH used) x (0.1M NaOH) x (1 mole HCL / 1 mole NaOH).
3. For each sample, determine the number of moles of HCL that reacted with $CaCO_3$ by taking the difference between the number of moles of HCL added and the number of moles of HCL remaining after the reaction is complete:
   Moles reacted = moles added – moles left
4. The moles of $CaCO_3$ in the sample is calculated by:
   Moles $CaCO_3$= (moles HCL) x (1 mole $CaCO_3$ / 2 moles HCL)
5. Calculate the percentage of $CaCO_3$ in each sample:

$$\% \, CaCO_3 = \frac{(\text{moles } CaCO_3) \times (100.09\text{g } CaCO_3 / \text{moles } CaCO_3)}{\text{Grams of sample}} \times 100$$

2.1.2.1.2 Determination of $MgCO_3$

2.1.2.1.2.1 Principle

$MgCO_3$ is practically insoluble in water. It dissolves in dilute acids with strong effervescence.

2.1.2.1.2.2 Procedure

0.15g of sample was dissolved 20ml of water to which has been added 2ml of 2M HCL. The solution was diluted to 50ml with water. 10ml of ammonium buffer pH 10.0 and 50mg mordant black II mixture were added to the resulting solution. The solution was heated to 40ºC and titrated with 0.1M disodium edetate until the colour changes from violet to blue.

2.1.2.1.2.3 Calculation

Each ml of 0.1M disodium edetate is equivalent to 0.004030g of $MgCO_3$.

$$\% \, MgCO_3 = \frac{\text{Gram weight}}{\text{Total weight}} \times \frac{100}{1}$$

2.1.2.1.3 Plate Preparation [12]

65g of powdered periwinkle shell was mixed in 100ml of chloroform/methanol (2:1) in a glass stoppered flask to homogeneity. Two clean and dry glass slides were placed together and dipped into the suspension above. The two slides were removed from the suspension and air-dried. The resultant plates were activated by heating in oven at 110ºC for about 30mins.

2.1.2.1.4 Preparation of Leaf Extract

10g of fresh cassava (*Mannihot utilisima*) leaves were washed with water and grinded with mortar and pestle to homogeneity. The pigments were extracted by adding 100ml of acetone. The suspension was filtered with whatman paper and the filtrate allowed to concentrate by leaving on the bench for a few minutes.

2.1.2.1.5 Spotting the Plate

20µl of filtrate was spotted at 1.5cm from the end of the plate, by use of a micropipette. The chromatogram was run by placing the spotted plate ends into a chromatographic tank filled with 5% methanol in benzene to a mark of 0.5ml from bottom. The tank was properly covered with a glass plate for 10 minutes after which the chromatogram was examined.

2.1.3 Statistical Analysis

All reported values were expressed as mean ± standard deviation. The statistical significance of all biochemical estimations between any two groups was estimated with the student T-test. The significant level was set at 95% confidence limit and P-value less than 0.05 ($P<0.05$) was considered significant.

3. Results

Table 1 shows results for $CaCO_3$ and $MgCO_3$ contents of the powdered shells of *Tympanotonus fuscatus* and *Littorina littorea*. The $CaCO_3$ and $MgCO_3$ of *Tympanotonus fuscatus* are significantly different (*P*<0.05) from that of *Littorina littorea.*

Table 1: $CaCO_3$ and $MgCO_3$ contents of Tympanotonus fuscatus and Littorina littorea.

Specie	*T. fuscatus*	*L. littorea*
% $CaCO_3$	88.22 ± 0.75a	85.38 ± 0.80b
% $MgCO_3$	10.25 ± 0.42a	9.43 ± 0.60b

Values are Mean ± SD (n=20)

Values with different letters within a row differ significantly from each other ($P \leq 0.05$).

3.1 Thin Layer Chromatography

Extraction and separation of pigments from cassava (*Mannihot utilisima*) leaf using powdered periwinkle shell as slurry material yielded two colour bands; greenish yellow indicating the presence of chlorophyll b, and yellow indicating the presence of carotenoid.

4. Discussion

Mollusks are an important group of invertebrates in the animal kingdom [6]. Molluscan research is taking place in the areas of parasitology, biochemistry, mathematics, archeology, paleobiography, paleontology, taxonomy, ecology and zoology [13]. The part of the periwinkle which builds the shell is the mantle which covers the body. The mantle contains cells which produce an external organic matrix that is rapidly mineralized with $CaCO_3$. It is the presence of $CaCO_3$ that makes the shells hard [6]. The level of $CaCO_3$ in 0.50g of *T. fuscatus* and *L. littorea* shells were probably higher than the pure calcium carbonate at 100% dry matter. This agrees with the work of Kleyn [14], who stated that calcium carbonate is the most important consideration and obviously has a direct influence on feed formulation. It has its greatest effect on layer diet and high density diet. Pure calcium carbonate at 100% dry matter would be 40.04% calcium while commercial limestone is available at (registered) levels of 38%, 36%, 34%, and 32% respectively [14].

In this study, the two species recorded high levels of $CaCO_3$ and they were significantly different ($P < 0.05$) from each other. There was also a substantial quantity of $MgCO_3$ in both species. This agrees with the work done by Malu and Bassey [9] on periwinkle (*T. fuscatus*) shell as alternative source of lime for glass industry. Their work showed that *T. fuscatus* shells contained a high level of CaO (38.40%) and MgO (18.70%). These findings suggest that due to the high content of $CaCO_3$, the periwinkle shells could be applied in agriculture and horticulture [15], paint industry [16], jewelry designs and pearl button industry [4] and construction industry. Shells, along with gravel are used for building roads, and lime from shell is a vital component in the production of concrete and plaster [13]. This work also demonstrated that the powdered shells of *T. fuscatus* and *L. littorea* may be applied in the production of slurry for thin layer chromatography as two colour bands were observed when cassava (*Mannihot utilisima*) leaf extract was spotted on the plate.

5. Conclusion

The periwinkle shells were found to contain high percentage of $CaCO_3$, making them a probable source of $CaCO_3$. The high $CaCO_3$ content is probably responsible for most of the applications of the shell listed above.

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