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Morphological and Spectroscopic Characterization of Costus afer Ker Gawl. Leaf on Surface Modification with Archachatina marginata Mucus as a Potential Carrier for Anti-diabetic Therapy

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Abstract. *FTIR and SEM analyses were conducted on Costus afer Methanol leaf extract (CAPExt), Snail mucus (GALSM), and a mixture of extracts to study surface modification of snail mucus. The FTIR of the plant extract and mixed extracts [CAPExt/GALSM] showed strong absorption bands. The absorption bands at 3363.97 – 3363.25, 2854.74 – 2962.74, 1381.08 – 1450.52, 1519.96 – 1558.54, 1651.12 – 1712.85, 1242.20 – 1265.35, and 1033.88 – 1165.04 cm-1 were characteristic absorption peaks of O – H stretching vibrations, C – H deformation, aromatic rings, esters, acetyl groups, and carboxylate groups. SEM images reveal a smooth plant extract surface with a rough area containing bioactive molecules, while snail mucus morphology reveals crystalline cube-shaped molecules. SEM images reveal rocky, swollen, roughened, and bulging CAPExt/GALSM molecules, suggesting a new method for structural development of anti-diabetic drug carriers, potentially releasing plant material intermittently upon absorption.*

Keywords: *Morphological, diabetic therapy, SEM, Archachatina marginata, Snail mucus*

1. Introduction

In nature plants has manifold uses which provides some benefits to human beings like shelter, flavour, food, fragrances, medicines etc. Most of these benefits are associated with the treatment of specific ailments and the management of conditions such as abnormal blood pressure and diabetes mellitus, particularly in situations where access to conventional medications is limited (Balde et al.,2006). Generally, plant origin is the pedestal on which African medicines, traditional medicine, Chinese medicine etc. stand. Medicinal plants is used globally though strongly pronounced in countries like Nigeria, China, Pakstan, India Japan, Thailand, and Sri Lanka. At present, developed nations are increasingly adopting and supporting the inclusion of plant-based natural medicines in their healthcare practices (Fransworth et al., 1994; Gaur et al., 1987). The aim of this study is to investigate the morphological and spectroscopic study of Bush Cane (*Costus afer* Ker Gawl.) on surface

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modification with Snail (*Archachatina marginata*) mucus as a potential chemical carrier for health remedies through an objective which is to: Perform morphological and spectrophotometric measurements on *Costus afer* methanol leaf extract, snail mucus and their mixture using SEM and FTIR spectrometer.

With the advancement of Ayurvedic tradition in recent time as well as its scientific evolvement, researchers has carried out studies on different classes and parts of plant species in order to appraise and isolate their curative potentials and compounds contained respectively. The presence of the secondary plant metabolites in medicinal plants has been ascribed to their medicinal and pharmacological activeness (Bruneton et al., 1999; Heinrich et al., 2004). To buttress the activity of medicinal plants, the recent studies carried out by (Agu et al., 2020; Agu et al., 2018) on the anti-diabetic effects of *Costus afer* leaves, snail mucus and their mixture using Albino Rats showed an impressive results of reduction in blood glucose level. Therefore, this study is carried out to elaborate on the analytical representation using SEM and FTIR to determine the morphological and spectroscopic modification as may be expected.

The Giant African Land Snails studied belong to the phylum Mollusca and the family Achatinidae, which includes around 200 species native to Africa (Bequaert, 1950; Pilsbry, 1919; Agu et al., 2015). Known by various local names such as Ejula, Katantanwa, Igbin, and Ekwong across Nigerian ethnic groups, this snail—often called 'Congo meat'—is a valued source of animal protein (Fagbuaro et al., 2006). While Achatina species are found in humid forests of West and Central Africa, Archachatina species inhabit less humid areas, with some restricted to untouched rainforests (Raut & Barker, 2002; Van, 1986).

Generally, the mechanisms of action in most plants correlated to the presence of the plant's bioactive ingredient. In the work of (Tanira, 1994) there were several possible mechanisms through which anti-diabetic medicinal plants can act to control the blood glucose level. More to the observation of (Agu et al., 2018) the regenerative capacity of the snail slime on damaged shells of snails and with the fact that diabetes is characterized by damage of the pancreatic beta cells may in one way or the other be associated with the reduction of blood glucose level observed in the use of Costus afer methanol leaf extract and snail slime for possible anti-diabetic treatment.

Anti-diabetic plants lower blood glucose by stimulating insulin release, enhancing glucose uptake in fat and muscle cells, altering glucose metabolism enzymes, or reducing sugar absorption in the digestive system (Cheng et al., 2005; Tanira, 1994). This therapeutic potentials of plants in a combined medium were also established in the works of (Agu, 2020; Agu et al., 2015; Barminas et al., 2017) resulting to blood glucose lowering effect on Albino rats. Ordinarily, these effects were accomplished through examination of the consultations with African herbal workers by testing the therapeutic potentials of some African medicinal plants (Elujoba et al., 2005; Van et al., 1997).

2. Materials and Methods

2.1 Plant Material and collection

Costus afer Ker Gawl was collected from Umuewi village of Ihebinowere Autonomous Community in Njaba Local Government Area of Imo State. It was properly covered and rapped with paper and plastic materials in a sack and transported over night to Bali, Taraba

State, Nigeria where it was sorted to remove the spoilt leaves as preliminary activity towards plant preparation.

2.2. Extraction of Snail mucus from Giant African Land Snail

Snail mucus extraction from Giant African Land Snails (*Archachatina marginata*) began by thoroughly washing the snails with clean water to remove dirt and dust from their shells and foot surfaces. The fleshy body of the snail was continuously pressed to exert some pressure which forced the snail to release some mucus. The mucus was scraped, collected, and precipitated with chilled acetone. After freeze-drying at -4°C, the extract was ground into a fine powder and kept in a refrigerator for future analysis. This procedure was according to the modified method employed by (Adikwu and Nnamani, 2005).

2.3. Homogenous mixture of bioactive component of Costus afer methanol leaf extract (CAPExt) and giant African land Snail mucus (GALSM) for anti-diabetic remedy

Equal parts of Costus afer methanol leaf extract and snail mucus were combined and stirred magnetically at 1500 rpm, with the mucus gradually added every 10 minutes over 20 minutes to achieve a uniform mixture, followed by continued stirring for 30 minutes. The final mixture was then dried in an oven at 40 °C for 12 hours and stored in a desiccator for later use (Agu et al., 2018).

2.4. Instrumentation analysis

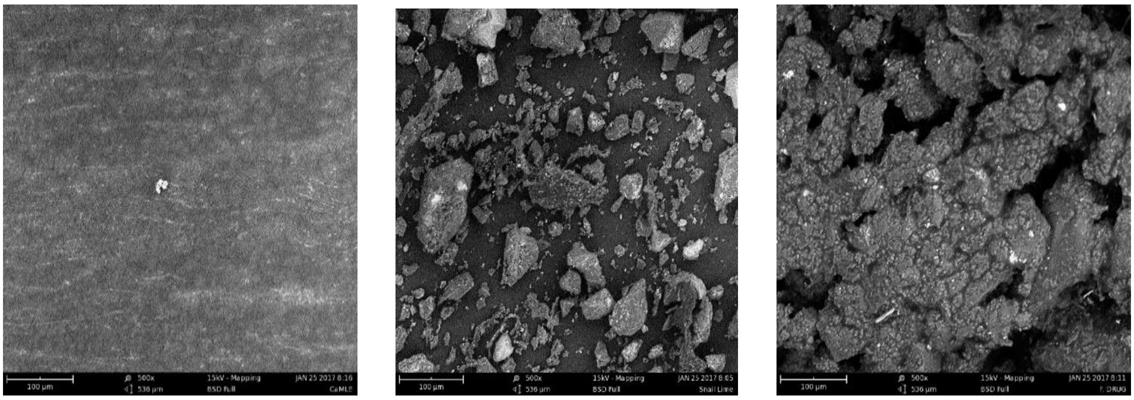
Morphological analysis of the Costus afer extracts, snail mucus, and their mixture was conducted using SEM and FTIR spectroscopy. The SEM used was a Phenom World desktop model with a magnification of 5000, while the FTIR analysis was performed using a Shimadzu FTIR-8400S spectrometer (Tokyo, Japan).

3. Result and Discussion

The results obtained from the Scanning electron microscope analysis was used for morphological study of the *Costus afer*, Snail mucus and mixed extracts (CAPExt/GALSM).

Figure 1 shows the SEM micrograph of (a) plant extract (b) snail mucus and (c) mixed extracts (CAPExt/GALSM). The SEM images of the plant extract showed almost a smooth surface with some rough portion which could possibly be the site of the bioactive molecules in the plant extract. The morphology of the snail mucus are crystalline in the form of cubes. The SEM image for the mixed extracts (CAPExt/GALSM) showed that the molecules of the mixed extracts were amorphous. The surface could be seen as rocky, swollen, roughened and bulged with various sizes of wrinkled shapes.

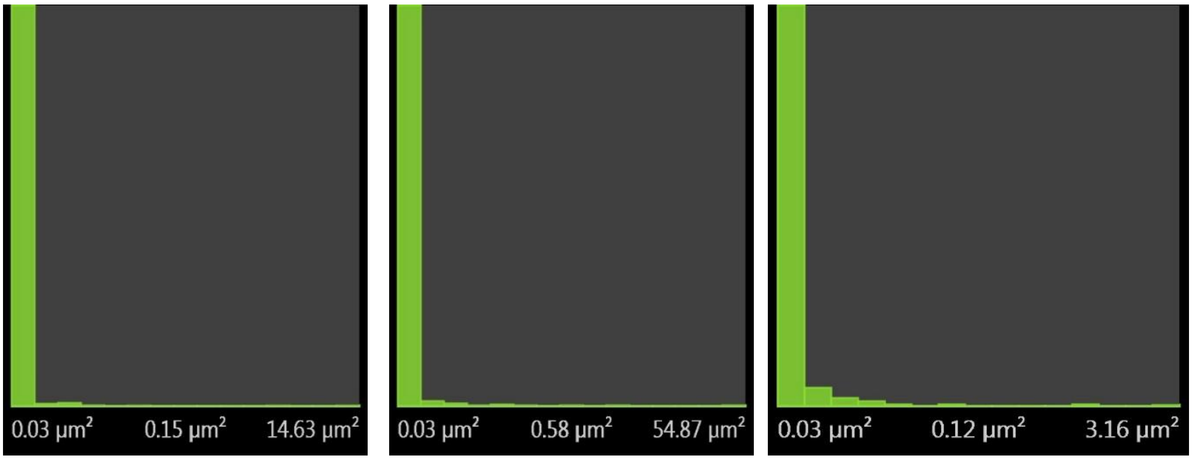
Figure 2 (a,b,c) shows the sample sizes of pores in the bioactive component of the plant extract CAPExt, snail mucus (GALSM) and the mixed extracts (CAPExt/GALSM). The sample pores were grouped into three marked discrete sizes; small, middle and large pores. As illustrated on the bar chart the majority of the sample pore size are small. The middle sample pore size in the snail mucus are bigger in both the plant extract (CAPExt) and mixed extracts (CAPExt/GALSM).



(a) (b) (c)

Figure 1. SEM Images (a) Plant Extract (b) Snail Mucus and (c) Mixed extracts (CAPExt/GALSM)

Figure 1 shows the SEM micrograph of (a) plant extract (b) snail mucus and (c) mixed extracts (CAPExt/GALSM). The SEM images of the plant extract (**Figure 1 a**) showed almost a smooth surface except for a distinct rough portion which could possibly be the site of the bioactive molecules in the plant extract. From the morphology of the snail mucus (Figure 1 b), it was observed that the molecules of the snail mucus are crystalline in the form of cubes. However, the SEM image for the mixed extracts (CAPExt/GALSM) (Figure 1 c) showed that the molecules of the mixed extracts are irregular and shapeless. The surface are rocky, swollen, roughened and bulged with various sizes of wrinkled shapes as reported. This could be as a result of absorption of the bioactive molecules of the plant into the pores of the crystalline snail mucus. These suggest successful absorption of the bioactive molecules of the plant extract(CAPExt) by the snail mucus(GALSM) which results in the swelling of the snail mucus molecules as in **Figure 1c**.



(a) (b) (c)

Figure 2. Pore Size Analysis for (a) Plant Extract (CAPExt) (b) Snail Mucus (GALSM) (c) Mixed extracts (CAPExt/GALSM)

Figure 2 (a,b,c) shows the sample sizes of pores in the bioactive component of the plant extract CAPExt, snail mucus (GALSM) and the mixed extracts (CAPExt/GALSM). The sample pores are grouped into three marked distinct sizes; small, middle and large pores as reported by (Agu, 2020). As illustrated on the bar chart the majority of the sample pore size are small. The middle sized sample pore in the snail mucus are bigger in both the plant extract (CAPExt) and mixed extracts (CAPExt/GALSM). The presence of pores in the snail mucus suggests that the plant extract could be absorbed, facilitating surface modification. This high absorption may enable the snail mucus to intermittently release the plant materials for medical and pharmaceutical use, as indicated by the reduction in pore sizes after absorption, similar to findings reported by Onwuka et al. (2016) using an acetylation method.

Figure 3 (a-d) showed the FTIR spectra of the plant extract (red), snail mucus (green) and mixed extracts [CAPExt/GALSM] (black). The FTIR spectra of the snail mucus (green spectra) showed a very weak absorption bands. The FTIR of the plant extract (red) and mixed extracts [CAPExt/GALSM] (black) showed strong absorption bands at 3363.95, 2924.18, 1651.12, 1558.54, 1519.96, 1450.52, 1381.08, 1242.20, 1157.33, 1080.17 and 1049.31 cm^{-1}. The FTIR spectra show strong absorption bands for bioactive molecules like alkaloids, flavonoids, and others. The weak absorption bands observed in the snail mucus were found to be enhanced or intense in the mixed extracts (CAPExt/GALSM) (which is plant extract absorbed by snail mucus).

Table 1 showed the frequencies and band assignments as obtained from the results of FTIR of the Costus afer (CAPExt), snail mucus (GALSM) and the mixed (CAPExt/GALSM) extracts. The absorption bands observed at 3363.97 – 3363.25, 2854.74 – 2962.74, 1381.08 – 1450.52, 1519.96 – 1558.54, 1651.12 – 1712.85, 1242.20 – 1265.35, and 1033.88 – 1165.04 cm^{-1} are characteristic absorption peaks of O – H stretching vibrations, C – H stretch (methyl and methylene groups), C – H deformation in – O – C = O – CH_3 group, – C=O stretching vibrations of aromatic rings, C=O of esters, C = O stretch of acetyl group and C – O stretching from carboxylate groups, respectively.

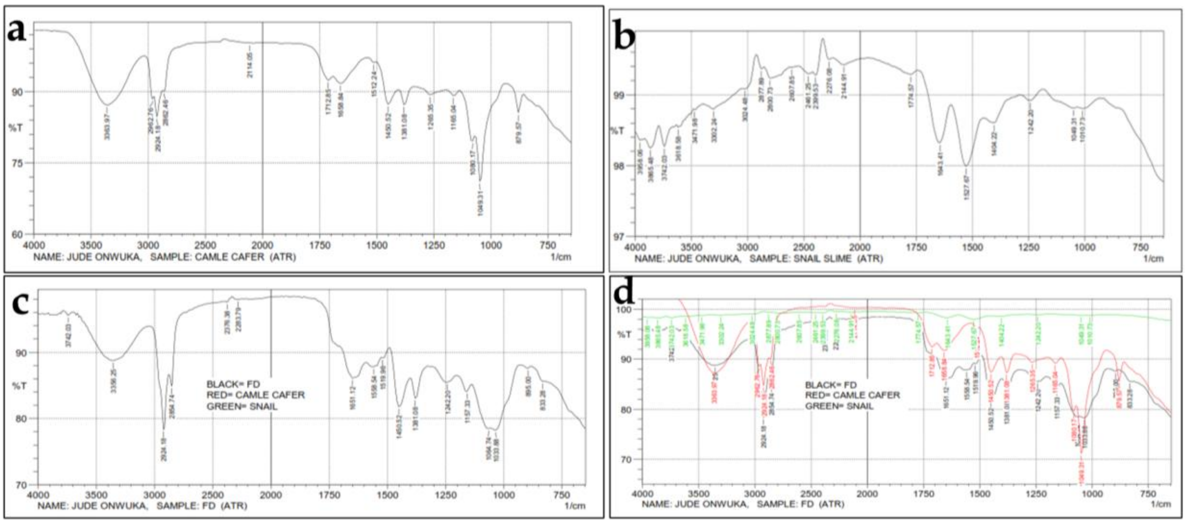


Figure 3. Shows the Highlighted FTIR Spectral Presentation of (a) CAPExt (Costus afer extract), (b) Giant African Snail Mucus Extract, (c) mixed extract (CAPExt/GALSM), and (d) CAPExt (red), Giant African Land Snail Mucus extract GALSM (green) and Mixed extract CAPExt/GALSM (black)

Table 1. FTIR results of the C. afer (CAPExt), snail Mucus (GALSM) and the mixed (CAPExt/GALSM) extracts

Frequencies	Band Assignments
3363.97 – 3363.25	O-H Stretching Vibrations
2854.74 – 2962.74	C-H Stretch(methyl) and methylene groups
1381.08 – 1450.52	C-H Deformation in –O-C=O-CH_3 group
1519.96 – 1558.54	-C=O Stretching Vibrations of aromatic rings
1651.12 – 1712.85	C=O of esters
1242.20 – 1265.35	C=O Stretch of acetyl group
1033.88 – 1165.04	C-O Stretching from carboxylate groups

Figure 3 (a-e) showed the FTIR spectra of the plant extract (red), snail mucus (green) and mixed extracts [CAPExt/GALSM] (black). A very weak absorption bands were generally observed from the FTIR spectra of the snail mucus (green spectra). However, the FTIR of the plant extract (red) and mixed extracts [CAPExt/GALSM] (black) showed strong absorption bands at 3363.95, 2924.18, 1651.12, 1558.54, 1519.96, 1450.52, 1381.08, 1242.20, 1157.33, 1080.17 and 1049.31 cm^{-1}. These strong absorption bands in the FTIR spectra of the plant extract indicates the stretching vibrational bands for bioactive molecules like alkaloids, anthraquinones, glycosides, phenols, tannins, saponins, flavonoids, and terpenoids (Shameli et al., 2012; Dhesi et al., 2016). The weak absorption bands observed in the snail mucus were found to be increased or intensified in the mixed extracts (CAPExt/GALSM) (which is plant extract absorbed by snail mucus). This indicated that the bioactive molecules in the plant extract were successfully absorbed into the pores of the snail mucus and thus modifies the surface of the snail mucus.

The absorption bands observed at 3363.97 – 3363.25, 2854.74 – 2962.74, 1381.08 – 1450.52, 1519.96 – 1558.54, 1651.12 – 1712.85, 1242.20 – 1265.35, and 1033.88 – 1165.04 cm^{-1} are characteristic absorption peaks of O – H stretching vibrations, C – H stretch (methyl and methylene groups), C – H deformation in – O – C = O – CH_3 group, – C=O stretching vibrations of aromatic rings, C=O of esters, C = O stretch of acetyl group and C – O stretching from carboxylate groups, respectively (Morrison et al., 2002; Dhesi et al., 2015; Onwuka et al., 2016). The FTIR result obtained indicated successful absorption of the bioactive molecules of the plant extract by the snail slime.

Conclusion

The study found that snail mucus, which shows slight solubility in both acidic and alkaline environments, could act as a carrier for chemical and biological substances in the medical and pharmaceutical fields, with the ability to release drugs gradually, while its regenerative effects on the snail shell may contribute to the observed hypoglycemic impact in the research on Costus afer leaf methanol extract, snail slime, and their combination (CAPExt/GALSM), which may help repair pancreatic beta cells damaged in diabetes. The weak absorption bands observed in the snail mucus were found to be enhanced or intensified in the mixed extracts (CAPExt/GALSM) (which is plant extract absorbed by snail mucus). Hence the instrumental study of the morphological and spectroscopic characterization of Costus afer, snail mucus and their mixture convincingly approves that the bioactive molecules in the plant extract were successfully absorbed into the pores of the snail mucus and thus modifies the surface of the snail mucus. The FTIR result obtained indicated successful absorption of the bioactive molecules of the plant extract by the snail mucus.

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Conflicts of Interest

The authors declare no conflict of Interest.

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