Comparative evaluation of the mucoadhesive strengths of *Abelmoschus esculentus* and *Irvingia gabonensis* gums for possible applications in veterinary mucoadhesive vaccine delivery systems.

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INTRODUCTION

- Studies have shown that mucosal vaccination can successfully induce both **systemic** and **mucosal immune responses,** thus preventing the invasion and colonization of pathogens on the mucosal surfaces.
- Mucosal vaccination in livestock has faced varying limitations due to certain factors of which includes; **low contact time** resulting in poor antigen uptake and possible **enzyme degradation** with regard to the oral or gastrointestinal route
- **Mucoadhesive Vaccine Delivery Systems** (NMVDS) for existent livestock vaccines to maximize their effectiveness in terms of optimal cellular and humoral immune response and disease control is fast gaining popularity.
- Antigen charged mucoadhesive can bind temporarily unto mucus membranes thus decreasing the transit time, potentially forms a temporary depot system for gradual continuous antigen release, activation and recruitment of antigen presenting cells (APCs) with subsequent formation of high affinity antibodies.

- The potentials of *Abelmoschus esculentus* (Okra) and *Irvingia gabonensis* (Bush mango) plant gums for mucoadhesive for vaccine delivery have not been explored.
- This study was designed to evaluate ex vivo, the effects of different methods of gum extraction on the mucoadhesive strengths of *AE* and *IG* gums.

RESEARCH QUESTIONS

- Do the plant extracts have substancial mucoadhesive strength on cattle and goat mucous membranes ex-vivo?
- Does the method of plant gum extraction affect the mucoadhesiveness on cattle and goat tissues ex-vivo?

METHODS

Gum extraction and preparation

- ***Abelmoschus esculentus* plant preparation and gum extraction**

5kg weight of *Abelmoschus esculentus (okra)* fruits were purchased from the local market. The fruits were washed in distilled water and the seeds were carefully removed. The mesocarp were homogenized using a mechanical blender and filtered with muslin cloth. The resultant mucilaginous material was divided into two equal parts and the gums were extracted with acetone and subsequently oven-dried and freeze-dried as described by Sharma et al., (2013) and Tawari et al., (2018), respectively.

- ***Irvingia gabonesis* seed preparation and gum extraction**

Irvingia gabonensis (bush mango) seeds were purchased from the local fruit market. The seed were pulverized and dried under room temperature. The pulverized seed materials were divided into two equal parts. The gums were extracted using sodium chloride (NaCl) and acetone and subsequently freeze-dried and oven-dried as described by Ogaji et al., (2012) and Onyishi and Chime (2013), respectively.

Tissue preparation

- About 5cm x 4cm each of freshly excised nasal mucosa, trachea and duodenum from cattle and goat collected from the local abattoir were used for the experiment. The tissues were collected within 15 minutes of slaughter.

Quantification of mucin-polymer bioadhesive strength

- This was determined as describe by Emikpe et al., (2016). A tablet dissolution machine (Copley dissolution apparatus) (Figure 1a) was adaptively used to quantify the mucin polymer bioadhesive strength.
- The machine was used to quantify the mucin-polymer adhesive strength by determining the peak adhesion time in a hydrated environment (physiologic buffer, pH and temperature) while the revolution of the machine probe provided a measure of the shear stress of the gum on the biologic tissue (Odeniyi et al., 2013).
- The peak adhesion time was the time it takes for a mucoadhesive material compressed into tablet to detach from an animal tissue mounted on the probe of the dissolution machine under physiological conditions which simulates the interplay of the in vivo condition.

Experimental procedure

- The freshly excised and trimmed animal tissues were firmly mounted on a cassette and attached to the probe of the tablet dissolution machine.
- The gum tablets were carefully placed until it becomes adhered to the mucosal surface of the tissues (Figure 1b) which was then lowered slowly into a beaker containing phosphate buffered solution at pH 6.8. The beaker sits within a large water bath maintained at 37°C.
- The machine was then set at 50 revolutions per minute to mimics in vivo physiologic fluid, pH and/or peristaltic movement of the contractile smooth muscles.
- The time it took for the gum tablet to detach fully from the tissue is recorded as the peak adhesion time (PAT). This adhesion time is a reflection of the strength of interaction between the gum and mucosal layers of the tissues under simulated in vivo conditions.
- The procedure was conducted in replicates for the different extraction methods on the same tissues from the different animals as mentioned above. The average peak adhesion time was evaluated using Graphpad prism software, version 7.0

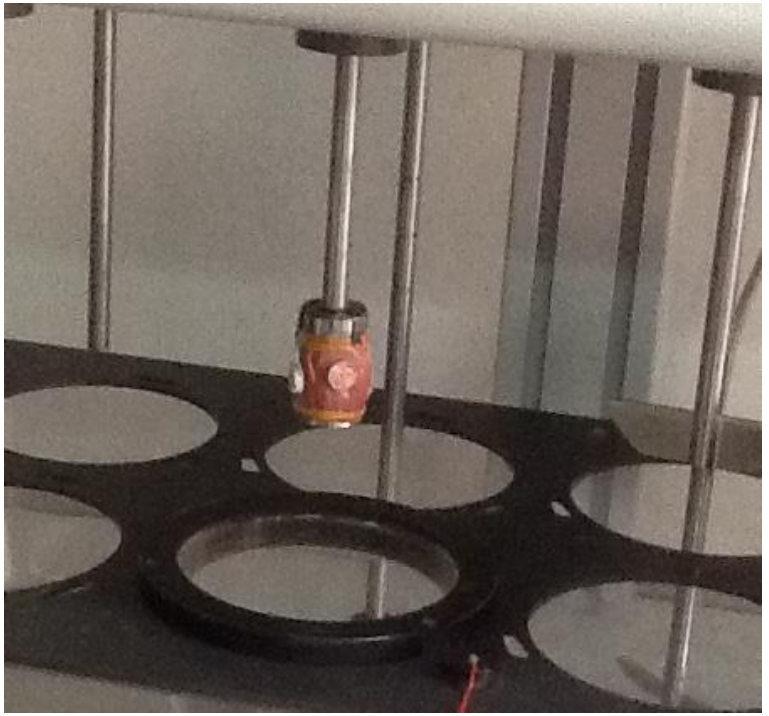

Figure 1a: Tablet dissolution machine

Figure 1b: Gum extract tablet on tissue, attached to the probe of the tablet dissolution machine

RESULTS

Mucosal Tissue		Acetone Precipitated (Sharma et al., 2013)	Acetone precipitated and lyophilized (Talawi et al., 2018)
Goat Mucosa	Nasal	192 ± 21 sec.	316 ± 33 sec.
	Trachea	358 ± 34 sec.	612 ± 46 sec
	Intestine	334 ± 42sec.	504 ± 44 sec
Cattle Mucosa	Nasal	212 ± 31sec.	237 ± 22 sec
	Tracheal	585 ± 45 sec.	1053 ± 87sec
	Intestine	475 ± 39 sec.	651 ± 55 sec

Table 1. Peak adhesion time (PAT) of ***Abelmoschus esculentus*** gum polymer on the nasal mucosa, tracheal and gastrointestinal mucosa of cattle and goat.

Mucosal Tissue		Acetone precipitated (Onyishi & Salome, 2013)	NaCl + lyophilisation (Ogaji et al., 2012)
Goat Mucosa	Nasal	57 ± 12 sec	1766 ± 73 sec
	Trachea	83 ± 23 sec	2116 ± 101 sec
	Intestine	167 ± 45 sec	7044 ± 117 sec
Cow Mucosa	Nasal	97 ± 37 sec	1508 ± 77 sec
	Tracheal	110 ± 32 sec	1721 ± 98 sec
	Intestine	183 ± 33 sec	3212 ± 145 sec

Table 2. Peak adhesion time (PAT) of ***Irvingea gabonensis*** gum polymer on the nasal mucosa, tracheal and gastrointestinal mucosa of cattle and goat.

DISCUSSION AND CONCLUSION

- The means Peak adhesion times (PAT) of both extraction methods used for ***A. esculentus*** study were moderately low in all the test mucous membranes in both species which reflects a relatively poor mucoadhesiveness. Thus, both extraction methods may not be ideal for the production of mucoadhesive gums for use as mucosal vaccine or drug delivery system in ruminants.
- The **acetone precipitated and oven-dried gum polymer extract** of ***I. gabonensis*** showed a transiently low mean PAT on all the test mucous membranes in both cattle and goat.
- In comparison with the **NaCl extracted and freeze-dried method**, Pearson's chi-squared analysis showed a significantly higher PAT ($p<0.05$) on the nasal, tracheal and intestinal mucosae of both cattle and goat.
- This result suggest that *I. gabonesis* (bush mango) gum is more suitably for use in mucoadhesive delivery in ruminants more than *A. esculentus* (okra) gum.
- The findings of this work have also shown that the methods of polymer extraction, plays a very important role in the mucoadhesiveness.

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