



IMAGE: A MAP OF THE STARS OF THE ORION CONSTELLATION

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Schauffer-like Theorems for Medical and Paramedical Algebras

Yu. M. Movsisyan & D.N. Harutyunyan

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ABSTRACT

In [1], the endoliness of regular division binary algebras satisfying second-order associativity identities was shown. Furthermore, schauer-like theorems were proven for these algebras. This paper aims to establish similar results for regular division binary algebras satisfying second-order identities of mediality or paramediality. MSC 2010: 03C05, 03C85, 20N05.

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Schauffer-like Theorems for Medial and Paramedical Algebras

Yu. M. Movsisyan^o & D.N. Harutyunyan^o

ABSTRACT

In [1], the endlinearity of regular division binary algebras satisfying second-order associativity identities was shown. Furthermore, schauer-like theorems were proven for these algebras. This paper aims to establish similar results for regular division binary algebras satisfying second-order identities of mediality or paramediality. MSC 2010: 03C05, 03C85, 20N05.

Keywords: 89 (8)- identities; division regular groupoids; schauer theorem; quasiendomorphisms.

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I. INTRODUCTION

We call $Q(\cdot)$ the division(cancellation) groupoid if for any $a \in Q$ the left and right multiplications are surjections(injections), if the groupoid is both division and cancellation then it's called quasi-group. If $Q(\cdot)$ is a division(cancellation) groupoid, then its operation is called a divisible(cancellable) operation. A binary algebra $(Q; \Sigma)$ is called division(cancellation) if each operation $A \in \Sigma$ is a divisible operation and it's called invertible algebra if it's both division and cancellable algebra. We call a groupoid $(Q; \cdot)$ left-regular if $ca = cb \implies Ra = Rb$, where $a, b, c \in Q$. Similarly, we define the right-regular groupoid. We call a groupoid regular if it is simultaneously left-regular and right-regular. If $Q(\cdot)$ is a regular groupoid, then its operation is called regular. A binary algebra $(Q; \Sigma)$ is referred to as regular if each operation $A \in \Sigma$ is a regular operation. We say that a groupoid $(Q; A)$ is homotopic to a groupoid $(Q; B)$ if there exist such mappings $\alpha, \beta, \gamma : Q \implies Q$ that the equality $\gamma A(x, y) = B(\alpha x, \beta y)$ holds for any $x, y \in Q$ [2, 3]. Then the triple (α, β, γ) is called a homotopy from $(Q; A)$ to $(Q; B)$. If $\gamma = id_Q$, then the groupoids are called principally homotopic. If α, β, γ are surjective mappings, then the groupoids are called epitopic or principally epitopic, respectively. We say that an algebra $(Q; \Sigma)$ is homotopic (epitopic) to a groupoid $(Q; \cdot)$ if for each $A \in \Sigma$ the groupoid $(Q; A)$

is homotopic (epitopic) to the groupoid $(Q; \cdot)$. In the same manner we define the principal homotopy (epitopy) of an algebra $(Q; \Sigma)$ to a groupoid $(Q; \cdot)$. An algebra $(Q; \Sigma)$ is referred to as r-algebra if it is regular, division, and there exists at least one invertible operation $A \in \Sigma$. A binary algebra $(Q; \Sigma)$ is called left(right)-linear on a groupoid $(Q; \cdot)$ if each its operation is left (right) linear on the groupoid $(Q; \cdot)$, that is, for each operation $A \in \Sigma$ there exists an automorphism ϕ_A of the groupoid $(Q; \cdot)$ and a permutation α_A of the set Q such that:

$$A(x, y) = \phi x \cdot \alpha y,$$

$$(A(x, y) = \alpha x \cdot \phi y)$$

binary algebra $(Q; \Sigma)$ is called linear (endoliner) on a groupoid $(Q; \cdot)$ if each its operation is linear (endoliner) on the groupoid $(Q; \cdot)$, that is, for each operation $A \in \Sigma$ there exist automorphisms (endomorphisms) ϕ_A and α_A of the groupoid $(Q; \cdot)$ and an element $t_A \in Q$ such that

$$A(x, y) = (\phi_A x \cdot t_A) \cdot \alpha_A y$$

for any $x, y \in Q$.

During World War II, while working at the German cryptographic center, Schaufler obtained applications In Cryptography using invertible algebras that satisfy second-order identities, through proving the following theorem. [4–6]

Theorem 1.1 (Schaufler). *Let Q be a non-empty set. The following propositions are equivalent:*

- For all $(Q; X), (Q; Y)$ quasigroups, there exist $(Q; X'), (Q; Y')$ quasigroups, such that following $\forall \exists (\forall)$ -identity holds:

$$\forall X, Y \exists X', Y' \forall x, y, z (X(Y(x, y), z) = X'(x, Y'(y, z))), \quad (1.1)$$

- For all $(Q; X), (Q; Y)$ quasigroups, there exist $(Q; X'), (Q; Y')$ quasigroups, such that following $\forall \exists (\forall)$ -identity holds:

$$\forall X, Y \exists X', Y' \forall x, y, z (X(x, Y(y, z)) = X'(Y'(x, y), z)), \quad (1.2)$$

- $|Q| \leq 3$

In the [7] proved schaufler-like theorem for other second-order identities and hyperintensities (see [8–10]).

Theorem 1.2. (Movsisyan) *Let Q be non empty set. The following propositions are equivalent:*

- for all $(Q; X), (Q; Y)$ quasigroups, there exist $(Q; X'), (Q; Y')$ quasigroups, such that (1.1) identity holds,
- for all $(Q; X), (Q; Y)$ quasigroups, there exist $(Q; X'), (Q; Y')$ quasigroups, such that (1.2) identity holds,
- for all $(Q; X), (Q; Y)$ loops, there exist $(Q; X'), (Q; Y')$ quasigroups, such that (1.1) identity holds,
- for all $(Q; X), (Q; Y)$ loops, there exist $(Q; X'), (Q; Y')$ quasigroups, such that (1.2) identity holds,
- for all $(Q; X), (Q; Y)$ loops, there exist $(Q; X'), (Q; Y')$ loops, such that (1.1) identity holds,
- for all $(Q; X), (Q; Y)$ loops, there exist $(Q; X'), (Q; Y')$ loops, such that (1.2) identity holds,
- following hyperidentity holds in the $(Q; L_Q)$ algebra:

$$X(x, Y(y, z)) = X(Y(x, y), z),$$

- following hyperidentity holds in the $(Q; L_Q)$ algebra:

$$X(x, Y(y, z)) = X(Y(x, y), z),$$

- For all $(Q; X)$ quasigroup, there exist $(Q; X'), (Q; Y')$ quasigroups, such that following $\forall \exists (\forall)$ -identity holds:

$$\forall X \exists X', Y' \forall x, y, z (X(X(x, y), z) = X'(x, Y'(y, z))), \quad (1.3)$$

- For all $(Q; X)$ quasigroup, there exist $(Q; X'), (Q; Y')$ quasigroups, such that following $\forall\exists(\forall)$ -identity holds:

$$\forall X\exists X', Y'\forall x, y, z(X(x, X(y, z)) = X'(Y'(x, y), z)), \quad (1.4)$$

- $|Q| \leq 3$,

where L_Q is the set of all loop-operations over the set Q .

In [11] it was proved that an invertible algebra $(Q; \Sigma)$ with the formula (1.1) or (1.2) is linear on the group. In this paper, we will prove that r-algebras with second-order formulas of mediality and paramediality are endolinear on the group.

II. PRELIMINARY RESULTS

Definition 1. A mapping $\phi : Q \implies Q$ is called quasiendomorphism of a group $(Q; \cdot)$ if $\phi(x \cdot y) = \phi x \cdot (\phi 1)^{-1} \cdot \phi y$ for all $x, y \in Q$, where 1 is the unity of the group $(Q; \cdot)$. If ϕ is also a bijection from Q to Q , then ϕ is called the quasi-automorphism of the group $(Q; \cdot)$.

Lemma 2.1. Each quasiendomorphism ϕ of a group $(Q; \cdot)$ has the form $\phi = L_a\phi'$, where $L_ax = a \cdot x$, $a \in Q$, and ϕ' is an endomorphism of the group $(Q; \Delta)$. The converse is valid: if ϕ is an endomorphism of a group $(Q; \cdot)$, then an arbitrary mapping $\phi' = L_a\phi$ from Q to Q is a quasiendomorphism of the group $(Q; \cdot)$.

Proof. Suppose that $\phi 1 = k$. We show that $\phi' = L_{k^{-1}}\phi$ is an endomorphism. We have

$$\phi'(ab) = L_{k^{-1}}\phi(ab) = k^{-1} \cdot \phi a \cdot (\phi 1)^{-1} \cdot \phi b = (k^{-1} \cdot \phi a) \cdot (k^{-1} \cdot \phi b) = \phi' a \cdot \phi' b.$$

Lemma 2.2. Suppose that $(Q; \cdot)$ is a group and α is the principal epitopy of this group. Then α is a surjective quasiendomorphism of this group; moreover, if

$$\alpha(x \cdot y) = \beta x \cdot \gamma y, \tag{2.5}$$

then β and γ are also quasiendomorphisms.

Proof. Making the successive replacements in the (2.5): (1) $x = 1$, (2) $y = 1$, and (3) $x = y = 1$, we obtain

$$\alpha y = \beta 1 \cdot \gamma y, \quad x = \beta x \cdot \gamma 1, \quad \alpha 1 = \beta 1 \cdot \gamma 1. \tag{2.6}$$

We transform equality (2.5), taking into account equalities (2.6):

$$\alpha(x \cdot y) = \alpha x \cdot (\gamma 1)^{-1} \cdot (\beta 1)^{-1} \cdot \alpha y = \alpha x \cdot (\beta 1 \cdot \gamma 1)^{-1} \cdot \alpha y = \alpha x \cdot (\alpha 1)^{-1} \cdot \alpha y,$$

that is, α is a quasiendomorphism.

From (2.5) we also have

$$\begin{aligned} \beta(x \cdot y) &= \alpha(x \cdot y) \cdot (\gamma 1)^{-1} = \alpha x \cdot (\alpha 1)^{-1} \cdot (\alpha y \cdot (\gamma 1)^{-1}) = \\ &= (\alpha x \cdot (\gamma 1)^{-1}) \cdot (\beta 1)^{-1} \cdot \beta y = \beta x \cdot (\beta 1)^{-1} \cdot \beta y. \end{aligned}$$

In the same manner, we prove that γ is a quasiendomorphism of the group $(Q; \cdot)$.

Lemma 2.3. [12] *If a loop $(Q; \circ)$ is principally homotopic to a group $(Q; \cdot)$, then they are isomorphic. If a group $(Q; \circ)$ is principally homotopic to a group $(Q; \cdot)$, then they are isomorphic.*

Theorem 2.3. [12] *Let the set Q form a division groupoid under the six operations $A_i(x; y)$ (for $i = 1, \dots, 6$) and A_1 or A_4 is regular operation. If these operations satisfy the following equation:*

$$A_1(A_2(x, y), A_3(u, v)) = A_4(A_5(x, u), A_6(y, v)), \tag{2.7}$$

for all elements $x, y, u, v \in Q$, then there exists an operation (\cdot) under which Q forms an abelian group and all these six division

groupoids are epitopic to the group $(Q; \cdot)$ and there exist eight surjective mappings $\alpha, \beta, \gamma, \delta, \lambda, \sigma, \phi, \psi$ of Q onto itself such that:

$$\begin{aligned} A_1(x, y) &= \alpha x \cdot \phi y, \\ \alpha A_2(x, y) &= \gamma x \cdot \delta y, \\ \phi A_3(x, y) &= \lambda x \cdot \beta y, \\ A_4(x, y) &= \psi x \cdot \sigma y, \\ \psi A_5(x, y) &= \gamma x \cdot \lambda y, \\ \sigma A_6(x, y) &= \delta x \cdot \beta y. \end{aligned}$$

The abelian group $(Q; \cdot)$ is unique up to isomorphisms.

Theorem 2.4. [12] Let the set Q form a division groupoid under the six operations $A_i(x; y)$ (for $i = 1, \dots, 6$) and A_1 or A_4 is regular operation. If these operations satisfy the following paramedial equation:

$$A_1(A_2(x, y), A_3(u, v)) = A_4(A_5(v, y), A_6(u, x)) \quad (2.8)$$

for all elements $x, y, u, v \in Q$, then there exists an operation (\cdot) under which Q forms an abelian group, and all these six division groupoids are epitopic to the group $(Q; \cdot)$ and there exist eight surjective mappings $\alpha, \beta, \gamma, \delta, \lambda, \sigma, \phi, \psi$ of Q onto itself such that:

$$\begin{aligned} A_1(x, y) &= \alpha x \cdot \phi y, \\ \alpha A_2(x, y) &= \gamma x \cdot \delta y, \\ \phi A_3(x, y) &= \lambda x \cdot \beta y, \\ A_4(x, y) &= \sigma x \cdot \psi y, \\ \sigma A_5(x, y) &= \beta x \cdot \delta y, \\ \psi A_6(x, y) &= \lambda x \cdot \gamma y. \end{aligned}$$

The abelian group $(Q; \cdot)$ is unique up to isomorphisms.

III. ENDO LINEAR REPRESENTATIONS

Theorem 3.5. *Suppose that $(Q; \Sigma)$ is an r -algebra. If for arbitrary $X, Y \in \Sigma$ there exist $X', Y', Z' \in \Sigma$ such that the following identity of mediality holds:*

$$X(Y(x, y), Y(u, v)) = X'(Y'(x, u), Z'(y, v)), \quad (3.9)$$

then there exist an abelian group $(Q; \cdot)$ such that an arbitrary operation $X \in \Sigma$ is endolinear over the group $(Q; \cdot)$. The group $(Q; \cdot)$ is determined uniquely up to isomorphism.

Proof. Let $X \in \Sigma$ be invertible operation, then from the theorem 2.3 we will have that exists operations $X_1, X_2, X_3 \in \Sigma$ and abelian group $(Q; \cdot)$ such that following identities hold:

$$\begin{aligned} X(x, y) &= \alpha x \cdot \phi y, \\ \alpha X(x, y) &= \gamma x \cdot \delta y, \\ \phi X(x, y) &= \lambda x \cdot \beta y, \\ X_1(x, y) &= \psi x \cdot \sigma y, \\ \psi X_2(x, y) &= \gamma x \cdot \lambda y, \\ \sigma X_3(x, y) &= \delta x \cdot \beta y. \end{aligned}$$

Since X is invertible operation, then we will have that α and β are bijections, moreover:

$$\alpha(\alpha x \cdot \phi y) = \gamma x \cdot \delta y,$$

from which we will obtain:

$$\alpha(x \cdot y) = \gamma \alpha^{-1} x \cdot \delta \phi^{-1} y,$$

This means that α is quasiautomorphism of the group $(Q; \cdot)$.

Lets fix operation X , for every operation Y exists operations $X', Y', Z' \in \Sigma$ such that(3.9) identity holds, and then from the theorem 2.3 we will have that exist abelian group $(Q; \cdot_Y)$ such that those identities hold:

$$\begin{aligned}
 X(x, y) &= \alpha_Y x \cdot_Y \phi_Y y, \\
 \alpha_Y Y(x, y) &= \gamma_Y x \cdot_Y \delta_Y y, \\
 \phi_Y Y(x, y) &= \lambda_Y x \cdot_Y \beta_Y y, \\
 X'(x, y) &= \gamma_Y x \cdot_Y \sigma_Y y, \\
 \gamma_Y Y'(x, y) &= \gamma_Y x \cdot_Y \lambda_Y y, \\
 \sigma_Y Z'(x, y) &= \delta_Y x \cdot_Y \beta_Y y.
 \end{aligned}$$

From the proof of the theorem 2.3 we can construct the group $(Q; \cdot_Y)$ in such way that $\alpha_Y = \alpha$.

We have that: $X(x, y) = \alpha x \cdot_Y \phi_Y y = \alpha x \cdot \phi y$, which is the same as: $x \cdot_Y y = x \cdot \phi h_{\phi_Y} y$, where h_{ϕ_Y} is right inverse of ϕ_Y .

We have for every operation $Y \in \Sigma$ the following identity is true:

$$\begin{aligned}
 \alpha Y(x, y) = \gamma_Y x \cdot_Y \delta_Y y = \gamma_Y x \cdot \delta_Y \phi h_{\phi_Y} y &\implies \\
 Y(x, y) = \alpha^{-1}(\gamma_Y x \cdot \delta_Y \phi h_{\phi_Y} y). &
 \end{aligned}$$

Since the set of all quasiautomorphisms of the group is also a group, then α^{-1} will also be quasiautomorphisms, which means:

$$Y(x, y) = \alpha^{-1} \gamma_Y x \cdot (\alpha^{-1} e)^{-1} \cdot \alpha^{-1} \delta_Y \phi h_{\phi_Y} y = \alpha^{-1} \gamma_Y x \cdot L_{(\alpha^{-1} e)^{-1}} \alpha^{-1} \delta_Y \phi h_{\phi_Y} y,$$

where $e \in Q$ is the identity element of the group $(Q; \cdot)$, $L_{(\alpha^{-1} e)^{-1}}$ is left translation of the group $(Q; \cdot)$ with the $(\alpha^{-1} e)^{-1}$ element.

We obtained that for every operation $Y \in \Sigma$ there exists surjections ν_Y and μ_Y , such that $Y(x, y) = \nu_Y x \cdot \mu_Y y$. This means we can rewrite the representations of the operations X, Y, X', Y', Z' in the following way.

$$\left\{ \begin{aligned}
 X(x, y) &= \alpha_X x \cdot \beta_X y, \\
 Y(x, y) &= \alpha_Y x \cdot \beta_Y y, \\
 X'(x, y) &= \alpha_{X'} x \cdot \beta_{X'} y, \\
 Y'(x, y) &= \alpha_{Y'} x \cdot \beta_{Y'} y, \\
 Z'(x, y) &= \alpha_{Z'} x \cdot \beta_{Z'} y,
 \end{aligned} \right.$$

where $\alpha_X, \alpha_Y, \alpha_{X'}, \alpha_{Y'}, \alpha_{Z'}, \beta_X, \beta_Y, \beta_{X'}, \beta_{Y'}, \beta_{Z'}$ are surjections.

By doing the replacements in the identity (3.9) we will obtain:

$$\alpha_X(\alpha_Y x \cdot \beta_Y y) \cdot \beta_X(\alpha_Y u \cdot \beta_Y v) = \alpha_{X'}(\alpha_{Y'} x \cdot \beta_{Y'} u) \cdot \beta_{X'}(\alpha_{Z'} y \cdot \beta_{Z'} v).$$

Replacing $x = h_{\alpha_Y} h_{\alpha_X} e$, $y = h_{\beta_Y} e$, $u = h_{\alpha_Y} u$ and $v = h_{\beta_Y} v$, where $h_{\alpha_X}, h_{\alpha_Y}, h_{\beta_Y}$ respectively are the right inverses of the $\alpha_X, \alpha_Y, \beta_Y$ and e is the identity element of the group $(Q; \cdot)$, we will have:

$$\beta_X(u \cdot v) = \alpha_{X'}(\alpha_{Y'} h_{\alpha_Y} h_{\alpha_X} e \cdot \beta_{Y'} h_{\alpha_Y} u) \cdot \beta_{X'}(\alpha_{Z'} h_{\beta_Y} e \cdot \beta_{Z'} h_{\beta_Y} v) = \mu u \cdot \nu v,$$

where $\mu = \alpha_{X'} L_{\alpha_{Y'} h_{\alpha_Y} h_{\alpha_X} e} \beta_{Y'} h_{\alpha_Y}$ and $\nu = \beta_{X'} L_{\alpha_{Z'} h_{\beta_Y} e} \beta_{Z'} h_{\beta_Y}$. We showed that β_X is quasiendomorphism of the group $(Q; \cdot)$ and from lemma 2.1 we know that there exists ϕ_X endomorphism of the group $(Q; \cdot)$ such that $\beta_X = L_a \phi_X$, where L_a is left translation of the group $(Q; \cdot)$ with the element $a \in Q$. We will have following representation of the arbitrary operation $X \in \Sigma$: $X(x, y) = \alpha_X x \cdot L_a \phi_X y = R_a \alpha_X x \cdot \phi_X y = \sigma_X x \cdot \phi_X y$ where $\sigma_X = R_a \alpha_X$.

By doing following replacements in the identity $x = h_{\alpha_Y} x$, $y = h_{\beta_Y} y$, $u = h_{\alpha_Y} h_{\beta_X} e$ and $v = h_{\beta_Y} e$, we will obtain that σ_X is also a quasiendomorphism of the group $(Q; \cdot)$ and from lemma 2.1 we know that there exists ϕ_X endomorphism of the group $(Q; \cdot)$ such that $\sigma_X = R_b \phi_X$, where R_b is right translation of the group $(Q; \cdot)$ with the element $b \in Q$, so we will have:

$$X(x, y) = \phi_X x \cdot b \cdot \phi_X y,$$

for every $x, y \in Q$.

Corollary 1. *Suppose that $(Q; \Sigma)$ is an r -algebra. If for arbitrary $X, Y, Z \in \Sigma$ there exist $X', Y', Z' \in \Sigma$ such that the following identity holds:*

$$X(Y(x, y), Z(u, v)) = X'(Y'(x, u), Z'(y, v)), \tag{3.10}$$

then there exist an abelian group $(Q; \cdot)$ such that an arbitrary operation $X \in \Sigma$ is endolinear over the group $(Q; \cdot)$. The group $(Q; \cdot)$ is determined uniquely up to isomorphism.

Corollary 2. *Suppose that $(Q; \Sigma)$ is an r -algebra. If for arbitrary $X, Y \in \Sigma$ there exist $X', Y' \in \Sigma$ such that the following identity holds:*

$$X(Y(x, y), Y(u, v)) = X'(Y'(x, u), Y'(y, v)), \quad (3.11)$$

then there exist an abelian group $(Q; \cdot)$ such that an arbitrary operation $X \in \Sigma$ is endolinear over the group $(Q; \cdot)$. The group $(Q; \cdot)$ is determined uniquely up to isomorphism.

Similarly, we can prove the following results.

Theorem 3.6. *Suppose that $(Q; \Sigma)$ is an r -algebra. If for arbitrary $X, Y \in \Sigma$ there exist $X', Y', Z' \in \Sigma$ such that the following identity of paramediality holds:*

$$X(Y(x, y), Y(u, v)) = X'(Y'(v, y), Z'(u, x)), \quad (3.12)$$

then there exists an abelian group $(Q; \cdot)$ such that an arbitrary operation $X \in \Sigma$ is endolinear over the group $(Q; \cdot)$. The group $(Q; \cdot)$ is determined uniquely up to isomorphism.

Corollary 3. *Suppose that $(Q; \Sigma)$ is an r -algebra. If for arbitrary $X, Y, Z \in \Sigma$ there exist $X', Y', Z' \in \Sigma$ such that the following identity holds:*

$$X(Y(x, y), Z(u, v)) = X'(Y'(v, y), Z'(u, x)), \quad (3.13)$$

then there exists an abelian group $(Q; \cdot)$ such that an arbitrary operation $X \in \Sigma$ is endolinear over the group $(Q; \cdot)$. The group $(Q; \cdot)$ is determined uniquely up to isomorphism.

Corollary 4. *Suppose that $(Q; \Sigma)$ is an r -algebra. If for arbitrary $X, Y \in \Sigma$ there exist $X', Y' \in \Sigma$ such that the following identity holds:*

$$X(Y(x, y), Y(u, v)) = X'(Y'(v, y), Y'(u, x)), \quad (3.14)$$

then there exists an abelian group $(Q; \cdot)$ such that an arbitrary operation $X \in \Sigma$ is endolinear over the group $(Q; \cdot)$. The group $(Q; \cdot)$ is determined uniquely up to isomorphism.

Theorem 3.7. *Suppose that $(Q; \Sigma)$ is an r -algebra. If for arbitrary $X, X' \in \Sigma$ there exist $Y, Z, Y', Z' \in \Sigma$ such that (3.10) identity of mediality satisfies, then there exists an abelian group $(Q; \cdot)$ such*

that an arbitrary operation $X \in \Sigma$ is endolinear over the group $(Q; \cdot)$. The group $(Q; \cdot)$ is determined uniquely up to isomorphism.

Proof. Lets fix $X' = X$, from the theorem 2.3 we will have that there exists operations $Y_1, Z_1, Y_2, Z_2 \in \Sigma$ and abelian group $(Q; \cdot)$ such that following identities hold:

$$\begin{aligned} X(x, y) &= \alpha x \cdot \phi y, \\ \alpha Y_1(x, y) &= \gamma x \cdot \delta y, \\ \phi Z_1(x, y) &= \lambda x \cdot \beta y, \\ X(x, y) &= \psi x \cdot \sigma y, \\ \psi Y_2(x, y) &= \gamma x \cdot \lambda y, \\ \sigma Z_2(x, y) &= \delta x \cdot \beta y. \end{aligned}$$

Lets fix operation X , and for every operation $X' \in \Sigma$ we have that there exists operations $Y, Z, Y', Z' \in \Sigma$ such that identity (3.10) holds, and from the theorem 2.3 we will have that there exists $\alpha_{X'}, \beta_{X'}, \gamma_{X'}, \delta_{X'}, \lambda_{X'}, \sigma_{X'}, \phi_{X'}, \psi_{X'} : Q \rightarrow Q$ surjections and abelian group $(Q; \cdot_{X'})$ such that following identities hold:

$$\begin{aligned} X(x, y) &= \alpha_{X'} x \cdot_{X'} \phi_{X'} y, \\ \alpha_{X'} Y(x, y) &= \gamma_{X'} x \cdot_{X'} \delta_{X'} y, \\ \phi_{X'} Z(x, y) &= \lambda_{X'} x \cdot_{X'} \beta_{X'} y, \\ X'(x, y) &= \psi_{X'} x \cdot_{X'} \sigma_{X'} y, \\ \psi_{X'} Y'(x, y) &= \gamma_{X'} x \cdot_{X'} \lambda_{X'} y, \\ \sigma_{X'} Z'(x, y) &= \delta_{X'} x \cdot_{X'} \beta_{X'} y. \end{aligned}$$

By doing following replacements $x = h_{\alpha_{X'}} x$ and $y = h_{\phi_{X'}} x$ we will obtain:

$$x \cdot_{X'} y = \alpha h_{\alpha_{X'}} x \cdot \phi h_{\phi_{X'}} y,$$

where $\alpha h_{\alpha_{X'}}$ and $\phi h_{\phi_{X'}}$ are surjections.

We showed that arbitrary operations $X' \in \Sigma$ has following representation:

$X'(x, y) = \nu_{X'}x \cdot \mu_{X'}y = \alpha h_{\alpha_{X'}} \nu_{X'}x \cdot \phi h_{\phi_{X'}} \mu_{X'}y = \nu_{X'}x \cdot \mu_{X'}y$,
 where $\nu_{X'}$ and $\mu_{X'}$ are surjections.

From which we have that there exists abelian group $(Q; \cdot)$ that following identities hold:

$$\begin{cases} X(x, y) = \nu_X x \cdot \mu_X y, \\ Y(x, y) = \nu_Y Y x \cdot \mu_Y y, \\ Z(x, y) = \nu_Z x \cdot \mu_Z y, \\ X'(x, y) = \nu_{X'} x \cdot \mu_{X'} y, \\ Y'(x, y) = \nu_{Y'} x \cdot \mu_{Y'} y, \\ Z'(x, y) = \nu_{Z'} x \cdot \mu_{Z'} y, \end{cases}$$

where $\nu_X, \mu_X, \nu_Y, \mu_Y, \nu_Z, \mu_Z, \nu_{X'}, \mu_{X'}, \nu_{Y'}, \mu_{Y'}, \nu_{Z'}, \mu_{Z'}$ are surjections.

By doing replacements in the identity (3.9) we will have:

$$\nu_X(\nu_Y x \cdot \mu_Y y) \cdot \mu_X(\nu_Z u \cdot \mu_Z v) = \nu_{X'}(\nu_{Y'} x \cdot \mu_{Y'} u) \cdot \mu_{X'}(\nu_{Z'} y \cdot \mu_{Z'} v).$$

Replacing $x = h_{\nu_Y} h_{\nu_X} e$, $y = h_{\mu_Y} e$, $u = h_{\nu_Z} u$ and $v = h_{\mu_Z} v$, where $h_{\nu_X}, h_{\nu_Y}, h_{\mu_Y}, h_{\nu_Z}, h_{\mu_Z}$ respectively are the right inverses of the $\nu_X, \nu_Y, \mu_Y, \nu_Z, \mu_Z$ and e is the identity element of the group $(Q; \cdot)$, we will have:

$\mu_X(u \cdot v) = \nu_{X'}(\nu_{Y'} h_{\nu_Y} h_{\nu_X} e \cdot \mu_{Y'} h_{\nu_Z} u) \cdot \mu_{X'}(\nu_{Z'} h_{\mu_Y} e \cdot \mu_{Y'} h_{\mu_Z} v) = \theta u \cdot \gamma v$,
 where $\theta = \nu_{X'} L_{\nu_{Y'} h_{\nu_Y} h_{\nu_X} e} \mu_{Y'} h_{\nu_Z}$ and $\gamma = \mu_{X'} L_{\nu_{Z'} h_{\mu_Y} e} \mu_{Y'} h_{\mu_Z}$. We showed that μ_X is quasiendomorphism of the group $(Q; \cdot)$ and from lemma 2.1 we know that there exists ϕ_X endomorphism of the group $(Q; \cdot)$ such that $\mu_X = L_a \phi_X$, where L_a is left translation of the group $(Q; \cdot)$ with the element $a \in Q$. We will have following representation of the arbitrary operation $X \in \Sigma$: $X(x, y) = \nu_X x \cdot L_a \phi_X y = R_a \nu_X x \cdot \phi_X y = \sigma_X x \cdot \phi_X y$ where $\sigma_X = R_a \nu_X$.

By doing following replacements in the identity $x = h_{\nu_Y}x$, $y = h_{\mu_Y}y$, $u = h_{\nu_Z}h_{\mu_X}e$ and $v = h_{\mu_Z}e$, we will obtain that σ_X is also a quasiendomorphism of the group $(Q; \cdot)$ and from lemma 2.1 we know that there exists ϕ_X endomorphism of the group $(Q; \cdot)$ such that $\sigma_X = R_b \phi_X$, where R_b is right translation of the group $(Q; \cdot)$ with the element $b \in Q$, so we will have:

$$X(x, y) = \phi_X x \cdot b \cdot \phi_X y,$$

Theorem 3.8. *Suppose that $(Q; \Sigma)$ is an r -algebra. If for arbitrary $X, X' \in \Sigma$ there exist $Y, Z, Y', Z' \in \Sigma$ such that (3.13) identity of paramediality satisfies, then there exists an abelian group $(Q; \cdot)$ such that an arbitrary operation $X \in \Sigma$ is endolinear over the group $(Q; \cdot)$. The group $(Q; \cdot)$ is determined uniquely up to isomorphism.*

Let denote by Ω_Q all the regular division operations of the set Q .

Theorem 3.9. *One of the following $\forall\exists(\forall)$ -identities of mediality*

$$\begin{aligned} \forall X, Y \exists X', Y', Z' \forall x, y, u, v X(Y(x, y), Y(u, v)) &= X'(Y'(x, u), Z'(y, v)), \\ \forall X, Y, Z \exists X', Y', Z' \forall x, y, u, v X(Y(x, y), Z(u, v)) &= X'(Y'(x, u), Z'(y, v)), \\ \forall X, Y \exists X', Y' \forall x, y, u, v X(Y(x, y), Y(u, v)) &= X'(Y'(x, u), Y'(y, v)), \\ \forall X, X' \exists Y, Y', Z, Z' \forall x, y, u, v X(Y'(x, y), Y'(u, v)) &= Y(X'(x, u), X'(y, v)), \end{aligned}$$

or paramediality

$$\begin{aligned} \forall X, Y \exists X', Y', Z' \forall x, y, u, v X(Y(x, y), Y(u, v)) &= X'(Y'(v, y), Z'(u, x)), \\ \forall X, Y, Z \exists X', Y', Z' \forall x, y, u, v X(Y(x, y), Z(u, v)) &= X'(Y'(v, y), Z'(u, x)), \\ \forall X, Y \exists X', Y' \forall x, y, u, v X(Y(x, y), Y(u, v)) &= X'(Y'(v, y), Y'(u, x)), \\ \forall X, X' \exists Y, Y', Z, Z' \forall x, y, u, v X(Y(x, y), Z(u, v)) &= X'(Y'(v, y), Z'(u, x)), \end{aligned}$$

holds in the algebra $(Q; \Omega_Q)$, if and only if $|Q| \leq 3$.

Proof. Let us prove it for the first identity; the rest are proved similarly. It follows from Theorem 3.6 that there exists a group $(Q; \cdot)$ such that any operation $X \in \Omega_Q$ is endolinear over this group, which implies that all loops in Ω_Q are endolinear over this group; therefore,

they are principally homotopic to this group. From Lemma 2.1 it follows that they are isomorphic to this group; however, the Albert theorems ([2], [3]) imply that a nonassociative loop is not isomorphic to a group; therefore, $|Q| < 5$. It is well-known that on a finite set each surjection is a bijection; so, each regular division operation is an invertible operation, that is, any operation in Ω_Q is invertible this means if we fix $u = a$ in the identity we will have:

$$X(Y(x, y), \alpha v) = X'(\beta x, Y'(y, v)),$$

which is same

$$X(Y(x, y), v) = X'(\beta x, Y'(y, \alpha^{-1}v)),$$

where α, β are bijections and α^{-1} is inverse of the α .

This means $(Q; \Omega_Q)$ satisfies to the following second-order identity of associativity:

$$\forall X, Y \exists X'', Y'' \forall x, y, z X(Y(x, y), z) = X''(x, Y''(y, z)),$$

and from the Theorem 1.1 we have that $|Q| \leq 3$.

The sufficiency follows from the [7].

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ABSTRACT

The evaluation of the relationship between morphological predictors and home range size in aquatic mammals has been a tool used to understand the ecological requirements of the species, as well as provide relevant information for the construction of conservation actions and management of populations and aquatic habitats. We evaluated the relationship between the home range size of 34 Amazon River dolphin individuals (*Inia* spp.), and three explanatory variables reported in the scientific literature (1) body mass, (2) body length, and (3) sex. Home range sizes were calculated as the univariate kernel density estimates at 95% (K_{95}) for the *Inia* spp. individuals monitored through satellite telemetry across four rivers of the Amazon basin (Bolivia, Brazil, Colombia, and Peru) and five rivers of the Colombian Orinoco basin. Out of three home range predictors evaluated, only the sex predictor showed statistical significance in the Kruskal – Wallis test ($p = 0,037$).

Keywords: body length, body size, flood pulse kernel density estimates, neotropical rivers, top predator.

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Existing Relationship Between Morphological Predictors and Home Range Size of the Amazon River Dolphin (*Inia* spp.) in the Amazon and Orinoco Basins?

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ABSTRACT

*The evaluation of the relationship between morphological predictors and home range size in aquatic mammals has been a tool used to understand the ecological requirements of the species, as well as provide relevant information for the construction of conservation actions and management of populations and aquatic habitats. We evaluated the relationship between the home range size of 34 Amazon River dolphin individuals (*Inia* spp.), and three explanatory variables reported in the scientific literature (1) body mass, (2) body length, and (3) sex. Home range sizes were calculated as the univariate kernel density estimates at 95% (K_{95}) for the *Inia* spp. individuals monitored through satellite telemetry across four rivers of the Amazon basin (Bolivia, Brazil, Colombia, and Peru) and five rivers of the Colombian Orinoco basin. Out of three home range predictors evaluated, only the sex predictor showed statistical significance in the Kruskal – Wallis test ($p = 0,037$). This research also calls attention to the vulnerability of the *Inia* spp. to human impacts on aquatic landscapes such as the massive construction of dams on a regional scale regulating the flooding pulse and limiting the access of Amazon river dolphins to the different habitat types mainly in the Amazon basin.*

Keywords: body length · body size · flood pulse kernel density estimates · neotropical rivers · top predator.

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I. INTRODUCTION

Home ranges are defined as areas in which animals carry out their day-to-day activities and acquire the necessary resources to survive (e.g. Burt 1943; Buskirk 2004; Dahle et al. 2006; Péron 2019). Tucker et al. (2014) analyze the home range of 429 mammalian species including two coastal cetacean species (*Orcaella heinsohni*; McGowen 2011), and *Sotalia guianensis* (Caballero et al. 2008). They conclude that home range size is influenced by a range of factors such as body mass, diet, and the environment. Among the various potential consequences of allometry, an animal's home range size provides valuable information on a variety of ecological determinants, including resource use, social behavior and predator avoidance (Knight et al. 2009).

Home range analyses have been historically biased towards terrestrial mammals. Over the last two decades knowledge has grown about the spatial estimates of aquatic species, as represented in the scientific literature (Mosquera-Guerra et al. 2021). It is also unclear if factors driving home range size among mammals are the same in terrestrial and aquatic environments (Tucker et al. 2014). The size of an animal's home range is usually correlated with productivity, the species' biological requirements, and habitat heterogeneity (McNab 1963). Most animals do not use their entire home range with equal intensity but tend to concentrate their time in core areas (Dixon and Chapman 1980; Samuel et al. 1985; Oshima et al. 2010).

Previous studies on the spatial ecology of terrestrial mammals have suggested that home range sizes can be influenced by several factors as follow (1) mating system, (2) spatial distribution of resources (e.g. Boutin 1990; Dahle et al. 2006), (3) body mass (e.g. Harestad and Bunnell 1979; Dahle et al. 2006), (4) age and reproductive status (e.g. Dahle and Swenson 2003a, 2003b; Dahle et al. 2006); (5) population density and interactions with same-species neighbors (e.g. Dahle and Swenson 2003a; Buskirk 2004; Dahle et al. 2006); (6) morphological variation between subspecies (e.g. Kie et al. 2002); (7) intra- or (e.g. Riley and Dood 1984) interspecific competition (e.g. Loft et al. 1993); (8) foraging and predation avoidance (e.g. Krebs and Kacelnik 1991; Tufto et al. 1996; Powell et al. 1997; Relyea et al. 2000); (9) trophic level (e.g. Harestad and Bunnell 1979); (10) season (e.g. Nicholson et al. 1997); and (11) anthropogenic landscape fragmentation (e.g. Kie et al. 2002; Bejder et al. 2006; Pirota et al. 2013; Bas et al. 2014).

In the case of aquatic mammals, studies of home range variation in the coastal bottlenose dolphin (*Tursiops truncatus*) have been focused on the influence of environmental predictors such as productivity levels and tidal cycles (see, e.g., Wells et al. 1980; Defran et al. 1999; Wells and Scott 1999; Connor et al. 2000; Wells et al. 2017), highlighting the spatial complexity of the three-dimensional nature of aquatic environments (see, e.g., Alexander 2003; Shurin et al. 2006; Rip and McCann 2011; Pawar et al. 2012). Aquatic organisms experience high physiological challenges related to greater individual health decline (Tucker et al. 2014) as well as difficulties in detecting resources (see, e.g., Jetz et al. 2004; McGill and Mittelbach 2006; Pawar et al. 2012) that can result in variations of their home range.

The quantification of those elements that influence the selection and use of spatial mammalian ecology is technically challenging for aquatic species, and particularly for those inhabiting river systems characterized by greater hydrological dynamics. Home range studies in small cetaceans have been successfully conducted using different techniques and analyses for several coastal and oceanic species, including (1) the harbor porpoise (*Phocoena phocoena*, Sveegaard et al. 2011), (2) Hector's dolphin (*Cephalorhynchus hectori*, Rayment et al. 2009; Bräger and Bräger, 2018), (3) common bottlenose

dolphin (*Tursiops truncatus*, Mazzoil et al. [2008](#); Martínez-Serrano et al. [2011](#); Nekolny et al. [2017](#); Passadore et al. [2017](#); Wells et al. [2017](#); Genoves et al. [2018](#)), (4) Atlantic spotted dolphin (*Stenella frontalis*, Hill [2014](#)), (5) the franciscana dolphin (*Pontoporia blainvillei*, Bordino et al. [2008](#)), and (6) Guiana dolphin (*Sotalia guianensis*, Flores and Bazzalo [2004](#); Rossi-Santos et al. [2006](#); Azevedo et al. [2007](#); Wedekin et al. [2007](#); Oshima et al. [2010](#)). Currently, home range estimates are available only for the *Inia geoffrensis* with data derived from different methods (1) satellite tracking of adult individuals in the Amazon (Colombia and Peru), San Martín (Bolivia), Jurueña (Brazil), and Orinoco (Colombia and Venezuela) rivers (e.g. Mosquera-Guerra et al. [2021](#)); (2) VHF radio-tagging and capture, recapture, and marking of adult individuals in the Japurá and Solimões rivers (Mamirauá Sustainable Development Reserve, Brazil) e.g. Martin and da Silva, [1998](#), [2004a,b](#); Mintzer et al. [2016](#)); (3) photo-identification techniques of previously identified individuals (Pacaya-Samiria Reserve in Peru, e.g. McGuire and Henningsen [2007](#)), and the Cuyabeno and Lagartococha rivers (Cuyabeno Reserve, Ecuador; e.g. Denkinger [2010](#)).

Inia geoffrensis is the largest of the river dolphins (Martin and da Silva [2006](#)) and is also considered one of the top predators of the aquatic trophic webs of the Amazon, Orinoco, and Araguaia-Tocantins basins (McGuire and Winemiller [1998](#); Gómez-Salazar et al. [2012](#)). The species has the smallest group sizes known among cetaceans (Gómez-Salazar et al. [2011a](#), [2011b](#); Mosquera-Guerra et al. [2018a](#)), and its use of space is strongly conditioned by its ecological position as the top predator inhabiting dynamic systems characterized by seasonal variability (Frère et al. [2010](#)) and food availability (Mares et al. [1982](#)).

Inia geoffrensis is subdivided into two subspecies (Committee on Taxonomy [2021](#)); *I. g. geoffrensis* distributed across the Amazon, and Orinoco basins and *I. g. boliviensis*, found along the Mamoré, Iténez, and Madeira rivers (Aliaga-Rossel [2002](#); Aliaga-Rossel et al. [2006](#); Gravena et al. [2014](#); da Silva and Martin [2014](#); da Silva et al. [2018](#); Aliaga-Rossel and Guizada, [2020](#)). *Inia geoffrensis* is listed as Endangered (da Silva et al. [2018](#)) by the International Union for Conservation of Nature (IUCN), and is included in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES; da Silva and Martin [2018](#)). The species is considered among the most threatened aquatic mammals globally (Reeves et al. [2003](#); Trujillo et al. [2010](#)). In recent years, threats affecting aquatic ecosystems such as the construction of hydropower dams, mining, and bycatch of individuals river dolphins have increased in the major river basins of South America (e.g. Trujillo et al. [2010](#); Mitzer et al. [2014](#); da Silva et al. [2018](#); Mosquera-Guerra et al. [2019a](#); Barbosa et al. [2021](#); Campbell et al. [2020](#); Brum et al. [2021](#)).

Understanding the way in which *I. geoffrensis* home range size is influenced by morphological factors constitutes an important tool for planning management and conservation actions for populations and habitats. This study represents an important collaboration between researchers from multiple organizations from four South American countries. We aimed to evaluate the relationship between a set of morphological predictors and the home range sizes for the *I. geoffrensis* individuals in the four major rivers in Amazon and five rivers in Orinoco basins.

II. MATERIALS & METHODS

2.1 Study area

This study was conducted from October 2017 to June 2021 in the Amazon and Orinoco river basins. A total of 34 (♀: 11 and ♂: 23) *I. geoffrensis* individuals were tagged with SPOT-299A and SPOT6-F single-point fin mounted satellite tags (Wildlife Computers, Redmond, WA) in four rivers of the Amazon basin and five rivers of the Colombian Orinoco basin (Fig. 1).

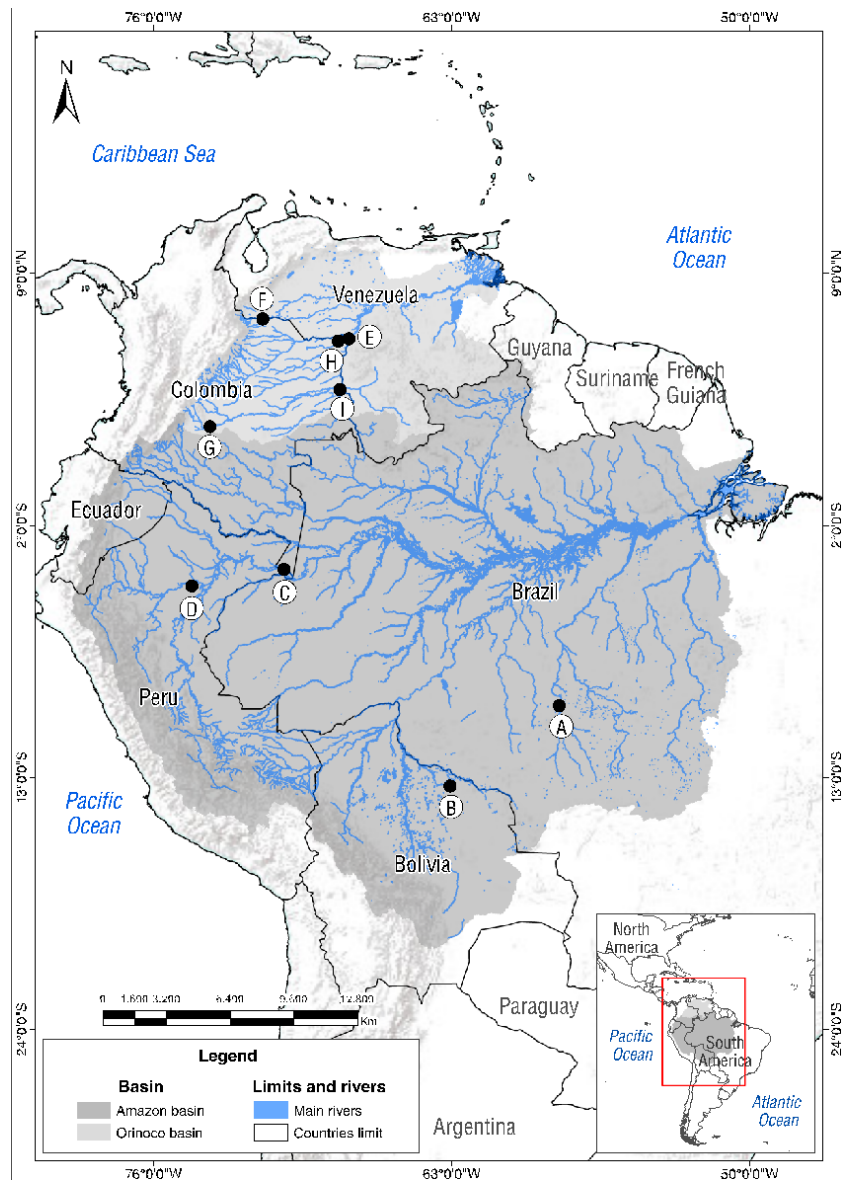


Figure 1: Amazon River dolphins satellite tagging: Amazon basin (A) Juruena River, Brazil, (B) San Martín River, Bolivia, (C) Amazon River, Colombia, and (D) Marañón River, Peru and rivers in the Colombian Orinoco basin (E) Orinoco River, (F) Arauca River, (G) Guayabero River, (H) Bitá River, and (I) Guaviare River.

2.2 Dolphin capture protocol and measurement recording

Only adult individuals were selected for tagging, and their ages classes were estimated based on body length, following the methods of da Silva (2009) and Martin and da Silva (2018), and avoiding females with calves. As part of our protocol, a veterinary team was present throughout the capture procedure to monitor the health of the animals according to cardiac and respiratory rates. There was no evidence that individuals experienced excessive stress. No increase in heart and respiratory rates, or sudden movements of head or caudal fins were noted that had been previously documented as signs of stress (Martin et al. 2006). In the event of excessive stress, our safety protocol required that the capture operation would immediately be halted and the dolphin released, but in none of the procedures was it necessary to do this. Our sample size was limited by the number of satellite tracking devices.

2.3 Spatial Analyses

The spatial analyses of the home range sizes of the *I. geoffrensis* followed the process of Mosquera-Guerra et al. (2021). The locations of the tagged river dolphins were determined using the ARGOS satellite system, maintained by ARGOS Service. Locations were classified by the ARGOS system into one of six location classes (LCs) based on the level of accuracy, measured in kilometers of uncertainty for latitude and longitude. ARGOS classifies location quality relative to an estimated error radius in the following location classes: 3 (accurate to <250 m), 2 (accurate to 250–500 m), 1 (accurate to 500–1500 m), and A and B (1–2 messages received but no accuracy estimation). In our study, we used only the most accurate data, LCs 3 and 2, after filtering the data with SAS-routine and the ARGOS-Filter (Witt et al. 2010; Wells et al. 2017; Dolton et al. 2020). Data with low accuracy, LC1 (500–1500 m), and data in classes A and B with no accuracy estimations were not used in our analyses (Mosquera-Guerra et al. 2021). Kernel density estimates at a 95% probability utilization distribution (UD) (K_{95}) were used to calculate home ranges (Powell 2000; Oshima et al. 2010; Wells et al. 2017; Mosquera-Guerra et al. 2021).

2.4 Predictors

In our study, the statistical analyses included three morphological explanatory predictors reported in the scientific literature for large terrestrial and aquatic mammals (Table 1). The morphological predictors were obtained from the 34 individuals tagged (10 individuals in this study and 24 Mosquera-Guerra et al. 2021; Supplementary material), and the Table 2 list the range (mean +/- SE) of these predictors. Satellite tagging was conducted under research permits for each country (Bolivia: DGBAP/MEG No. 0515/2017; Brazil: SISBIO 60171-1; Colombia: No. DTA 0898/2018; Peru: RD 515-2018 PRODUCE, RJ 003-2018).

Table 1: Summary of morphological explanatory predictors used in the Kruskal – Wallis test and significance values of the Shapiro-Wilk test

Category	Explanatory	Type	p Value	References
Morphological	Body mass (kg).	Continuous	0,001*	Harestad and Bunnell (1979); Dahle et al. (2006); Knight et al. (2009), and Tucker et al. (2014).
	Body length (cm).	Continuous	0,05*	Knight et al. (2009), and Tucker et al. (2014).
	Sex (♀ and ♂).	Categorical	-	Dahle and Swenson (2003a; b), and Dahle et al. (2006).

Those significant for the explanatory predictors are marked with an asterisk ().*

2.5 Statistical analyses

We used a Shapiro-Wilk normality test performed on the explanatory variables (Table 1) and subsequently a Kruskal-Wallis to assess the interactions between K_{95} and the three predictors (Table 3). Statistical analyses were carried out with the open-source software R.4.0.3 (R Core Team 2020) for the

graphical representation using the R software package ggplot2. In all cases, a value of $p < 0,05$ was considered statistically significant.

III. RESULTS

3.1 Home range (K_{95}) and explanatory predictors

The calculated values of the dependent variable and the morphological predictors considered in the statistical analyses are presented in Table 2 (Supplementary material).

Table 2: Range (mean \pm SE) and description of the values calculated for the dependent variable and explanatory predictors for the Kruskal – Wallis test.

Variable / predictors	Range (mean \pm SE) / Description
Dependent variable	
Amazon River dolphins home range sizes (K_{95}).	5,59–234 km ² (mean = 53 \pm 57,53 km ²).
Morphological predictors	
Body mass (kg).	49– 200 kg (mean = 102 \pm 37).
Body length (cm).	162– 227 cm (mean = 192 \pm 20).
Sex (♀ and ♂).	Males analyzed (n = 23, 68%) and females (n = 11, 32%).

The results of the statistical significance values for the interaction between the dependent variable (K_{95}) and the three predictors considered are presented in Table 3. These results show the influence of the sexual condition of the individuals on the size of the home range (Figures 2a-c, 3).

Table 3: Statistical significance values of the Kruskal-Wallis test

Interactions	X^2	df	p Value
$K_{95} \sim$ Body mass (kg)	2,65	3	0,44
$K_{95} \sim$ Body length (cm)	3,11	3	0,37
$K_{95} \sim$ Sex (♀ and ♂)	4,32	1	0,037*

Those significant for the explanatory predictors are marked with an asterisk ().*

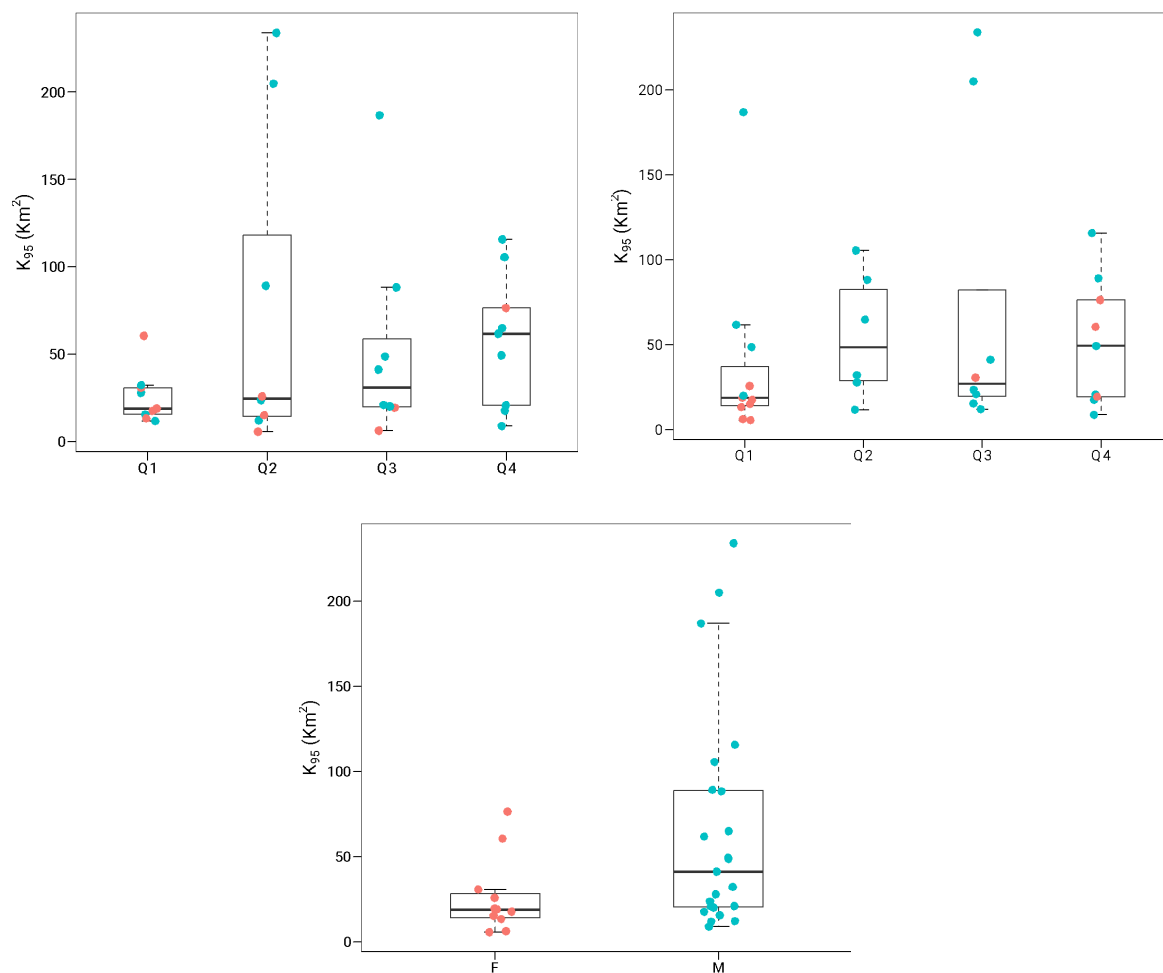


Figure 2: Box plot of the variation in home range size (K_{95}) by sex for the three predictors assessed: (A) body mass (kg), (B) body length (cm), and (C) sex (♀ and ♂). The red circles correspond to females and the blue circles to males.

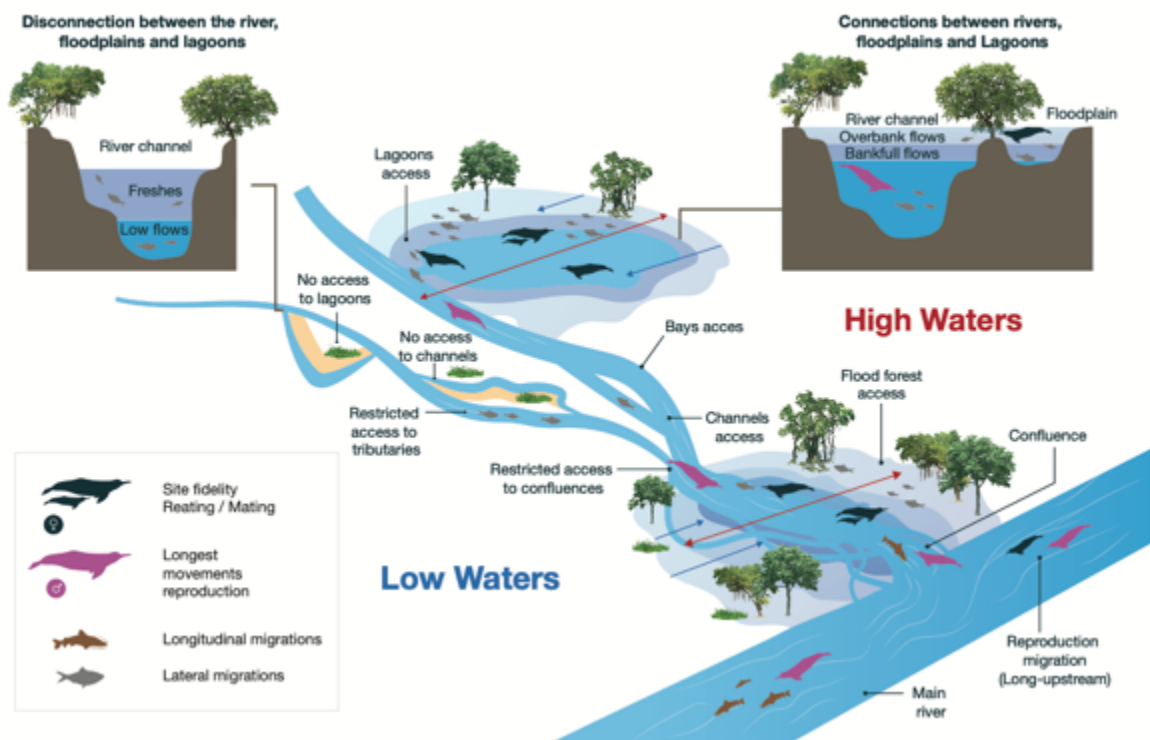


Figure 3: Flood pulse effects on *Inia geoffrensis* spatial ecology, highlighting variations in density, habitat availability, and fish prey migrations. During the high-water season, the rise in water levels causes the connection between the main river and the floodplains and lagoons. During this period fish and individuals of *I. geoffrensis* gradually access these new environments. In the season of the flood pulse, fission behaviors of dolphin groups occur, i.e., females with calves settle in the lagoons and confluences and males instead use more habitat types such as the main river and channels behind fish that make upstream breeding migrations. Conversely, during the low water season of flood pulse, the decrease in water levels disconnects the floodplains and lagoons from the main river, in this season dolphin groups merge again and individuals of *I. geoffrensis* converge in the confluence and channel habitat types to breed; some adult males move hundreds of kilometers to look for groups of females settled in other confluences along the basin.

IV. DISCUSSION

Our study had two limitations: the first related to small sample size, a condition that is a common limitation in satellite tracking studies due to factors such as the number of satellite devices available, and the low success rate in the capture process of the individuals (Taczanowska et al. 2008), especially in aquatic organisms that inhabit in contrasting ecosystems and are strongly influenced strongly by climatic variability. The second limitation is the representativeness of the individuals monitored by satellite in relation to the *Inia* populations size in the evaluated areas; for example in the case of the upper Amazon river basin of the 1,763 individuals that have been reported (Trujillo et al. 2019; Paschoalini et al. 2021) only 12 individuals were monitored, or for the upper Orinoco river basin of 1,573 individuals (Trujillo et al. 2019; Mosquera-Guerra et al. 2019b; Paschoalini et al. 2021) only 8 individuals were studied. The relevance of this study in data collection is the high resolution of data collected about *I. geoffrensis* individual home range size behavior obtained at a regional scale that allowed ecological analyses in an important part of the *Inia*'s distribution (Mosquera-Guerra et al. 2018b). In addition, the satellite monitoring of individuals provided fundamental information for spatial analyses (e.g. locations used in the calculation of the home range size) that contribute to an understanding of the home range sizes for this threatened mammals.

4.1 Body mass and length

The evolution of home range size appears to have been driven mainly by the energetic requirements and costs or benefits associated with a given body mass (see *e.g.*, Nagy [2005](#)). Ecologists have sought to understand the principles underlying how mammals optimize their space requirements (Buskirk [2004](#)). The body-size hypothesis has been used to explain differences in home range size among different species (see, *e.g.*, Harestad and Bunnell [1979](#); Kelt and Van Vuren [2001](#); Dahle et al. [2006](#)). Many ecological studies on terrestrial and aquatic mammals employ linear models to establish the relationships between home range size and body mass (see, *e.g.*, Buskirk [2004](#); Tucker et al. [2014](#); Kelt and Van Vuren [2001](#)). Early attempts to understand the relation between body mass and home range size suggest that home range increases at the same rate as metabolism (Kleiber, [1961](#)). The strong positive relationship between home range size and body mass reflects the balance between the cost of locomotion and metabolic requirements with increasing body mass (McNab [1963](#)). In the most taxonomically comprehensive analyses of home range size predictors, body mass is the principal predictor in mammals, accounting for 53–85% of the observed variation in home range size among species (Tucker et al. [2014](#)). However, in neotropical mammals, environmental ecosystemic variables such as seasonality, productivity levels, prey supply, sexual condition, gestation status, and presence of young, are perhaps greater influential predictors of home range size. Our statistical analyses failed to establish any relationship between mass and home range size, confirming Fahrig ([2013](#)).

In addition, the non-relationship between home range size and allometry of monitored river dolphins could also be explained by morphological variations among subspecies of the genus *Inia* reported in the scientific literature, even to the point of currently proposing three species (1) *I. geoffrensis* (Amazonas and Orinoco basins); da Silva and Martin [2014](#); da Silva et al. [2018](#)), (2) *I. boliviensis* (Madeira, Iténez, Mamoré, Blanco, and Grande rivers); Banguera-Hinestroza et al. [2002](#); Ruíz-García et al. [2008](#)), and (3) *I. araguaiaensis* (Araguaia-Tocantins river basins); Hrbek et al. [2014](#); Brum et al. [2021](#)). However, the Taxonomy Committee of the Marine Mammal Society only recognizes to date the subspecies considered in this study (Committee on Taxonomy [2021](#)).

Among the main morphological features that differentiate these two subspecies are the total length and the number of teeth. *Inia. g. geoffrensis* has an overall total length of 219–255 cm (males) and 182–225 cm (females), and the total number of upper jaw teeth range from 23-35 (mean = 26.6) while the lower jaw has from 24-35 (mean = 27.1). In the case, *I. g. boliviensis* the total length is 230 cm (males) and 208–216 cm (females), and the total number of upper jaw teeth ranges from 31-35 (mean = 33.3) while the lower jaw ranges from 31-34 (mean = 32.3); da Silva and Martin [2014](#)).

4.2 Sex and reproductive status

Sexual dimorphism has been documented in *I. geoffrensis* with males weighing between 113.5–207 kg, while females weigh between 72–154 kg (da Silva and Martin [2014](#)). Males are likely to be growing marginally faster than females of the same age in the months after birth (Martin and da Silva [2006](#); [2018](#)). We found a statistical relation between *I. geoffrensis* sex and home ranges (Table 3, Fig. 2c). Our results are in line with those proposed by Lindstedt et al. ([1986](#)) and Folkens et al. ([2008](#)) in relation to the differences in home range sizes between males and females for both marine cetaceans (bottlenose dolphin) and terrestrial carnivorous mammals. The documented pattern is concordant with those in fission-fusion societies, such as those seen in many dolphin species, where individuals in the same population may have greatly different ranging patterns (*e.g.* Defran et al. [1999](#)); and individuals may alternate between local site-fidelity and longer ventures away from the site of their first identification (*e.g.* Rako-Gospic et al. [2017](#)).

Our mean values for male home range values are also within the *I. geoffrensis* home ranges reported by Martin and da Silva (1998; 2004a,b) and Mosquera-Guerra et al. (2021), and may represent dominant male individuals in their search for females throughout the basin. Mosquera-Guerra et al. (2021) suggest that sexual status may determine home range sizes and the most extensive movements for individuals of the populations distributed in the Amazon (Colombia), San Martín (Bolivia), and Marañón (Peru) rivers in the Amazon and Orinoco river basins.

Similar results have been reported for male bottlenose dolphins that presumably have larger home ranges than females, a situation that is thought to allow males to increase their reproductive access to females (see, e.g., Eisenberg 1966; Wells et al. 1987; Wells 1991; Sprogis et al. 2016). Previous studies on the behavioral ecology of common bottlenose dolphins (*T. truncatus*) have revealed great variability in their home range characteristics (see, e.g., Connor et al. 2000; Defran et al. 1999; Wells et al. 1999) that may be due to habitat heterogeneity or to differences in the use of space between the genders (see, e.g., Connor 2000; Wells et al. 1980). Some authors also suggest that the period of parental care of the calf (1.5-5.8 years, Martin and da Silva 2018) for Amazon River dolphins of the genus *Inia* limits the movement of the female during this period of time when the calf reaches the right physical condition to perform the different movements along the different aquatic landscapes (see, e.g., Mosquera-Guerra et al. 2021). Additionally established correlations are known between age and reproductive status with home range size (see, e.g., Dahle and Swenson 2003a, b; Dahle et al. 2006; Rako-Gospic et al. 2017).

4.3 Conservation implications

In terms of conservation, Mintzer et al. (2016) and Mosquera-Guerra et al. (2022a) report on the relationship between spatial use, sexual status and the level of exposure to hazards for individuals of *Inia* spp. This sexual segregation differentially exposes males and females to targeted or incidental captures as well as other types of threats (see, Mintzer et al. 2016). Interactions between Amazon River dolphins and fisheries generally occur in highly productive habitats, such as (1) confluences, (2) channels, and (3) lagoons, where capturing mostly sexually mature individuals and possibly larger numbers of females that have minor movements and are restricted to specific habitats where they care for their calves (see, Mosquera-Guerra et al. 2022a). These factors contribute strongly to the drastic population decline of *Inia* spp. in its occurrence range of occurrence (see, Williams et al. 2016; Martin and da Silva 2021).

Also, a major human activity that affecting the spatial and movement ecology of *I. geoffrensis* and the possible adaptation of their populations to climate change is the presence of hydroelectric dams throughout the dolphins' range. In the Amazon basin, 158 hydroelectric dams are either in operation or under construction; and 351 more dams have been proposed (Forsberg et al. 2017; Almeida et al. 2019; Anderson et al. 2019). Many aquatic species are affected by hydroelectric dams (Lees et al. 2016). Among the direct effects caused by hydroelectric dams is the fragmentation of both *I. geoffrensis* and fish prey populations, resulting in disruptions to gene flow that cause diminishing genetic variability and increased vulnerability (Trujillo et al. 2010; Gravena et al. 2014; Pavanato et al. 2016; Mosquera-Guerra et al. 2018b; Paschoalini et al. 2020; Mosquera-Guerra et al. 2022b). Indirectly, the dams can decrease limiting nutrients for aquatic food webs such as nitrogen and phosphorus downstream of the dam, decreasing levels of productivity and biomass, thus reducing prey availability for the dolphins (Trujillo et al. 2010; Mosquera-Guerra et al. 2018b; Araújo and Wang 2015).

Fragmentation of river networks by hydroelectric dams and other infrastructure constrains potential range shifts of aquatic species for coping with expected temperature rise under climate change (Myers et al. 2017; Mosquera-Guerra et al. 2019b). Range shifts of fish to higher altitudes as a result of climate changes have already been documented, but river fragmentation by hydroelectric dams will block this

form of adaptation (Herrera-R et al. [2020](#)). Aquatic species, distributed in the Andean countries such as the *I. geoffrensis* populations in Peru and Bolivia are thought to be particularly impacted because most hydroelectric dams have been built or planned on Andean tributaries such as the Maderia river basin (Forsberg et al. [2017](#); Mosquera-Guerra et al. [2018b](#); Anderson et al. [2019](#); Tognelli et al. [2019](#)). The ecology and conservation of *I. geoffrensis* are complex in the Amazonian and Tocantins basins in the current context of transformation, and this urgently requires transforming the energy matrix of the Amazonian countries from hydropower to sustainable production based on solar, wind, and geothermal energies.

Our results do not support the previously established allometric relationship between the body length and mass on the home range sizes of large aquatics mammals, possibly due to the morphological variations of the subspecies of the genus *Inia* and the influence of the sexual status of monitored individuals on this spatial metric. We suggest the possible influence of non-evaluated predictors such as productivity levels of the aquatic ecosystems assessed (*e.g.*, white, black, clear, and mixed water systems), availability of prey, and the presence of highly productive habitats (*e.g.*, lagoons and confluences).

This research also calls attention on the possible vulnerability of the Amazon River dolphins to human impacts at a regional scale, such as the regulation of the flood pulse and its influence on the lateral and longitudinal movements of these cetaceans in the Amazon mainly. Finally, this paper warns about the negative effects of dam construction as physical barriers that alter prey migration and artificially regulate hydrological processes. These limit species' adaptations to climate change, increasing the risk of extinction of some populations that are located in the areas of influence of these infrastructure projects such as the individuals monitored in this study on the Tapajos River in Brazil.

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Availability of data and material: The datasets generated during the current study are available from the corresponding author on reasonable request.

Code availability: The R code used during the current study is available from the corresponding author on reasonable request.

Data availability: Data are available upon request.

Declarations

Ethics approval: All procedures involving animals were conducted with the appropriate permits and following ASM guidelines (Sikes [2016](#)).

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SUPPLEMENTARY MATERIAL

Appendix S1: Summary of the ecological predictors and home range sizes of the *Inia geoffensis* used in the GAMLSS, including as follow: locations, subspecies, platform transmitter terminal identification number (PPT ID); *I. geoffensis* home range sizes: K_{95} : home range 95% utilization distribution (UD) sizes. Morphological predictors: body mass (kg), body length (cm), and sex: ♀: female and male: ♂.

Locations / Subspecies	PPT ID	K_{95} (km ²)	Body Mass (kg)	Body Length (cm)	Sex	References
Amazon River (Colombia)						
<i>I. g. geoffensis</i>	40679	30,7	78,4	199	♀	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffensis</i>	40691-1	61,7	118	176	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffensis</i>	181675	48,7	109	174	♂	This study satellite tracking data
<i>I. g. geoffensis</i>	181676	105,5	112	178	♂	This study satellite tracking data
Jurueña River (Brazil)						
<i>I. g. geoffensis</i>	171926	23,6	89	197	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffensis</i>	40663	17,6	49	162	♀	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffensis</i>	40687	27,9	60	182	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffensis</i>	40681	15,5	56	193	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffensis</i>	40693	12,1	91	205	♂	Mosquera-Guerra <i>et al.</i> , 2021
Marañón River (Peru)						
<i>I. g. geoffensis</i>	55305	20,9	97	197	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffensis</i>	175587	32,2	63,9	177	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffensis</i>	55298	11,8	72,5	182	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffensis</i>	55306	6,2	101,9	173	♀	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffensis</i>	55309	20,1	92,6	170	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffensis</i>	55312	64,9	111,7	182	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffensis</i>	55319	186,8	99	176	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffensis</i>	175588	88,3	97	180	♂	Mosquera-Guerra <i>et al.</i> , 2021

Locations / Subspecies	PTT ID	K ₉₅ (km ²)	Body Mass (kg)	Body Length (cm)	Sex	References
<i>I. g. geoffrensis</i>	40688	8,8	180	214	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffrensis</i>	40691	18,9	68	159	♀	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffrensis</i>	171927	41,2	107	188	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffrensis</i>	40641	76,4	200	227	♀	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. geoffrensis</i>	181019	15,1	83	176	♀	This study satellite tracking data
<i>I. g. geoffrensis</i>	181020	13,3	82	175	♀	This study satellite tracking data
<i>I. g. geoffrensis</i>	181015	17,59	149	235	♂	This study satellite tracking data
<i>I. g. geoffrensis</i>	201339	20,75	129	224	♂	This study satellite tracking data
Bita River (Colombia)						
<i>I. g. geoffrensis</i>	181017	5,59	85	174	♀	This study satellite tracking data
Arauca River (Colombia)						
<i>I. g. geoffrensis</i>	181018	25,8	88	175	♀	This study satellite tracking data
Guayabero River (Colombia)						
<i>I. g. geoffrensis</i>	40641	49,38	180	214	♂	This study satellite tracking data
<i>I. g. geoffrensis</i>	181013	115,7	176	223	♂	This study satellite tracking data
Guaviare River (Colombia)						
<i>I. g. geoffrensis</i>	181016	19,43	110	210	♀	This study satellite tracking data
San Martín River (Bolivia)						
<i>I. g. boliviensis</i>	40640	233,9	91,2	204	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. boliviensis</i>	40662	89,2	83	209	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. boliviensis</i>	40674	204,9	85,4	208	♂	Mosquera-Guerra <i>et al.</i> , 2021
<i>I. g. boliviensis</i>	171928	60,5	69,2	211	♀	Mosquera-Guerra <i>et al.</i> , 2021

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Yield Response of Carrot to Cowpea -Carrot Rotation

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ABSTRACT

Carrot is a valued exotic vegetable in Ghana, mostly used in combination with other vegetables in preparing soups, stews, salads and drinks. Demand for carrots therefore, remains high especially in urban centres. A major constraint to carrot production is poor soil fertility. Carrot farmers generally experience high production costs as a result of inorganic fertilizer application. The use of legumes such as cowpea for nitrogen fixation leading to soil fertility enhancement provides a viable alternative for sustainable crop production. This chapter is an output of research that examined the contribution and improvement of cowpea-based Biological Nitrogen Fixation (BNF) in carrot production in the Ashanti Mampong Municipality in the Ashanti Region of Ghana to propel carrot production to sustainable levels. Carrot production is a widespread economic activity within the Municipality which supplies other urban centres such as Kumasi within the Ashanti Region of Ghana. The study highlights salient carrot production and handling practices which are expected to provide some relevant information to farmers, agricultural-service providers and other actors along the carrot value chain

Keywords: cowpea-carrot rotation, crop rotation, bnf, incorporation, nitrogen fixation.

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Listowel Aditwin Akologo^α, Harrison Kwame Dapaah^σ & Julius Yirzagla^ρ

ABSTRACT

Carrot is a valued exotic vegetable in Ghana, mostly used in combination with other vegetables in preparing soups, stews, salads and drinks. Demand for carrots therefore, remains high especially in urban centres. A major constraint to carrot production is poor soil fertility. Carrot farmers generally experience high production costs as a result of inorganic fertilizer application. The use of legumes such as cowpea for nitrogen fixation leading to soil fertility enhancement provides a viable alternative for sustainable crop production. This chapter is an output of research that examined the contribution and improvement of cowpea-based Biological Nitrogen Fixation (BNF) in carrot production in the Ashanti Mampong Municipality in the Ashanti Region of Ghana to propel carrot production to sustainable levels. Carrot production is a widespread economic activity within the Municipality which supplies other urban centres such as Kumasi within the Ashanti Region of Ghana. The study highlights salient carrot production and handling practices which are expected to provide some relevant information to farmers, agricultural-service providers and other actors along the carrot value chain.

Keywords: cowpea-carrot rotation, crop rotation, bnf, incorporation, nitrogen fixation.

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I. INTRODUCTION

Vegetable production is an important crop sub-sector of Ghana's agriculture with a great potential for both local and international markets. Carrot is a valued exotic vegetable in Ghana, mostly used in combination with other vegetables in preparing soups, stews, salads and drinks [1]. One of the constraints to carrot production is poor soil fertility. The nutrient status of the soils is usually depleted when farmers engage in practices such as continuous cropping and diminutive mineral and organic fertilizer use [2].

Cowpea is a major component of the cropping systems of the drier parts of the tropics, particularly sub-Saharan Africa. It is mainly grown in mixtures with other crops and a great diversity of crop mixtures has been reported. Because of its soil improvement ability (fixing nitrogen into the soil), it has the beneficial effects on subsequent crops in the rotation and also on the crops grown in association with it. In any cropping system research, it is useful to understand how the components which make up the cropping system interact. Cropping cowpea generally improves soil fertility through nitrogen fixation and addition of soil organic matter. It compensates for the loss of nitrogen absorbed by cereals and has a positive impact on soil properties. The use of legumes such as cowpea for nitrogen fixation leading to soil fertility enhancement provides a viable alternative for sustainable crop production [3]. This is due to its unique capacity to fix atmospheric nitrogen and good performance

even in poor soils [4]. The common practice of integrating legumes into cropping systems include crop rotation, simultaneous intercropping, improved fallows, green manuring, and alley cropping [5].

This chapter is an output of research that examined the contribution and improvement of cowpea-based biological nitrogen fixation (BNF) in carrot production in the Ashanti Mampong Municipality in the Ashanti Region of Ghana to propel carrot production to sustainable levels. The study highlights salient carrot production and handling practices which are expected to provide some relevant information to farmers, agricultural-service providers and other actors along the carrot value chain. Such pieces of information can be updated as new research information and recommendations are generated in future studies.

II. AGRONOMY AND CROPPING PRACTICES AGRONOMY AND CROPPING PRACTICES

2.1 Land preparation

Carrots give highest production on well drained, light sandy soils as well as deep, loose, loamy soils with pH between 6 - 6.5. For effective BNF, cowpeas are grown on well-drained sandy loams. Since organic manure releases both major and minor nutrients, carrot and vegetable farmers are advised to use any available manure including cowpea residuals for effective growth and yield. The land should be cleared of debris, ploughed, and leveled before planting. The biomass or stubble should be incorporated into the soil to decompose. After the incorporation of the biomass or stubble, the land should be cleared of weeds, raked, loosened and leveled after it is thoroughly watered. For soils with poor structure, high run-off and low water infiltration, the physical properties can be improved markedly and cowpea yields increased if the land is ploughed. Zero tillage (for example using Roundup spray prior to planting) may be used only where drainage is good. Beds should be of uniform size of 3 m² and raised at 1.5 cm wide and 2 cm long for the sowing of carrots (Plate 1).



a: Seed bed of cowpea



b: Vegetative stage of cowpea

Plate 1: Photographs of cowpea fields at different management stages

III. PLANTING OF COWPEA

Generally, for early maturing cowpea varieties, planting at the beginning of the rains is advised so that the sensitive stages of the crop could avoid the peak activity of insect pests (Plate 2). Soronko variety (80-85 days) should be planted at 60 cm x 20 cm, while the Asetenapa variety (70-75 days) should be 50 cm x 20 cm. After harvesting the cowpea, the cowpea residue should be incorporated into the soil to decompose before sowing of carrot seed. Application of fresh leguminous organic matter to the soil during cultivation is detrimental to carrot root development [8]. The seeds should be planted ¼ inch deep and thinned out to 3 inches apart within rows and 9 inches apart between rows. About 3 lbs of seeds is required to plant 1 acre. Heavy rains should be avoided during planting as it can result in heavy loss of seeds and seedlings.



a: Podding stage

b: immature pods stage

c: mature pods stage

Plate 2: Photographs of carrot fields at different developmental stages

IV. WEED CONTROL

Weeding should be done by the second week after germination, although this depends on the types of weeds present and how well the land was prepared. If a pre-emergence herbicide (e.g. Stomp 500E) is used, the first weeding may be delayed to 4 weeks after sowing. It is important to complete weeding by the end of the 6th week when the crop is establishing ground cover. Weeds should be controlled manually when needed and should be done twice manually with a hoe. Loosening of the soil (forking) should be done periodically to enhance water percolation and air circulation and also to aid carrot root development.

V. FERTILIZER AND PESTICIDE APPLICATION

Generally, cowpea varieties especially Asetenapa and Soronko combine effectively with 60 kg N/ha in amending the soil for optimum carrot yield (Table 1). Carrot root forking and cracking is generally low in cowpea amended plots as these plots record minimal cracked and forked carrot roots compared with non-amended plots. The 60 kg N/ha fertilizer application rate gives significant performance in plant height, number of leaves per plant, dry matter accumulation, low nematodes ratings and carrot root length. Cymethoate 2.5EC (Cymethoate + Dimethoate) should be applied at 40 ml per 15 litres of water using a Knapsack sprayer at 30, 40 and 50 DAS to control pest in Asetenapa, and at 35, 45, 55 and 65 DAS for Soronko. The last spraying should be applied to control post flowering pests.

VI. CARROT YIELD

Generally, the soil amendment using cowpea varieties leads to higher carrot yield. Soil physical composition is of special significance for carrot yield. In the soil amendment studies in Asante Mampong, [6] it was observed that increased carrot yield and other yield components such as root length, root diameter and root weight in treated plots, and improved soil chemical and physical properties are influenced by the application of organic matter (Plate 3). Application of *Mucuna pruriens* green manure, chicken manure, and their combinations as well as the combination of *Mucuna pruriens* and NPK fertilizer significantly improves soil physical conditions, particularly total porosity and gravimetric moisture content and reduced bulk density [7]. Resource-poor farmers can therefore benefit from the cowpea grain as well as higher carrots yields to increase their household incomes.



a: Vegetative growth stage



b: Fresh tubers after harvest

Plate 3: Photographs of carrot field at different stages of growth

VII. YIELD AND YIELD COMPONENTS OF PRECEDING CROPS (COWPEA)

In the soil amendment studies in Asante Mampong, it was also observed that Soronko and Asetenapa had similar number of pods per plant, number of seeds per pod, and hundred seed weight. This could be due to similarity in the number of branches and plant height between the cowpea varieties. The two varieties also had similar growth patterns, and although Soronko is medium maturing, it did not significantly produce higher yield components than Asetenapa (an early maturing variety) as it usually occurs. In terms of total grain yield however, Soronko had higher grain yield (793.0 kg/ha) than Asetenapa (365.8 kg/ha). Soronko's performance could be attributed to the high nodulation and high DM accumulated (Table 1).

Table 1: Dry matter accumulation, nodulation, yield and yield components of Soronko and Asetenapa as preceding crops

	Preceding crops	
	Soronko	Asetenapa
Dry biomass at 50 DAS (g/m ²)	0.67	0.51
Dry biomass at harvest (t/ha)	2.819	1.476
No. of effective nodule/plant	176	55
No. of non-effective nodules/plant	3	2
Dry weight of effective nodules	1.2	0.6
No. of pods per plant	11.00	9.67
No. of seeds per plant	15.00	12.00
100 seed weight	12.07	11.43
Grain yield (kg/ha)	793.0	365.8

VIII. EFFECT OF SOIL AMENDMENT ON NUMBER OF ROOTS FORKED, CRACKED AND NEMATODES INFESTATION

The number of forked carrot roots did not show any significant differences in both carrot seasons (Table 2). Root forking and cracking were generally low and this could largely be attributed to the improved soil conditions due to the cowpea amendment. [8], reported that organic soil amendments contribute macro and micro nutrients and also improve the soil structure for effective microbial activities and crop growth, increase the water holding capacity, porosity and decrease bulk density. [9] in a research conducted on growth and yield response of carrot (*Daucus carota* L.) to different rates of soil amendments and spacing in Ashanti Mampong, reported that amendments improve soil conditions such as moisture content, soil structure, texture and reduces soil compaction which

normally causes forking of carrot roots. There was minimal cracking among the carrot roots which could be due to the application of inorganic fertilizer as affirmed by [9], that well decomposed organic matter minimizes carrot root cracking. It could also be attributed to the soil amendment which probably improved the chemical and physical conditions of the soil for crop growth. Forking is a known problem in carrot production and if the organic matter is well decomposed forking will be reduced [8]. Thorough working into the soil promotes effective decomposition leading to less cracking of carrot roots [8]. However, nematode rating record showed significant differences with the non-amended plots, while carrots grown on the cowpea crop varieties showed no significant differences. This confirms that cowpea can be used to suppress the growth of nematodes [10]. The *meloidogyne incognita* species were suppressed by the cultivation of cowpea [11]. This confirms that cowpea can be used to suppress the growth of nematodes [11]. Rotating carrot with cereals alongside the application of organic manure minimizes the impact of nematodes [8]. The amendment also helps reduce the incidence of nematodes. Organic amendments procedures can also be practiced to control nematodes [8]. Certain lines of cowpea can also suppress the populations of the nematode *Scutellonema cavenessi* [12].

Table 2: Number of forked and cracked roots and nematodes infestation as influenced by preceding crops in Ghana

	No. of forked roots		No. of cracked roots		Nematodes Ratings	
	2020	2021	2020	2021	2020	2021
Variety						
Soronko	0.07	0.07	0.00	0.20	0.07	0.60
Asetenapa	0.07	0.07	0.13	0.07	0.00	0.13
Obatanpa	0.20	0.00	0.27	0.07	0.80	0.13
Mean	0.11	0.04	0.13	0.11	0.29	0.29

IX. HARVESTING

Matured, dried pods of cowpea should be harvested promptly, as delayed harvesting encourages weevil infestation in the field and seed shattering. In harvesting carrot, the mature plant is lifted gently by hand where the soil is loose. The soil should be loosened when it is heavy. This can be done using a spading fork and the roots lifted gently so that they do not break. In manual harvesting, hand tools such as hoes, spades, pick axes, sickles and crowbars (for loosening of soil) are used. Tractor-operated carrot harvester or animal drawn implement may also be used for harvesting carrots.



Plate 4: Fresh tubers after harvest

X. CONCLUSION

Cowpea used to amend the soil generally improves soil conditions leading to increased carrot productivity. Soil physical composition is of special significance for carrot yield. The application of organic and inorganic fertilizers promotes plant nutrient availability for crop growth, yield and yield components. There is also minimal root forking and cracking which could be due to the application of inorganic fertilizer as affirmed by [9] who reported that well decomposed organic matter minimizes carrot root cracking. Soil amendment improves the chemical and physical conditions of the soil for crop growth. The use of leguminous crop residues to amend soil has the potential of sustaining soil organic matter for increased yields of succeeding crops [9].

Competing Interests

Authors have declared that no competing interests exist.

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Walatimine (Vanillyl Butyl Imine): A New Ketimine from Schiff Base Synthesis and Evaluation of its Antioxidant, Antibacterial and Antifungal Properties

Olawale Hakeem Oladimeji, Joy Adesoji Olukoju, Samuel Ogayi Ogbu, Uforo Joseph Ubobo & Emmanuel Edet Attih

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ABSTRACT

Background of study: Vanillin is a white crystalline compound which is a phenolic aldehyde with a balsamic flavour. It is widely obtained from vanilla bean-pods amongst very many other sources. This compound and some of its derivatives possess diverse activities including anticancer, anti-inflammatory, antioxidant, antibacterial and antifungal amongst others. Hence, the import of this study.

Objectives: The growing concerns about the deleterious actions of free radical oxygenated species (FROS) in the human body have become a huge concern to scientific world. These chemical species continually devastate the human cells, tissues and organs leading to different patho-physiological conditions and neurodegenerative disorders. Also, the noticeable microbial resistance to antibiotics and antifungal drugs have prompted the search for lead compound(s) with the aim of chemically modifying its/their molecular structure(s) or synthesizing other compounds from reactions involving them such as Schiff base synthesis. The search for novel pharmaceutically active compounds with the aim of ameliorating these conditions led to the choice of vanillin.

Keywords: vanillin; vanillyl butyl imine; vanillin-2,4- dinitrophenylhydrazone; antioxidant; antibacterial; antifungal.

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Walatimine (Vanillyl Butyl Imine): A New Ketimine from Schiff Base Synthesis and Evaluation of its Antioxidant, Antibacterial and Antifungal Properties

Olawale Hakeem Oladimeji^a, Joy Adesoji Olukoju^a, Samuel Ogayi Ogbu^p, Uforo Joseph Ubobo^{co} & Emmanuel Edet Attih^{*}

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Background of study: Vanillin is a white crystalline compound which is a phenolic aldehyde with a balsamic flavour. It is widely obtained from vanilla bean-pods amongst very many other sources. This compound and some of its derivatives possess diverse activities including anticancer, anti-inflammatory, antioxidant, antibacterial and antifungal amongst others. Hence, the import of this study.

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*Methodology: Vanillin was subjected to the Schiff base synthesis. It was reacted separately with amines (aromatic and aliphatic amine) leading to the bases in the presence of acid. The melting points, refractive indices and optical rotations of the vanillin and the Schiff bases were obtained. The antioxidant activity (IC₅₀) of the lead compound and bases was determined employing the DPPH (2,2-diphenyl-1-picrylhydrazyl hydrate) bench-top assay test. Comparison of the obtained antioxidant activities was done to determine if any improvements could be noticed in the synthesized bases. Also, the agar-in-hole diffusion method was adopted for screening vanillin and the synthesized bases against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Candida albicans* for antibacterial and antifungal activities respectively.*

*Results: The identities of the synthesized Schiff bases have been revealed to be vanillyl butyl imine (W) a new ketimine referred to by the trivial name-Walatimine and vanillin-2,4-dinitrophenyl hydrazone (Vdnp) respectively using a combination of physico-chemical determinations and IR spectral technique. Vanillin and (W) demonstrated marginal antioxidant activity of IC₅₀ of 0.52 and 0.50 µg/mL respectively while Vdnp gave a remarkably significant IC₅₀ of 0.48 and which compare favourably with 0.46 µg/mL elicited by Vitamin C (a standard antioxidant drug). The antibacterial and antifungal activities elicited by both W and Vdnp were concentration-dependent. Furthermore, Vdnp was comparably more bacteriostatic than W against the test bacteria though it was inactive against *Ps. aeruginosa*. However, vanillyl butyl imine (W) was slightly more anti-candidal against *C. albicans* than vanillin-2,4-dinitrophenyl hydrazone (Vdnp).*

Conclusion: The results from this study indicate that vanillin-2,4-dinitrophenyl hydrazone (Vdnp) obtained from the condensation reaction of vanillin and an aromatic amine affords a comparably better antioxidant activity than W obtained from vanillin reacting with an aliphatic amine. Both W and Vdnp demonstrated remarkable antibacterial and antifungal activities hence, these two synthesized Schiff bases can be lead candidate compounds in the search for newer and more efficacious antioxidant and antimicrobial agents and in further structural activity relationship studies (SARS) and as well as in formulation studies in drug development.

Keywords: vanillin; vanillyl butyl imine; vanillin-2,4-dinitrophenylhydrazone; antioxidant; antibacterial; antifungal.

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I. INTRODUCTION

Vanillin is one of the most popular and widely used aromatic flavouring compounds found as a glycoside in the fruits of natural vanilla. The main source of vanilla is the bean or pod of the tropical vanilla orchid, *Vanilla planifolia* but also found in *Vanilla fragrans*, *Vanilla pompona* and *Vanilla tahitensis*. It was discovered amongst the Aztecs of Mexico by the Spaniards who introduced it to Europe in 1520 and is now cultivated around the world in countries such as Mauritius, Seychelles, Madagascar, Java, Ceylon, Tahiti, Guadeloupe, Martinique and Indonesia amongst many others. In 1874, Ferdinand Thompson and Wilhelm Haarman synthesized vanillin from coniferin, a glycoside of isoeugenol found in pine bark. Currently, synthetic vanillin is used as an intermediate in the chemical and pharmaceutical industries for the manufacture of herbicides, antifoam agents, drugs such as papaverine, L-methyldopa, L-dopa, trimethoprim and also in the production of fragrances and flavoring agents [1][2][3][4]. Many derivatives of vanillin have shown good antibacterial properties against gram (+) and gram (-) microbes.

Vanillin is also known to have antioxidant properties due to their strong free radical scavenging properties and derivatives obtained from its reduction, O-demethylation, oxidation and acetylation respectively gave between marginal and moderate antioxidant activities using the bench-top assay with DDPH reagent [5]. The increasing application of antibiotics in healthcare has thrown up the risk of growing resistance as multi-drug resistance bacterial pathogens are rising and there is a growing need for stronger and more effective antibiotics. Hence, the need to explore for novel alternatives. Similarly, for fungal infections therapy which also suffers from resistance occasioned by multi-drug resistant fungi thereby making a search for newer antifungal drugs/agents inevitable. Schiff base was first reported by Hugo Schiff in 1864. It is also known as imine which is a pharmacophore containing the azomethine group (-HC=N-). It can also be referred to ketimine or aldimine or hydrazone or aziridine. The ketimines in particular are used as anesthetic agents in animals after their introduction in 1970 by Federal Drug Administration (FDA) in America. This drug is employed by emergency responders on agitated patients or to calm them and as well as in treating depression and suicidal thoughts in patients.

Imines or Schiff bases are the products formed in condensation reactions of ketones or aldehydes with amines and generally take place in the presence of acid or base or heat. The formation of a Schiff base is a reversible reaction that is usually completed by the separation of product or removal of water or the both. It is best carried out at mild acidic pH. It can be hydrolyzed back to their aldehydes or ketones and amines by aqueous acid or base. The mechanism involves the addition of a nucleophile (amine) to the carbonyl group. The amine reacts with the aldehyde or ketone to form an unstable

compound called carbinolamine which undergoes dehydration catalyzed by acid, thus loses water. Schiff bases are one of the most widely used organic compounds in the production of pigments, dyes, catalysts and polymer stabilizers. Reports exist which show that these compounds exhibit a wide range of biological activities including antioxidant, antibacterial, antifungal, anti-malarial, antidepressant, anti-proliferative, anti-inflammatory, anticancer, antipyretic, anti-diabetic and antidepressant properties [6] [7] [8] [9][10]. Schiff bases with anthracene and pyrene units have reportedly been found to be antibacterial against *Bacillus cereus*, *Escherichia coli* and *Pseudomonas aeruginosa* in *in-vitro* studies while benzothiazole-based Schiff ligands were bacteriostatic against *Staphylococcus aureus* [11]. Furthermore, cinnamyl Schiff bases have shown antifungal activities against *Candida albicans* and *Aspergillus fonsceaea* [12] while phenylenediamine containing Schiff bases and those with metallic ions such as zinc, chromium, copper and manganese have demonstrated radical scavenging activity [13]. Consequently, this present research was designed to synthesize Schiff bases using vanillin, a compound with an aldehydic group (-HC=O) and two different amines separately. One, a straight chain aliphatic amine (butyl amine) while the other was aromatic (2,4-dinitrophenyl hydrazine). The synthesized Schiff bases were screened for antioxidant activity (IC₅₀) using the DPPH reagent and the agar-in-hole diffusion method was employed in determining the anti-bacterial and antifungal sensitivity properties. Comparison of results obtained was done with values given by vanillin and the synthesized Schiff bases and as well as the positive controls such as Vitamin C (antioxidant drug), chloramphenicol (antibiotic) and fluconazole (antifungal drug) with a view to determining if any improvements could be noticed in the targeted biological activities of the synthesized Schiff bases.

II. MATERIALS AND METHODS

2.1 Reagents/chemicals

Both DPPH (2, 2-diphenyl-1-picryl hydrazyl hydrate) and vanillin were sourced from Tianjin Kernel Chemical Reagent Company, China and Sigma Aldrich Chemicals, Germany respectively while chloramphenicol, fluconazole and Vitamin C tablets were obtained from Fidson Healthcare PLC, Nigeria. Reagents namely, acetic acid (glacial), acetone, butyl amine, ethanol, ethyl acetate, 2,4-dinitrophenyl hydrazine, hydrochloric acid, methanol, n-butanol, n-hexane, petroleum-ether and toluene were purchased as AnaLAR Grade Chemicals from British Drug House Chemicals Limited, Poole, England.

2.2 Solubility/Dissolution tests for vanillin

Vanillin (0.03 g) was added to 3 mL of each of the following solvents namely, dilute HCl, distilled water, ethyl acetate, ethanol, petroleum ether, n-hexane, n-butanol and methanol separately and observation was made for complete dissolution (solubility) or otherwise.

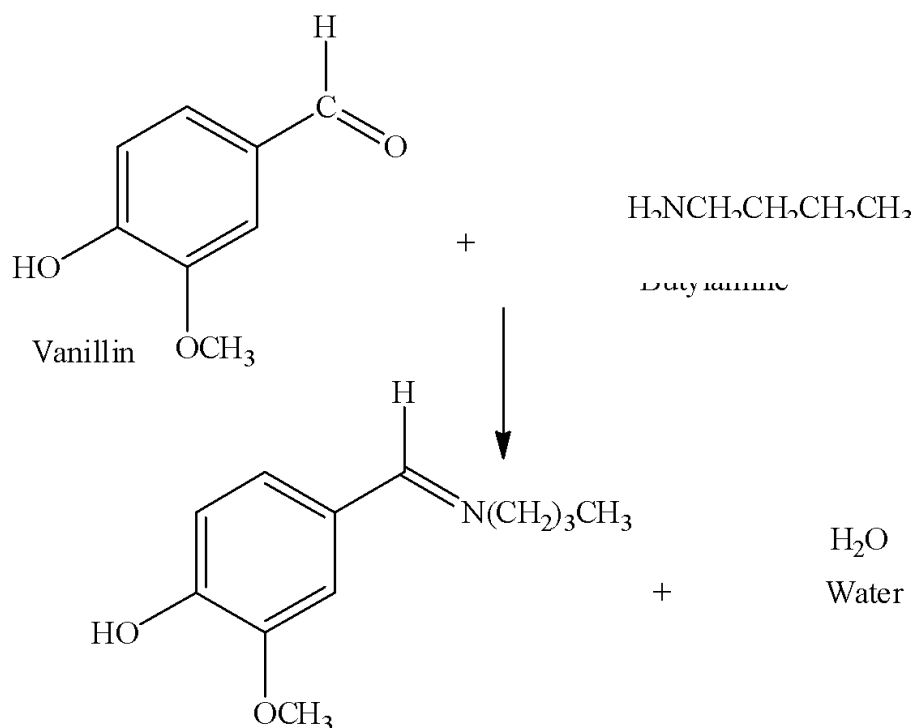
2.3 Determination of melting point

Vanillin (0.04 g) was filled to a quarter of the length of a micro-capillary tube and the melting point determined [14] using an Electro-thermal Melting Point apparatus (Electro-thermal Engineering Limited, England).

2.4 Synthesis of vanillyl butyl imine (Walatimine)

This Schiff base was synthesized as described by [15] with slight modifications. Butyl amine (3 mL) was added to 30 mL of glacial acetic acid to give a mixture. To this mixture was a solution of vanillin (3 g in 10 mL glacial acetic acid) added drop wise whilst stirring and the reaction mixture (green colour) was heated under reflux (glass chamber) for 8 h. At the completion of reaction, the yellow product mixture

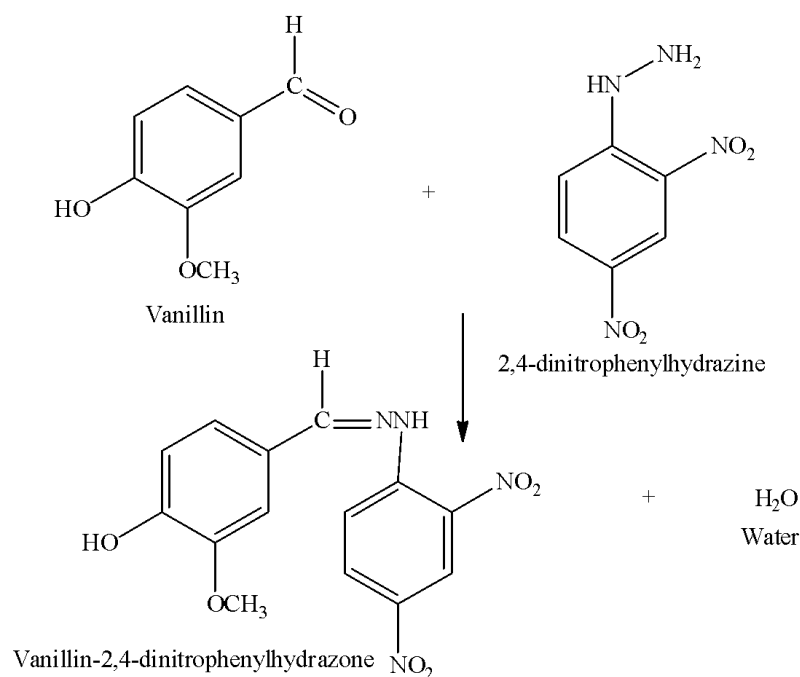
obtained was allowed to cool over crushed ice possibly for crystallization to occur. The mixture in the absence of the formation of crystals was then concentrated down *in vacuo* on a rotary evaporator (920H-Flaswk, Switzerland) at 40 °C to dryness.



W = Vanillyl butyl imine (Walatimine)

Synthesis of vanillin-2,4-dinitrophenyl hydrazone

This Schiff base was synthesized as described by [15] with slight modifications. 2, 4-dinitrophenyl hydrazine (2 g) was dissolved in 30 mL of glacial acetic acid to give a mixture. To this mixture was a solution of vanillin (3 g in 10 mL glacial acetic acid) added drop wise whilst stirring and the reaction mixture was heated under reflux for 8 h. At the completion of reaction, the pale brown-product mixture obtained was allowed to cool over crushed ice possibly for crystallization to occur. The mixture in the absence of the formation of crystals was then concentrated down *in vacuo* on a rotary evaporator (920H-Flaswk, Switzerland) at 40 °C to dryness.



Determination of optical rotation and refractive indices of vanillin and the synthesized vanillin Schiff bases.

This is done by using a polarimeter (ADP-220, Bellingham Stanley, England) and a refractometer (WAY-15, Abbe, England). Each sample (0.05 g) was dissolved in methanol (10 mL). The tube of the polarimeter was filled with distilled water and the machine subsequently zeroed. The tube was then refilled with 5 mL of sample and the optical rotation was measured at the wavelength (λ) of sodium D line (589.3nm) at 20.5 °C. Similarly, the refractive index of sample was obtained on a refractometer at the wavelength (λ) of sodium D line (589.3 nm) at 20.5 °C [16] [17].

2.5 Antioxidant activity

Spectrophotometric determination of antioxidant activity using DPPH reagent. Substances which are capable of donating electrons or hydrogen atoms can convert the purple-coloured DPPH radical (2, 2-diphenyl-1-picrylhydrazyl hydrate) to its yellow-coloured nonradical form; 1, 1-diphenyl-2-picryl hydrazine [18][19]. This reaction can be monitored by spectrophotometry.

2.6 Preparation of calibration curve for DPPH reagent

This experiment was carried out as described by both [5] [20] with some modifications. DPPH (4 mg) was weighed and dissolved in methanol (100 mL) to produce the stock solution (0.004 % w/v). Serial dilutions of the stock solution were then carried out to obtain the following concentrations *viz*, 0.0004, 0.0008, 0.0012, 0.0016, 0.0020, 0.0024, 0.0028, 0.0032 and 0.0036 % w/v. The absorbance of each of the sample was taken at λ_m 517 nm using the Ultra-Violet Spectrophotometer (Jenway 6405, USA). This machine was zeroed after an absorbance had been taken with a solution of methanol without DPPH which served as the blank. Determination of the antioxidant activity of vanillin, synthesized Schiff bases and Vitamin C 2 mg of sample was mixed with 50 mL of methanol. Serial dilutions were carried out to obtain the following concentrations; 0.0004 mg mL⁻¹, 0.0008 mg mL⁻¹, 0.0012 mg mL⁻¹, 0.0016 mg mL⁻¹ and 0.0020 mg mL⁻¹ using methanol. 5 mL of each concentration was incubated with 5 mL of 0.004 % w/v methanolic DPPH solution for optimal analytical accuracy. After an incubation period of 30 minutes in the dark at room temperature (25 ± 2 °C), observation was made for a change in the colour of the mixture from purple to yellow. The absorbance of each of the samples was then

taken at λ_m 517 nm. The Radical Scavenging Activity (RSA %) or Percentage Inhibition (PI %) of free radical DPPH was thus calculated:

$$RSA \% (PI \%) = [(A_{\text{blank}} - A_{\text{sample}}) / A_{\text{blank}}] \times 100$$

A_{blank} is the absorbance of the control reaction (DPPH solution without the test sample and A_{sample} is the absorbance of DPPH incubated with the sample. Vanillin /synthesized Schiff base / Vitamin C concentration providing 50 % inhibition (IC_{50}) was calculated from a graph of inhibition percentage against the concentration of the vanillin/ synthesized Schiff base /Vitamin C [21][22][23]. Vitamin C was used as a standard antioxidant drug.

2.7 Antimicrobial Tests

The micro-organisms used in this study, namely; *Bacillus subtilis* (NCTC 8432), *Staphylococcus aureus* (NCTC 4532), *Escherichia coli* (NCTC 1065), *Pseudomonas aeruginosa* (ATCC 4675) and *Candida albicans* (NCYC 2436) were clinically isolated from specimens of diarrheal stool, abscesses, necrotizing fasciitis, osteomyelitis, urine, wounds and vaginal swabs obtained from the Medical Laboratory, University of Uyo Health Centre, Uyo.

The clinical isolates were collected in sterile bottles, identified and typed by convectional biochemical tests [24][25] and then refrigerated at $-5\text{ }^{\circ}\text{C}$ at the Microbiology and Parasitology Unit, Faculty of Pharmacy prior to use. The hole-in-plate agar diffusion method was used observing standard procedures for bacterium and fungus respectively. The inoculum of each micro-organism was introduced into each petri-dish (Pyrex, England). Cylindrical plugs were removed from the agar plates by means of a sterile cork borer (Pyrex, England) to produce wells with diameter of approximately 5.00 mm. The wells were equidistant from each other and the edge of the plate [26][27]. Concentrations of 20 mg mL⁻¹ of vanillin, 10 mg mL⁻¹ and 20 mg mL⁻¹ of synthesized Schiff bases were introduced into the wells. Also, different concentrations of 5 $\mu\text{g mL}^{-1}$ chloramphenicol (Fidson Healthcare Chemicals, Nigeria), 1mg mL⁻¹ of fluconazole (Fidson Healthcare Chemicals, Nigeria) and aqueous methanol (1:1) were introduced into separate wells as positive and negative controls respectively [28][29][30][31]. The experiments were carried out in triplicates. The plates were left at room temperature for 2 h to allow for diffusion. The plates were then incubated at $37 \pm 2\text{ }^{\circ}\text{C}$ for 24 h. Zones of inhibition were measured in millimetre (mm).

2.8 Thin-layer chromatography of vanillin and synthesized Schiff bases

A portion of each solid sample (0.03 g) dissolved in methanol (2 mL) was applied on a 20 cm x 10 cm silica gel analytical plate (Merck, Germany) and then developed in a toluene : acetone : water (10:20:1) mixture in a chromatographic tank until optimal separation was observed [5].

The retardation factor (R_f) was then computed thus:

$$R_f = \frac{\text{distance moved by spot}}{\text{distance moved by solvent front}}$$

2.9 Infra-red spectroscopy of vanillin and synthesized Schiff bases

Each sample (0.03 g) was analyzed for IR characteristics using the FTIR 84005 Spectrophotometer (Shimadzu, Japan). Ultra-violet/visible spectroscopy of vanillin and synthesized Schiff bases A portion of each sample (0.03 g) was analyzed for UV/VS absorption characteristics using the Jenway 6405 UV/VS Spectrophotometer.

III. RESULTS

Table 1: Preparation of calibration curve for DPPH reagent at λ_{\max} 517 nm

Concentration	Average (± 0.004)	absorbance
0.0004	0.069	
0.0008	0.169	
0.0012	0.237	
0.0016	0.321	
0.0020	0.391	
0.0024	0.445	
0.0028	0.537	
0.0032	0.652	
0.0036	0.703	

Blank Absorbance of 0.004%w/v DPPH reagent: (0.911)

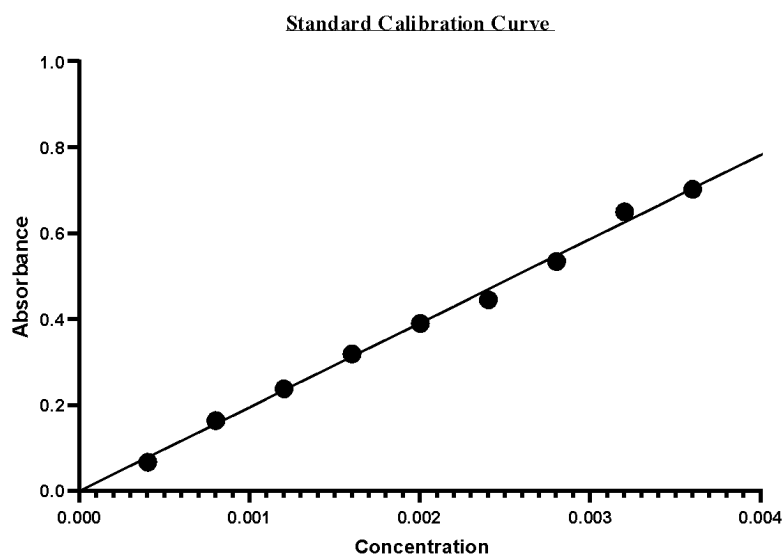


Figure: Graph of absorbance against concentration of methanolic solution of DPPH reagent

Table 2: Absorbance of samples incubated with DPPH at different concentrations at λ_{\max} 517 nm
(Blank absorbance of 0.004% DPPH reagent: 0.911) (± 0.004)

Sample	0.0008 mg mL ⁻¹	0.0016 mgmL ⁻¹	0.0024 mgmL ⁻¹
Vitamin C	0.066	0.064	0.062
Vanillin	0.272	0.270	0.269
W	0.251	0.230	0.164
Vdnp	0.082	0.073	0.071

Key: W = Vanillyl butyl imine (Walatimine)
Vdnp = Vanillin-2, 4-dinitrophenyl hydrazone
DPPH = 2, 2-Diphenyl-1-picrylhydrazyl hydrate

Table 3: Radical scavenging activity (percentage inhibition %) of samples at different concentrations and IC₅₀ of samples (±0.02)

Sample	0.0008 mgmL ⁻¹	0.0016 mgmL ⁻¹	0.0024 mgmL ⁻¹	IC ₅₀ (µgmL ⁻¹)
Vitamin C	92.65	92.76	92.86	0.46
Vanillin	70.14	70.36	70.47	0.52
W	72.23	74.25	82.11	0.50
Vdnp	91.20	91.78	91.99	0.48

Key: Refer to Table 2,

RSA % (PI %) = Radical Scavenging Activity

(Percentage Inhibition %)

IC₅₀ = Concentration at which 50 % of DPPH is scavenged or inhibited

Table 4: Antibacterial sensitivity screening of vanillin and synthesized Schiff bases at different concentrations on test microbes in MeOH/ H₂O(1:1) (± 0.01 mm)

Test microbe	vanillin 20 mg L ⁻¹	W 10 mg mL ⁻¹	W 20 mg L ⁻¹	Vdnp 10 mg mL ⁻¹	Vdnp 20 mg mL ⁻¹	Chloramphenicol 5 µg mL ⁻¹	MeOH/ H ₂ O (1:1)
<i>B. subtilis</i> (NCTC 8432)	32.21	16.23	30.16	21.26	38.31	46.01	5.00
<i>S. aureus</i> NCTC 4532)	29.23	15.11	32.19	25.32	35.22	45.23	5.00
<i>E. coli</i> (NCTC 1065)	30.65	18.20	29.12	26.22	37.21	47.45	5.00
<i>Ps. aeruginosa</i> (ATCC 4675)	27.24	19.45	30.64	5.21	5.10	26.12	5.00

Key: The zone diameter recorded is zone of inhibition + size of cup (zone of inhibition +5.00) mm Refer to Table 2.

NCTC - National Collection of Type Cultures, Central Public Health Laboratory, Colindale Avenue, London NW9, UK.

ATCC- American Type Culture Collection, Washington, DC.+

Table 5: Antifungal sensitivity testing of vanillin and synthesized Schiff bases at different concentrations on test microbes in MeOH/H₂O(1:1) (± 0.01 mm)

Test microbe	vanillin 20mg L ⁻¹	W 10 mg mL ⁻¹	W 20 mg L ⁻¹	Vdnp 10 mg mL ⁻¹	Vdnp 20 mg mL ⁻¹	Fluconazole 1 mg mL ⁻¹	MeOH/ H ₂ O (1:1)
<i>C. albicans</i> (NCYC 2436)	22.21	15.32	28.23	14.12	24.34	43.54	5.00

Key: The zone diameter recorded is zone of inhibition + size of cup (zone of inhibition +5.00) mm Refer to Table 2.

NCYC- National Collection of Yeast Cultures, UK.

Vanillin: $C_8 H_8 O_3$; mol. wt. (152.15 g/mol); white crystals (solid); m.pt. (81-83 °C); $[n]_D^{20}$ (1.5776); $[\alpha]_D^{20}$ (0 °); λ_{max} (246 nm); R_F (0.64); FTIR (cm^{-1}):1145 (-C-O-C), 1587 (-Ar-C=C), 1664 (-C=O), 2917 (-CH stretching) and 3289 (-Ar-OH).

Vanillyl butyl imine (Walatimine) W: $C_{12} H_{17} NO_2$; mol. wt. (207.08 g/mol); yellow compound; $[n]_D^{20}$ (1.5880); $[\alpha]_D^{20}$ (0 °), λ_{max} (412 nm); R_F (0.81); FTIR (cm^{-1}): 875 and 921 (finger print region, alkyl bending mode), 1145 (-C-O-C), 1583 (Ar-C=C), 1691 (-HC=N, of imine), 2917 (-CH stretching) and 3288 (-Ar-OH). Vanillin-2,4-dinitrophenyl hydrazone (Vdnp): $C_{14} H_{12} N_4 O_6$; mol. wt. (332.27 g/mol); pale brown resin; $[n]_D^{20}$ (1.5984); $[\alpha]_D^{20}$ (0 °); λ_{max} (308 nm); R_F (0.60); FTIR (cm^{-1}): 1145 (-C-O-C), 1355 (-O₂N group), 1567 and 1601 (-Ar-C=C), 2917 and 2918 (-CH stretching) and 3291 (-Ar-OH).

IV. DISCUSSION

4.1 Spectroscopic analyses

Vanillin is a white crystalline compound with pleasant and balsamic fragrance. Some monographic determinations were done in this study where the identity, purity, integrity and suitability substance were established. The compound was observed to be soluble in ethanol, ethyl acetate, n-butanol, n-hexane, methanol and water when heated. However, it was insoluble in petroleum ether, dilute hydrochloric acid and dilute sulphuric acid. The observations and the determined melting point and refractive index values are consistent with those in literature and those obtained in an earlier study [5]. The UV absorption characteristic at λ_{max} (246 nm) indicates the presence of electron clouds over -Ar-C=C, -OH, -OCH₃ and -HC=O pharmacophores while retardation factor R_F (0.64) shows that vanillin is moderately polar and likewise retarded on the silica gel. The IR spectrum of vanillin shows absorptions at 1145, 1587, 1664, 2917 and 3289 cm^{-1} which are diagnostically characteristic of -C-O-C, -Ar-C=C, -C=O, -CH and -Ar-OH groups respectively. The condensation reaction between vanillin and butyl amine led to the synthesis of a yellow compound which has been identified by as vanillyl butyl imine by a combination of physico-chemical determinations and the IR spectral technique and hereby coded as W. It has a slightly pleasant flavour. Comprehensive data-based library searches of organic compounds were done and hence it is safe to infer that the compound is novel and hereby referred to by a trivial name Walatimine. It is a Schiff base which belongs to the class of compounds known as ketimines or aldimines. These compounds are obtained by condensation reactions between amines and carbonyl moieties such as ketones or aldehydes in the presence of an acid or base and under heat. The UV absorption at λ_{max} (412 nm) which is comparably higher than that of the vanillin shows the presence of electrons de-localized over -Ar-C=C, -OCH₃, -HC=N (imine) and -Ar-OH chemical species while the retardation factor R_F (0.81) shows that this Schiff base is comparably non-polar on account of the butyl group $(-CH_2)_3CH_3$ which makes the compound more lyphophilic and hence weakly retarded on an apparently hydrophobic silica gel plate. The IR spectral matrix of W shows stretchings at 875 and 921, 1145, 1583, 1691, 2917 and 3288 cm^{-1} which are characteristically diagnostic of alkyl bending modes (finger print region), -C-O-C (ether linkage), -Ar-C=C, -HC=N (imine), -CH and -Ar-OH respectively. Also, in a somewhat similar fashion, the condensation reaction between vanillin and 2,4-dinitro phenyl hydrazine resulted in a pale brown product which has been identified to be vanillin-2,4-dinitro phenyl hydrazone (Vdnp) using similar techniques as mentioned above. This Schiff base belongs to the class of compounds referred to as hydrazones or aziridines. The UV absorption at λ_{max} (308 nm) is higher than that of vanillin at 246 nm but less than that of W at 412 nm. However, the electron densities are found over -O₂N, -Ar-C=C, -OCH₃, -HC=N and -Ar-OH chemical species inherent in the compound while the retardation factor R_F (0.60) is indicative of this compound being more adsorbed onto the silica gel than the vanillin at 0.64. This observation is not surprising because the two nitro groups (on the second benzene ring) and -OH (on the first benzene ring) both contribute to making the synthesized hydrazone more polar hence, more greatly retarded on the silica gel analytical plate. Furthermore, the IR spectral

matrix of vanillin-2,4-dinitro phenyl hydrazone (Vdnp) shows absorptions at 1145, 1355, 1587, 1601, 2917, 2918 and 3291 cm^{-1} which correspond to the presence of -C-O-C (ether linkage), -O₂N (nitro group), -Ar-C=C, -CH bending modes and -Ar-OH chemical entities respectively. Moreover, the presence of the two O₂N (nitro group) being electron withdrawing chemical entities will predispose the characteristic -Ar-C=C and -CH absorptions to higher IR peaks (especially in the second benzene ring) as can be seen in the IR spectrum of the synthesized hydrazone. The determinations of physical parameters are important in identifying compounds. Physical constants such as refractive index and optical rotation are used in the qualitative and quantitative analyses of substances. Also, these parameters are employed to confirm the purity, identity, integrity of active substances and as well as monitor the progress of reactions. In this study, both physical parameters were measured at the wavelength (λ) of Na-D light (589.3 nm) and a temperature of 20.5 °C. In addition, the refractive index of a substance is an indication of the number, type of atoms and chemical groups (species) in the substance. Each atom or group in the substance contributes to its refractivity which adds eventually to the refractive index of the substance. Furthermore, refractive index can be used to monitor the progress of chromatographic separation by measuring the refractive indices of the effluent solvents employed. The pro-drug, vanillin and both synthesized Schiff bases gave refractive indices of 1.5776, 1.5880 and 1.5984 respectively. Furthermore, it was observed that all the compounds demonstrated optical rotation $[\alpha]_D^{20}$ of 0 ° implying that the compounds do not have chiral centers hence, are optically inactive. In addition, none of these compounds will demonstrate laevorotation (-) (ability of a compound to rotate plane of light in anticlockwise direction) or dextro-rotation (+) (ability of a compound to rotate plane of light in clockwise direction)[16][17].

4.2 Antioxidant assays

In absorption spectrophotometry, it is germane that a calibration curve be prepared for the reagent to be employed in the assays. Hence, this was done for DPPH (2, 2-diphenyl-1-picryl hydrazyl hydrate) reagent with the aim of ascertaining its purity and suitability for use in the antioxidant determinations. The Beer-Lambert's Law is for such determinations [19][32].

The calibration curve obtained indicates that the underlying principles behind the aforementioned Law were fulfilled as the curve (Figure) shows a straight line which passes through the origin. The reduction of the DPPH radical was determined by taking its absorption at a wavelength of λ_m 517 nm. It was observed that the absorbance of DPPH decreased as the concentration of added free radical scavenger (vanillin /Schiff base/Vitamin C) increased which suggested that the DPPH reagent was being reduced (Table 2), On the other hand table 3 shows radical scavenging activity (RSA %) or percentage inhibition (PI %) and the computed IC₅₀ values of vanillin /Schiff base / Vitamin C. The RSA % is an indicator or a measure of the antioxidant activity of vanillin / Schiff base / Vitamin C. Interestingly, vanillin, and vanillyl butyl imine (W) both demonstrated moderate antioxidant activity (IC₅₀) of 0.52 and 0.50 $\mu\text{g mL}^{-1}$ respectively. However, W being more lyphophilic because of the presence of the butyl group -(CH₂)₃ CH₃ has a slightly better activity than the vanillin because the 4-carbon aliphatic long chain will ensure that the compound gets to the active or allosteric sites relatively faster where the pharmacological action of anti-oxidation is effectuated. Vanillin-2,4-dinitro phenyl hydrazone (Vdnp) showed a significantly remarkable antioxidant activity at 0.48 $\mu\text{g mL}^{-1}$ which compares favourably with that of Vitamin C (a standard antioxidant drug) at 0.46 $\mu\text{g mL}^{-1}$ It could be inferred that the product (Vdnp) of condensation reaction between vanillin and an aromatic amine such as 2,4-dintro phenyl hydrazine slightly enhances the antioxidant activity of vanillin than that it forms with a straight chain aliphatic amine such as butyl amine. Vanillin like most low molecular weight phenolic compounds possess weak or in some instances moderate antioxidant properties [5]. This sweet smelling fragrant compound also has proven to demonstrate anticancer, anti-inflammatory and neurone-protective activities [33]. Hydrazones have been reported to possess anti-infective activity [34]. Besides the DPPH

test for determining the antioxidant activity of compounds include the hydrogen peroxide, nitric oxide, conjugated diene, superoxide, phosphomolybdenum, peroxyxynitrile and xanthine oxidase assay methods amongst many others [35][36].

4.3 Antibacterial sensitivity tests

The microbes employed in the sensitivity tests reflected the antibacterial spectrum encompassing two (2) gram positive bacteria namely, *B. subtilis* and *S. aureus* and two (2) gram negative bacterial species namely, *E. coli* and *Ps. aeruginosa*. The results displayed on the Table 4 show that vanillin was remarkably suppressive or bacteriostatic of all the four microbes at 20 mg m L⁻¹ while the antibacterial activities recorded by both W (vanillyl butyl imine) and Vdnp (vanillin-2,4-dinitro phenyl hydrazone) were concentration-dependent at 10 and 20 mg m L⁻¹ respectively as reflected on Table 4. The higher the concentration, the better the activity.

Generally, both bases demonstrated good antibacterial activities as can be seen on Table 4. However, W was noticeably and remarkably suppressive of *Ps. aeruginosa* at both concentrations while Vdnp was inactive. This observation was surprising because *Ps. aeruginosa*, a gram negative bacterium is well known for its unique resistance to antibacterial agents. This resistance is believed to be due to the nature of the cell envelope of the organism which unlike gram positive organisms possess a sophisticated three-layered envelope which does not allow the permeation of external agents [37]. It can be inferred from these observations that both bases could be promising lead compounds in the search for newer antibacterial agents for treatment and management of bacterial infections.

4.4 Antifungal activity

The antifungal screening was done with *C. albicans* and generally the three compounds displayed somewhat remarkable anti-candidal activities at the concentrations tested. Vanillin was tested at 20 mg m L⁻¹ while W and Vdnp were screened at both 10 and 20 mg m L⁻¹ respectively. Similarly, the antifungal activity demonstrated by the two Schiff bases are concentration-dependent as can be seen on Table 5. However, it is noted that W was slightly more anti-candidal than Vdnp.

V. CONCLUSION

This study reports for the first time the synthesis of vanillyl butyl imine (W), a new ketimine from Schiff base synthesis. It has been given the trivial name Walatimine. This compound demonstrated a moderate antioxidant activity (IC₅₀) of 0.50 as vanillin at 0.52 µg mL⁻¹.

However, vanillin-2,4-dinitro phenyl hydrazone (Vdnp) gave a significant antioxidant activity (IC₅₀) of 0.48 µg mL⁻¹ which compare favourably with that shown by Vitamin C at 0.46 µg mL⁻¹. Vanillyl butyl imine (W) demonstrated remarkable antibacterial activities against the bacteria tested including *Ps. aeruginosa*, a organism noted for its resistance to anti-infective agents. Also it was slightly more anti-candidal than vanillin-2,4-dinitro phenyl hydrazone (Vdnp). It is noteworthy that both W and Vdnp could be lead compounds in the search for newer antioxidant and antimicrobial agents and in further structural activity relationship studies (SARS) and as well as in formulation studies in drug development.

Consent for publication

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None

Conflict of interest

The authors declare no conflict of interest financial or otherwise.

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