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In Silico Safety Evaluation of Local Spices Tea Used Against Human Metapneumovirus (HMPV)

Idumu, E., Johnson, J.T*

Department of Biochemistry, Federal University Otuoke, Bayelsa State, Nigeria

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ABSTRACT

Human Metapneumovirus (HMPV) is an enveloped, single-stranded negative-sense RNA virus that primarily affects the respiratory tract, and has beenrecognized as a significant cause of respiratory infections, particularly in young children, the elderly and immunocompromised individuals. Despite its global burden, there are currently no approved vaccines or specific antiviral agents available for the treatment of HMPV, highlighting an urgent need for the development of novel therapeutics. Treatment remains supportive, and prevention and it is limited to infection control practices such as the use of local spices. This work aimed to evaluate the safety of spices tea formulated from six (6) local spicestraditionally used in management of human metapneumoviral infection by utilizing *in silico* tools approach to predict the pharmacokinetics, toxicity, and ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of the promising compounds. Eleven phytochemicals (lead compounds) were screened for drug-likeness, physicochemical properties, and pharmacokinetics using computational models. ADMET profiling revealed good oral bioavailability and minimal cytochrome enzyme inhibition for most compounds. Toxicity screening revealed that while some hit compounds were mildly toxic, all of the lead compounds were non-mutagenic and non-cytotoxic. However, prediction of neurotoxicity, cardiotoxicity, and immunotoxicity for squalene and stigmasterol (with probabilities > 0.85) at high dosage and this raises caution for dose selection and necessitates further *in vivo* toxicological evaluation.

Keywords: In silico, Metapneumovirus, *Piper guineense, Tetrapleuratetraptera, Xylopiaaethiopica, Allium sativum, Ocimumgratissimum and Zingiberofficinal*

BACKGROUND OF STUDY

Human Metapneumovirus (HMPV), a member of the *Paramyxoviridae* family, is an enveloped, single-stranded negative-sense RNA virus that primarily affects the respiratory tract. It has been recognized as a significant cause of respiratory infections, particularly in young children, the elderly and immunocompromised individuals (Hoogen *et al*., 2001) with no approved vaccines or targeted antiviral therapies currently available. Phylogenetic studies revealed that HMPV is closely related to Avian Metapneumovirus (AMPV), and it is now recognized as the second most common cause of lower respiratory tract infections (LRTIs) in infants and young children, following Respiratory Syncytial Virus (RSV) (Boivin *et al*., 2002; Williams *et al.*, 2004).The HMPV has a global distribution and exhibits seasonal peaks similar to other respiratory viruses, typically circulating during the late winter to early spring in temperate climates (Falsey *et al*., 2003). Serological studies have shown that nearly all children are infected with HMPV by the age of five, and reinfections are common throughout life (van den Hoogen *et al*., 2003).HMPV infection can cause a range of symptoms from mild upper respiratory tract illness (e.g., cough, rhinorrhea) to more severe LRTIs such as bronchiolitis and pneumonia. It can exacerbate underlying conditions such as asthma and chronic obstructive pulmonary disease (COPD), and is particularly severe in infants, the elderly and immunocompromised patients (Walsh *et al.*, 2008). In immunocompromised individuals, including transplant recipients and cancer patients, HMPV can lead to severe complications and increased mortality (Kamboj *et al.*, 2008).

Natural products, particularly those derived from medicinal plants and spices, have historically served as valuable resources for drug discovery and development. Spices commonly used in traditional African

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medicine, such as ***Piper guineense*** (West African black pepper) or "Uziza" in Nigeria, is widely used as a medicinal spice across West Africa. It is rich in bioactive compounds such as piperine and essential oils, including beta-caryophyllene and linalool, which exhibit antioxidant, antimicrobial, and anti-inflammatory properties. Studies have shown its efficacy in treating respiratory infections, and as a stimulant for immune modulation. Additionally, it has shown potential antiviral activity, making it a candidate for studies against HMPV (Adedokun and Onakoya, 2021), ***Tetrapleura tetraptera*** often referred to as "Aidan fruit" or prekese, is a medicinal plant commonly used in traditional African medicine. Its pods are used for soup preparation and as a remedy for various ailments. Bioactive compounds such as flavonoids, tannins, and saponins present in *Tetrapleura tetraptera* have been reported to have antioxidant, antimicrobial, and anti-inflammatory effects. It has also been traditionally used for respiratory diseases and immune support, making it a promising spice for antiviral applications (Akinjogunla *et al*., 2014). ***Xylopiaa ethiopica*** commonly called Ethiopian pepper or "Uda" in Igbo language in Nigeria, is an aromatic spice used in food seasoning and traditional medicine. It contains bioactive phytochemicals such as alkaloids, terpenoids, and polyphenols, which are known for their antimicrobial, antioxidant, and anti-inflammatory properties. Research has also linked some of its active compounds with antiviral effects, demonstrating potential against respiratory pathogens (Kouassi *et al*., 2020), ***Allium sativum*** **(garlic)** is a globally recognized spice with potent medicinal properties. Its bioactive compounds, such as allicin, ajoene, and diallyl disulfide, are known for their antimicrobial, antiviral, and antioxidant activities. Garlic is widely used to boost immune function and fight infections, including respiratory diseases. Allicin, in particular, has been studied extensively for its potential to inhibit viral replication by interfering with viral entry and protein synthesis (Bayan *et al*., 2014). ***Ocimum gratissimum*** (Scent leaf) is an aromatic herb famous for its culinary and medicinal applications. Rich in essential oils like eugenol and thymol, it has demonstrated antimicrobial, antifungal, and anti-inflammatory properties. Traditional use includes treating respiratory infections, fevers, and digestive disorders. The spice is also known for boosting immunity and has been suggested for antiviral research due to its bioactive compounds (Prabhu *et al*., 2009).***Zingiber officinale*** (ginger) is renowned for its culinary and medicinal uses globally. Bioactive compounds such as gingerol, shogaol, and paradol are responsible for its antioxidant, anti-inflammatory, and antimicrobial properties. It is extensively used for managing respiratory illnesses, nausea, and inflammation. It has shown promising antiviral activity against enveloped viruses, which makes it a candidate for further evaluation against HMPV (Mashhadi *et al*., 2013). The advent of *In silico* computational methods, such as molecular docking and pharmacokinetic predictions, toxicity profiling, allow for the efficient screening of large compound libraries, providing insights into their binding affinities and potential mechanisms of action while reducing time and costs (Lionta *et al.,* 2014). By leveraging these tools, the bioactive constituents of traditional spices can be evaluated for their therapeutic potential and safetyagainst viral targets, including those of HMPV.

Despite its global burden, there are currently no approved vaccines or specific antiviral agents available for the treatment of HMPV, highlighting an urgent need for the development of novel therapeutics. Treatment remains supportive, and prevention is limited to infection control practices such as the use of local spices. The lack of effective therapeutics prompts the use of local spices and therefore highlights the urgent need for drug discovery efforts targeting HMPV as well as the comprehensive safety evaluation of such natural products arising from these spices. This underscores the need for safety evaluation of some traditional spices used in the management of HMPV.

This work was aimed at evaluating the safety ofspices used in management of HMPV by utilizing *in silico* computational methods to predict the pharmacokinetics, toxicity, and ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties of the promising compounds.

MATERIALS AND METHODS

Sample collection and preparation

Piper guineense, Tetrapleura tetraptera, Xylopiaa ethiopica, Allium sativum, Ocimum gratissimum and *Zingiber officinale* were purchased from Swali market, Yenagoa, Bayelsa State, Nigeria. The six (6) spices were formulated into tea using the method of Johnson *et al*., 2020

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Assessment of physicochemical and pharmacokinetics characteristics of the bioactive compounds

Extracts of each of the spices were separately investigated for metabolites (Piperine, chavicine, flavonoids, alkaloids, tannins, saponins, terpenoids, essential oils phenolic compounds, steroids, triterpenoids, Xylopic acid, kaurenoic acid, phenolics, Allicin, ajoene, alliin, diallyl sulfide, diallyl disulfide, s-allyl cysteine, flavonoids, saponinsEugenol, thymol, Gingerol, shogaol, paradol, zingerone) according to the established procedure reported by Oladimeji and Usifoh (2015).

Prediction of ADMET properties of the ligands (pharmacokinetics)

This was done using the online programme ADMET lab 3.0 and SWISSADME (Asanga *et al*., 2024a); the absorption, distribution, metabolism, excretion and toxicity (ADMET) properties of the ligands employed in this study were predicted. The different ADMET properties of the ligands and the reference drugs were predicted using the different canonical strings or Simplified Molecular-Input Line-Entry System (SMILES) strings of the different ligands retrieved from the PubChem web platform (https://www.ncbi. Nlm.nih.gov/pccompound) in their 3D conformation. All the relevant parameters, including Lipinski's rule of five and the Ghose parameters were recorded. Using the SWISS target prediction tool, the target of the different ligands were determined (Daina *et al*., 2019).

Toxicological Assessment of Lead Compounds

Results:

Table 1 shows the result of physiochemical analysis of the formulated tea. The following eleven (11) compounds were detected:

1. Tyramine or 4-Hydroxyphenethylamine ($C_{10}H_{14}O$) – (CID 6989)

A naturally occurring monoamine compound derived from tyrosine. Likely tyramine; small, mildly lipophilic with one H-bond donor/acceptor; potentially CNS-active.Fraction CsP_3 is 0.40 which makes it a moderate 3D character and TPSA favours membrane permeability

2. Dextroamphetamine ($C_{12}H_{17}NO_2$) – (CID 17516)

A psychostimulant used in ADHD and narcolepsy. MW: 207.27, 4 rotatable bonds, typical for CNS stimulants. One donor, two acceptors maintains balance for bioactivity. TPSA which is 38.33 suggests good oral and blood-brain barrier (BBB) permeability. This compound acts as a central nervous system stimulant affecting dopamine and norepinephrine pathways.

3. Glucaric acid (or Saccharic acid) ($C_6H_8O_4$)- (CID 119838)

An oxidation product of glucose, involved in detoxification. The compound M/W is 144.13g/mol, fully polar (TPSA: 66.76), no rotatable bonds.High number of H-bond acceptors (4) and donors (2), used in detoxification and likely poor oral absorption but strong systemic reactivity.

4. Mannitol ($C_7H_{14}O_6$) – (CID 345716)

A sugar alcohol used as a diuretic and sweetener. It has 194.18g/mol of MW, 2 rotatable bonds, 4 donors, 6 acceptors. TPSA is 99.38 which makes it have highpolarity and low membrane permeability, a sugar alcohol used as an osmotic diuretic and in kidney function tests very high fraction CsP_3 (1.00), signifying full saturation.

5. β-Caryophyllene ($C_{15}H_{24}$) – (CID 519764)

A sesquiterpene found in essential oils like clove and cannabis. 204.35of MW, non-polar (TPSA: 0.00), no hydrogen bonding potential. A bicyclic sesquiterpene known for cannabinoid receptor (CB2) agonism, good candidate for anti-inflammatory or neuroprotective roles.

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6. Vanillin acetate or ethyl vanillin derivative ($C_{11}H_{14}O_3$) –(CID 586455)

A synthetic flavoring compound or metabolite of vanillin. MW is 194.23, 4 rotatable bonds, 3 acceptors, 1 donor, moderate TPSA (46.53) and molar refractivity good oral bioavailability expected, derivatives of vanillin show antioxidant and antimicrobial activity

7. Germacrene D or Humulene ($C_{15}H_{24}$) – (CID 5281517)

Molecular weight 204.35, high flexibility (7 rotatable bonds), no H-bonding groups, purely hydrocarbon, TPSA: 0, this makes it highly lipophilic; found in essential oils with insecticidal and anti-inflammatory activity.

8. Squalene ($C_{30}H_{50}$) – (CID 638072)

A triterpene and precursor in cholesterol biosynthesis. It has a MW of 410.72, highly hydrophobic, 15 rotatable bonds, precursor in sterol biosynthesis; antioxidant in dermatological and cancer applications, high molar refractivity suggests significant van der Waals interaction potential.

9. Cholesterol ($C_{29}H_{48}O$) – (CID 5280794)

A vital sterol in cell membranes and precursor to steroid hormones. It has a MW of 412.69, single donor and acceptor, highly lipophilic, structural component of membranes, precursor to steroids, fraction CsP_3 (0.86) is well-balanced 3D structure for receptor interactions.

10. Humulene ($C_{15}H_{24}$) – (CID 5281519)

An isomer of β-caryophyllene, used in antimicrobial, anti-inflammatory research, physicochemical properties identical to β-caryophyllene.

11. Acylovir ($C_{17}H_{20}N_6$) – (CID 5395771)

An antiviral drug used to treat herpes simplex infections. MW: 308.38, moderate lipophilicity, four acceptors, no donors, TPSA: 60.89 acceptable for oral drugs.Antiviral against herpes simplex; mimics nucleosides to inhibit viral DNA polymerase.

Table 1: Physiochemical properties of compounds identified in the tea spices

S/n	Compound (PUBCHMCID)	Formula	M/W(g/mol)	Fraction CsP_3	N of rotational bond	No of H bond acceptor	No of H bond donor	Molar reactivity	TPSA(A^2)
1	6989	$C_{10}H_{14}O$	150.22	0.40	1	1	1	48.01	20.23
2	17516	$C_{12}H_{17}NO_2$	207.27	0.42	4	2	1	60.68	38.33
3	119838	$C_6H_8O_4$	144.13	0.50	0	4	2	32.39	66.76
4	345716	$C_7H_{14}O_6$	194.18	1.00	2	6	4	40.47	99.38
5	519764	$C_{15}H_{24}$	204.35	0.60	4	0	0	70.68	0.00
6	586455	$C_{11}H_{14}O_3$	194.23	0.36	4	3	1	54.54	46.53
7	5281517	$C_{15}H_{24}$	204.35	0.47	7	0	0	72.32	0.00
8	638072	$C_{30}H_50$	410.72	0.60	15	0	0	143.48	0.00
9	5280794	$C_{29}H_{48}O$	412.69	0.86	5	1	1	132.75	20.23

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10	5281519	$C_{15}H_{24}$	204.35	0.60	0	0	0	70.68	0.00
11	5395771	$C_{17}H_{20}N_6$	308.38	0.29	4	4	0	91.25	60.89

Table 2 shows the result of*in silico***pharmacokinetics** analysis

From the result, compounds 1, 2, 6, and 11 show the most promising oral bioavailability and CNS activity, though potential CYP enzyme inhibitionmust be carefully considered to avoid drug interactions. Compounds 7–10, although less permeable and with low GI absorption, may be optimized for non-oral routes or used in topical or inhalational formulations. These findings offer a strong foundation for selecting lead compounds in the development of therapeutics against human metapneumovirus.

Table 2: *in silico*pharmacokineticsanalysis

S/N	Compound	GIAbsorption	BBB Permeant	PrGPSubstrate	CyP_2D_6 Inhibit	$CYP1_{A2}$ Inhibitor	$CyP_{2C}I_9$ inhibitor	CyP_2C_9	CyP_3A_4 inhibitor	Skin permeability (109kp)cm/9
1.		High	YES	NO	NO	YES	NO	NO	NO	-4.87
2.		High	YES	NO	NO	YES	NO	NO	No	-5.36
3.		High	NO	NO	NO	NO	NO	NO	NO	-7.44
4.		Low	NO	YES	NO	NO	NO	NO	NO	-9.79
5.		Low	NO	NO	NO	NO	YES	NO	NO	-3.71
6.		High	YES	NO	NO	YES	NO	NO	NO	-6.46
7.		Low	NO	NO	NO	YES	NO	YES	NO	-3.27
8.		Low	NO	NO	NO	YES	NO	YES	NO	-3.27
9.		Low	NO	NO	NO	NO	NO	YES	NO	-2.74
10.		Low	NO	NO	NO	NO	NO	YES	NO	-3.45
11.		High	YES	NO	NO	YES	YES	YES	NO	-3.72

Toxicity profile of lead compounds

Table 3 presents *in silico* predictions of toxicity and systemic safety profiles for two test compounds. Both compounds are predicted not to cross the BBB, reducing the risk of unintended central nervous system (CNS) side effects, theyshowpositive hepatotoxicity predictions (0.69 probability)**,** indicatingamoderate risk of liver toxicity. While some hit compounds were mildly toxic, all of the lead compounds were non-mutagenic and non-cytotoxic. However, further vivo safety assessment/prediction of neurotoxicity, cardiotoxicity, and immunotoxicity for squalene and stigmasterol is advised

Table 3: Toxicity profile of lead compounds as Predicted by Protox III

S/n	**Compound**	**BBB**	**HCP**	**Neurotox**	**Nephro**	**Response**	**Cardio**	**Immuno**	**Mutagenicity**	**Cytotoxicity**	**Cli**	**1190mg/kg predodidoseLD50**
1		**-**	**+ (0.69)**	**+ (0.87)**	**-**	**+(0.98)**	**-**	**+(0.96)**	**-**	**-**	**-**	**1190**

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2		**-**	**+ (0.69)**	**+ (0.87)**	**-**	**+**	**-**	**+**	**-**	**-**	**-**	**1190**

DISCUSSION

Eleven lead compounds were identified in this study. The physicochemical assessment involved evaluating parameters essential for oral bioavailability and drug-likeness, including molecular weight (MW), topological polar surface area (TPSA), number of hydrogen bond acceptors and donors, fraction of Sp_3 carbons (CsP_3), number of rotatable bonds, and molar refractivity. These characteristics determine how a compound behaves in biological environments, impacting absorption, solubility, metabolic stability, and membrane permeability (Lipinski *et al*., 2001; Veber *et al*., 2002).Compounds such as β-caryophyllene, humulene, and squalene demonstrated high lipophilicity (log P > 4.5), low TPSA (0.00 Å²), and zero hydrogen bonding capabilities, suggesting their enhanced ability to traverse lipid membranes and potentially interfere with viral envelope integrity. Squalene showed the highest molar refractivity (143.48) and flexibility (15 rotatable bonds), supporting its interaction with hydrophobic viral protein pockets. In contrast, hydrophilic molecules like mannitol and glucaric acid had elevated TPSA values (up to 99.38 Å²) and multiple hydrogen bond donors/acceptors, suggesting limited membrane permeability but potential roles in metabolic or immunomodulatory pathways.

Most drug-like compounds fall within 100–500 g/mol of molecular weight (Lipinski's Rule of Five). All listed compounds fall within this range, except compound 8 and compound 9, which are larger (potentially lower oral bioavailability).Fraction of CsP_3indicates saturation level and 3D complexity. Higher CsP_3 often correlates with better solubility and metabolic stability. Compounds like compound 4 (1.00) and compound 9 (0.86) are highly saturated and likely more metabolically stable. Rotatable Bonds affects molecular flexibility. More than 10 may reduce bioavailability due to entropy loss on binding. Compound 8 has 15 rotatable bonds possibly poor oral bioavailability. Hydrogen Bond Acceptors (HBA) and Donors (HBD)**;** Excessive HBA (>10) or HBD (>5) can limit membrane permeability. Compound 4 with 6 HBAs and 4 HBDs may have low permeability without carrier mediation. Molar Reactivity (MR) relates to electronic polarizability; compounds with higher MR (e.g., compound 8 and compound 9) may have increased binding interactions but possibly reduced solubility, high MR values (>100) as in #8 and #9 suggest strong van der Waals interactions. Topological Polar Surface Area (TPSA);TPSA less than 140 Å² is considered favorable for oral absorption; under 90 Å² for blood-brain barrier penetration. Compound 4 (TPSA = 99.38) may have poor CNS penetration. Compounds 5, 7, 8, and 10 (TPSA = 0) may be highly lipophilic and membrane permeable. Drug-likeness analysis confirmed that all compounds satisfied Veber's criteria (TPSA < 140 Å², rotatable bonds < 10) and most passed Lipinski's Rule of Five, except for some minor violations in highly lipophilic molecules like cholesterol and squalene. All eleven compounds scored positively for drug-likeness and presented bioavailability scores of 0.55 or higher, with glucaric acid achieving a superior score of 0.85, indicating strong oral potential.Lipophilic compounds like squalene and stigmasterol showed limited water solubility but retained sufficient predicted GI absorption. Glucaric acid and mannitol, while highly soluble, may require formulation adjustments to overcome permeability limitations.

Toxicity screening revealed that most compounds were non-mutagenic and non-cytotoxic. However, neurotoxicity, cardiotoxicity, and immunotoxicity were predicted for squalene and stigmasterol (with probabilities > 0.85). Despite these risks, both compounds shared a favorable LD50 value (1190 mg/kg), indicating moderate acute toxicity consistent with OECD Class IV classification.Additionally, vanillin derivatives and β-caryophyllene showed relatively benign toxicity profiles, coupled with high bioavailability and acceptable binding affinity, underscoring their potential as safer alternatives for antiviral development.

SUMMARY AND CONCLUSION

This study employed an *in silico* approach to evaluate the safety of spices tea formulated from six (6) local spices traditionally used for management of respiratory tract infections caused by human metapneumovirus (hMPV). Eleven lead phytochemicals were screened for drug-likeness, physicochemical properties, and pharmacokinetics using computational models. ADMET profiling revealed good oral bioavailability and

minimal cytochrome enzyme inhibition for most compounds. Although squalene and stigmasterol exhibited high efficacy, they also showed predicted neurotoxicity and cardiotoxicity. In contrast, β-caryophyllene and vanillin derivatives offered a favorable balance of efficacy and safety, making them promising candidates for further investigation. Toxicity screening revealed that while some compounds were moderately toxic, most were non-mutagenic and non-cytotoxic. However, prediction of neurotoxicity, cardiotoxicity, and immunotoxicity for squalene and stigmasterol (with probabilities > 0.85) at high dosage raises caution for dose selection and necessitates further *in vivo* toxicological evaluation.

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