Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2017, 9(4):364-370

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Bioinformatics Analysis of Garcinia Kola Active Components and Glycoproteins of Ebola Virus (*Zaire ebolavirus*)

Sanni David*, Fatoki Toluwase and Omotoyinbo Oluwasegun

Enzyme Biotechnology and Bioinformatics Unit, Department of Biochemistry, Federal University of Technology, Akure, Nigeria

---

ABSTRACT

Ebola virus is an aggressive pathogen that causes a highly lethal hemorrhagic fever syndrome in humans and nonhuman primates. The Ebola virus (EBOV) genome is 19 kb long; with seven open reading frames encoding structural proteins and nonstructural proteins, and its glycoprotein (GP) is responsible for binding and viral entry. Garcinia kola which is commonly called bitter kola has been implicated with antiparasitic, antiviral, anti-inflammatory, anti-diabetic, anti-oxidant, and antihepatoxic activities. In this study, selected bioactive components of Garcinia kola were computationally evaluated for therapeutic potential in relevance to Ebola virus disease (EVD) using standard bioinformatics tools such as Blastp, Hmmer, ClustalO, Swisstargetprediction and Swissadme. The result showed that EBOV-GP is a distant homolog of three human endogenous retrovirus proteins (HERV), and the probable targets of G. kola include cannabinoid receptor 1 and 2, matrix metalloproteinases (e.g. Collagenase-3) and cyclin dependent kinases, of which they have been implicated in human pathology such as viral infections and cancer.

Keywords: Ebola virus disease (EVD); Glycoprotein; Garcinia kola; Bioinformatics analysis

---

INTRODUCTION

Ebola virus is an aggressive pathogen that causes a highly lethal hemorrhagic fever syndrome in humans and nonhuman primates. It was first recognized near the Ebola River valley during an outbreak in Zaire in 1976. Ebola virus and the related Marburg virus are members of the Filovirus family, which are pleomorphic, negative-sense RNA viruses whose genome organization is most similar to the *Paramyxoviridae* [1]. The genus Ebola virus is divided into five different species (the Zaire, Sudan, Tai Forest, Bundibugyo, and Reston viruses), which differ in their virulence for humans. Among EBOV species, *Zaire ebolavirus* seems to be the most virulent, with a case fatality rate of up to 90%.

According to Sullivan et al. [1], the Ebola virus (EBOV) genome is 19 kb long, with seven open reading frames encoding structural proteins, including the virion envelope glycoprotein (GP), nucleoprotein (NP), and matrix proteins VP24 and VP40; nonstructural proteins, including VP30 and VP35; and the viral polymerase. Unlike that of Marburg virus, the GP open reading frame of Ebola virus gives rise to two gene products, a soluble 60- to 70-kDa protein (sGP) and a full-length 150- to 170-kDa protein (GP) that inserts into the viral membrane, through transcriptional editing.

According to Ohimain [2], the glycoprotein is responsible for binding and viral entry; NP, VP35, VP30 and L are responsible for replication and transcription of viral RNA, while VP 40 and VP 24 are responsible for assembly, budding and release of virion particles. The NP encapsulates the genome and forms a complex with VP30, VP35 and L, which are required for both genomic replication and transcription of viral genes. The three other proteins, GP,

VP40, and VP24 are membrane associated proteins, which may be important in the expression of antibodies against Ebola virus antigens (Figure 1).

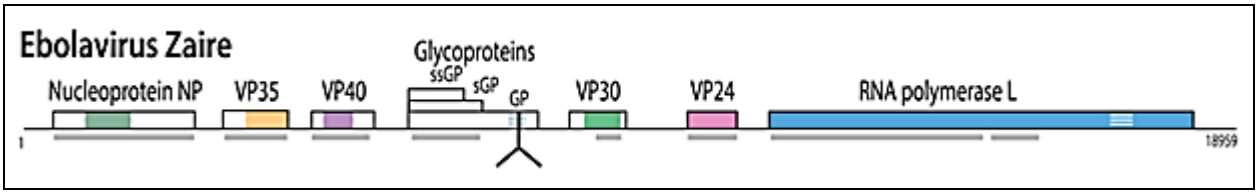

Figure 1: Genome of *Zaire Ebolavirus* [3]

Geisbert et al. [4] reported that VP24 and VP35 are potential targets for antiviral interventions since both genes have inhibitory effects against host type I interferon response, and that the L proteins which provides the RNA dependent RNA polymerase is a potential target for antiviral drugs because of two reasons, 1) suppression could result in the inhibition of viral replication and, 2) such protein is not found in mammalian systems.

Garcinia kola (Heckel) commonly called bitter kola, belongs to the family *Clusiaceae*, and the genus *Garcinia* includes more than 300 species. *Garcinia kola* is considered a wonder plant as every part of it has been found to be of medicinal importance. It has been implicated with antiparasitic, antiviral, anti-inflammatory, anti-diabetic, anti-oxidant and antihepatoxic activities [5]. The antiviral activity of *Garcinia kola* has been reported and Kolaviron has been identified as the specific antiviral bioflavonoid in bitter kola based on both *in vitro* and *in vivo* model systems [6]. Ten natural plants were reported as possible sources of anti-Ebola phyto-constituents [7], the complete viral genome sequences of Ebola virus (EBOV) directly from clinical samples was found with glycoprotein and polymerase genes showing the most sequence variation [8]. Comparative analysis of more than 100 currently available *ebolavirus* genomes to each other and to other viral genomes showed similar functions and gene order [3]. A benzodiazepine derivative was identified potent small-molecule entry inhibitor for filoviruses [9], and endosomal proteolysis of the Ebola virus glycoprotein has been found as necessary for infection [10].

In the post-genomic era, benefiting from the dramatic increase in bio-macromolecule and small molecule information, computational tools can be applied to most aspects of the drug discovery and development process, from target identification and validation to lead discovery and optimization; the tools can even be applied to preclinical trials, which greatly alters the pipeline for drug discovery and development. The use of computational tools could reduce the cost of drug development by up to 50% [11]. There are many sequences of *Zaria ebolavirus* that are available in the bioinformatics databases as at the time of this study. The most completed and reviewed sequence with Uniprot id: Q05320 was used in this study. In this study, selected bioactive components of *Garcinia kola* were computationally evaluated for therapeutic potential in relevance to Ebola virus disease (EVD).

MATERIALS AND METHODS

Sequence Retrieval and Homology Analysis

The glycoprotein sequence of *Zaria ebolavirus* (*strain Mayinga-76*) with *UniprotKB/Swiss-prot* ID: Q05320 which has 676 amino acid residues. Four other homolog strains with minimum of 98% identity were extracted by *blastp* from the *Uniprot* database. Thirty distant homologs with significant query matches were obtained from interactive database searching of Q05320 using *HMMER* [12]. The sequences of all the homologs were stored as FASTA format for further analysis.

Phylogenetic Analysis

Multiple sequence alignment was carried out by using *ClustalO*, at default setting and the phylogenetic tree was obtained. The phylogeny was visualized at *phylo.io*.

The Ligands Preparation

The components of *garcinia kola* were obtained from the available literature [5,13]. The three dimension structure in .sdf format and canonical SMILES of the ligands were obtained from NCBI PubChem Compound.

Target Analysis

The SMILES format of all the *garcinia kola* components was analyzed using *SwissTargetPrediction* server [14], and *Homo sapiens* was selected as the source of target. The possible potentials against Ebola infection were extrapolated from the literatures.

ADME/Tox Screening

ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) screening helps in detecting drug likeliness of compounds. The SMILES format of the ligands were loaded into the *SwissADME* server [15], and ADME screening was done at default parameters.

RESULTS AND DISCUSSION

Greater variation in oligosaccharide structure is exploited by nature through the combination of carbohydrates with proteins (glycopeptide/glycoprotein). This structural diversity has been termed glycocode [16]. The most conserved region of the Ebola glycoproteins studied (Figure 2), showed the identity conservation of cysteine (C) as component of Disulphide Bridge; conserved substitution of isoleucine (I), Aspartic acid (D) and Leucine (L), during the evolution. However, this region in human could be the location for cell-cell fusion, thus serve as possible target in human for antiviral drug as the case of anti-endogenous retrovirus glycoprotein [17]. The phylogeny showed the evolutionary closeness of the glycoproteins from strains of *Zaria ebolavirus*, and the divergence of other distant homologs, as showed in Figure 3.

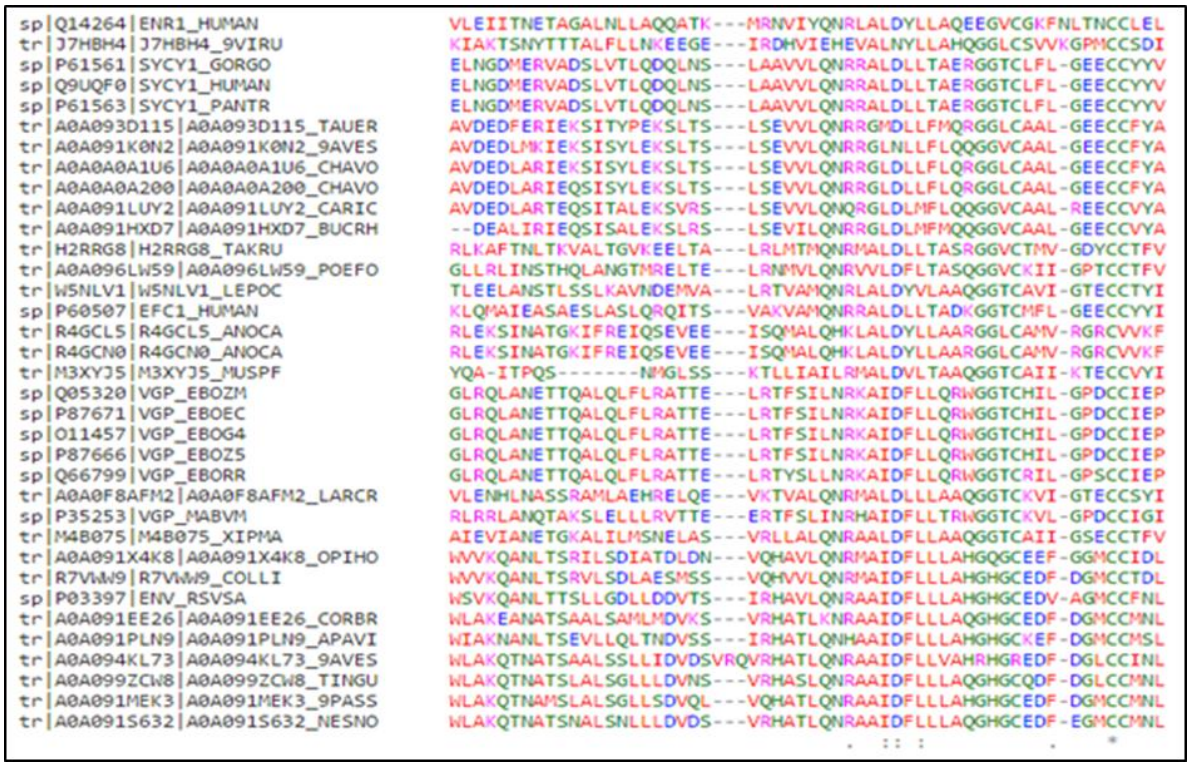

Figure 2: A segment of multiple sequence alignment of *Zaria ebolavirus* glycoproteins. (Red = small, hydrophobic, aromatic, not Y. Blue: acidic. Magenta: basic. Green: hydroxyl, amine, amide, basic. Gray: others. "*" = Identical, ":"= conserved substitutions (same colour group), "." = semi-conserved substitution (similar shapes)

Three human endogenous retrovirus proteins (HERV) with Uniprot ID: Q9UQF0, Q14264, and P60507, were found among distant homologs that matched the query Ebola glycoprotein (Q05320). About 8% of the human genome is human endogenous retrovirus (HERV) sequences. Retroviral envelope glycoproteins play an important role in the viral life cycle: they contain the recognition site required for entry and mediate cell fusion [18]. HERV-Wenv protein (Q9UQF0) which is called Syncytin-1, was reported to possess properties that may generates pathogenesis, as fusogenicity, induction of T mediated immunopathology along with induction of pro-inflammatory cytokines and T-cell responses [17]. Syncytin-1 expression was originally detected in the placenta and testis but subsequent studies have revealed the presence of syncytin-1 also in the brain and in breast, colon and endometrial cancers. Agents increasing cellular levels of cAMP or cAMP analogues have been known to elevate protein and mRNA levels of syncytin-1 in isolated cytotrophoblasts [19]. Moreover, experimentally induced truncations in the cytoplasmic tail of syncytin-1 increases its fusogenicity, similar to what has been observed for some virally derived Env proteins.

Proteolytic cleavage of the cytoplasmic tail regulates fusions and also cellular signaling events, such as tyrosine kinase activity, may be involved in regulation of virally induced cell fusions [19].

As showed in Table 1, there are thirteen components of *garcinia kola* that were active against targets ranging from membrane proteins, metallo proteases, and Ser_Thr Kinase. The three most featured targets; cannibanoid receptors, cyclin-dependent kinases, and matrix metallo-proteinases, were further investigated in alignment with the available reports and their potential against Ebola virus were hypothesized. The cannabinoids (CBs) are a group of terpenophenolic compounds, phytocannabinoids present uniquely in the cannabis plant. The broader definition of cannabinoids refers to a group of substances that are structurally related to Δ^9-tetrahydrocannabinol (Δ^9-THC) or that bind to cannabinoid receptors. The psychoactive effects of cannabinoids are associated with the CB1 receptor; the CB2 receptor mainly mediates anti-inflammatory and immunomodulatory actions [20]. Cannabinoid receptors are integral membrane proteins whose amino acid sequences are characterized by seven hydrophobic segments containing -helical patterns. CB receptors belong to α group of rhodopsin-like seven-transmembrane (7TM) GPCRs [21]. The induction of cannabinoid receptor 1 (CB1R) by hepatitis C Virus has been reported [22], and Costantino et al. [23] found that agonism of CB2R, but not CB1R, reduced infection in primary CD4+T cells following cell-free and cell-to-cell transmission of CXCR4-tropic HIV. CB2 agonism decreased CXCR4-activation mediated G-protein activity and MAPK phosphorylation. Furthermore, CB2 agonists altered cytoskeletal reorganization, by decreasing F-actin levels, impairing productive infection. We infer that *garcinia kola* could act as a CB2R agonist during EBOV infections. Matrix metalloproteinases (MMPs) are a family of structurally related, zinc dependent endopeptidases with a combined activity to degrade essentially all components of the extracellular matrix including collagens, elastins, matrix glycoproteins, and proteoclycans. They not only have an important function during development and in many physiological events but are implicated for much of the excessive matrix degradation in numerous pathological conditions, ranging from arthritis to cancer invasion and metastasis. According to Joronen et al. [24], MMPs participate in cell-cell and cell-matrix signalling by modulating the activity of growth factors, cytokines, and chemokines such as tumour necrosis factor α (TNF α), transforming growth factor b, fibroblast growth factors (FGFs), and monocyte chemoattractant protein-3 (MCP-3). Collagenase-3 (MMP-13) is the most efficient to degrade type II collagen and it also cleave other cartilage collagens, such as type IX collagen and aggrecan, the most abundant proteoglycan in articular cartilage [24]. Collagenase has been identified as a crucial virulence factor in the invasiveness and transmission of *L. interrogans* [25].

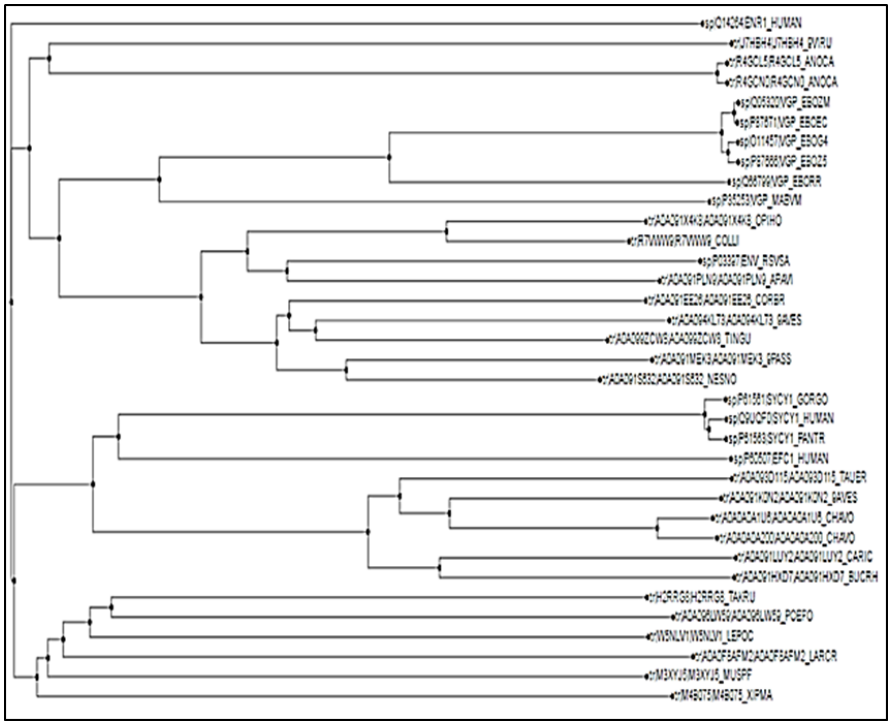

Figure 3: Phylogeny of *Zaria ebolavirus* glycoproteins visualized at *phylo.io*

Table 1: Summary of predicted targets of active components of *Garcinal kola*

TARGET	UNIPROT ID	TARGET CLASS	1	2	3	4	5	6	7	8	9	10	11	12
Dopamine receptor D(2), D(3), D(4)	P14416, P35462, P21917	Membrane receptor	*											
5-hydroxytryptamine receptor 2A, 2B, 2C	P28223, P41595, P28335	Membrane receptor	*											
Opioid receptor (Mu-type, Delta-type, Kappa-type	P35372, P41143, P41145	Membrane receptor	*											
Nociceptin receptor	P41146	Membrane receptor	*											
Beta-secretase 1 and 2	P56817, Q9Y5Z0	Aspartic Protease	*							*				
Adenosine receptor A1	P30542	Membrane receptor			*	*								
Adenosine receptor A2a	P29274	Membrane receptor			*	*								
Adenosine receptor A3	P33765	Membrane receptor			*									
Transitional endoplasmic reticulum ATPase	P55072	Unclassified	*											
Spermatogenesis-associated protein 5	Q8NB90	Unclassified	*											
Cyclin-dependent kinase 1	P06493	Ser_Thr Kinase			*	*		*						
Cyclin-dependent kinase 2	P24941	Ser_Thr Kinase			*	*		*						
Cyclin-dependent kinase 3	Q00526	Ser_Thr Kinase			*			*						
Cyclin-dependent kinase 4	P11802	Ser_Thr Kinase			*	*		*						
Cyclin-dependent kinase 6	Q00534	Ser_Thr Kinase			*			*						
Estrogen receptor	P03372	Transcription Factor				*					*	*		
Renin	P00797	Aspartic Protease					*							
Cathepsin D	P07339	Aspartic Protease					*							
Napsin-A	O96009	Aspartic Protease					*							
M-phase inducer phosphatase 1 and 2	P30304, P30305	Ser_Thr_Tyr Phosphatase		*										
Complex	P47870/P18507/P14867	Ion channel	*											
Complex	Q15078/Q00535, Q8WWL7/ P14635/P06493/O95067	Ser_Thr Kinase						*						
Cannabinoid receptor 1 and 2	P21554, P34972	Membrane receptor							*					*
22 kDa interstitial collagenase	P03956	Metallo Protease									*	*	*	
Stromelysin-1 and 2	P08254, P09238	Metallo Protease									*	*	*	
Macrophage metalloelastase	P39900	Metallo Protease									*	*	*	
Collagenase 3	P45452	Metallo Protease									*	*	*	
Matrix metalloproteinase-27 and 20	Q9H306, O60882	Metallo Protease									*	*	*	

* = 80-100% probability on the target. 1= Amentoflavone. 2 = Anthraquinone. 3 = Apigenin trimethyether. 4 = Apigenin. 5 = Arylbenzofuran (SCHEMBL8338618). 6 = Fisetin. 7 = Garcinioic acid. 8 = Garcinianin. 9 = Kolaviron (Kolaflavanone). 10 = Garcinal GB1. 11 = Garcinal GB2. 12 = δ-tocotrienol

According to Weeks et al. [26], HIV-1-infected human lymphocytes secrete increased amounts of the human 92-kDa type IV collagenase when compared to uninfected lymphocytes, and that HIV-1-infected lymphocytes degrade the extracellular matrix proteins collagen IV and fibronectin, and they are more invasive through a reconstituted basement membrane when compared to uninfected cells. It has been reported that endosomal proteolysis of the Ebola virus glycoprotein implicated cathepsin as necessary for infection has been reported [10], and a component of *garcinia kola* targeted this cathepsin, collagenase and other matrix metalloproteinase, as showed in Table 1. We infer that *garcinia kola* could prevent abnormal expression of MMPs during viral infection.

Deregulation of protein kinase activity plays a central role in many human diseases. The critical role of protein kinases in central biological processes is exemplified by the cyclin-dependent kinases (CDKs), which are heterodimeric complexes composed of a catalytic kinase subunit and a regulatory cyclin subunit, and comprise a family divided into two groups based on their roles in cell cycle progression and transcriptional regulation. Cyclin-dependent kinase pathways as targets for cancer treatment has been well explored [27].

It was reported by Schang [28] that CDKs are required for replication of many clinically important viruses, such as papillomaviruses, human immunodeficiency virus type 1 (HIV-1), human cytomegalovirus (HCMV) and herpes simplex virus (HSV) types 1 and 2. CDKs are a family of serine/threonine kinases involved in regulation of cell division (CDKs 1, 2, 3, 4, 6 and 7), transcription (CDKs 7, 8 and 9) or maintenance of the structure of the cytoskeleton (cdk5). CDKs have recently been reported to regulate human immunodeficiency virus type 1 (HIV-1) transcription [29]. Based on the result of this study, we infer that *garcinia kola* could inhibits CDKs thereby preventing viral replication. This supports the earlier report that *garcinia kola* extract stopped replication of the Ebola virus in lab test [30].

Furthermore, coumarin and quercetin which are also components of *garcinia kola* (not reported in Table 1), have carbonic anhydrases as probable target, similar targets was reported for paradol and zingerone which are active components of *Zingiber officinale* [15]. The summarized pharmacokinetics (ADME) result in Table 2 showed that not all these active components of *garcinia kola* in this study obeyed the Lipinski rule of drug-

likeness [31]. Although, those components that showed one violation could be waived, in that in a successful marketed drug, one parameter can compensate for another. Drug-like compounds must contain enough functionality to interact in a meaningful way with a protein [32]. Lipinski rule applies only to compounds that are delivered by the oral route, and to compounds that are absorbed by passive mechanisms. Natural products are another important exception to this rule, more likely that these compounds have been optimized by nature to take advantage of active transport or have developed special conformational features that are beneficial for passive transport [32]. Therefore, components that have low gastrointestinal absorption due to oral route could be administered via other routes such as intravenous injection. [33]

Table 2: ADME parameters of the main active components of *Garcinia kola*

Components	MW	HA	AH	FC	RB	HBA	HBD	MR	TPSA	XLogP	LogS	ESOL C	GA	LV	BS	L
Amentoflavone	538.46	40	32	0	3	10	6	146.97	181.8	5.04	-6.75	Poorly soluble	Low	2	0.17	2
Anthraquinone	208.21	16	12	0	0	2	0	59.75	34.14	3.39	-3.82	Soluble	High	0	0.55	1
Apigenin trimethyether	312.32	23	16	0.17	4	5	0	87.4	57.9	3.45	-4.2	Moderately soluble	High	0	0.55	0
Apigenin	270.24	20	16	0	1	5	3	73.99	90.9	3.02	-3.94	Soluble	High	0	0.55	0
Arylbenzofuran	500.63	37	21	0.35	12	6	2	150.08	64.89	4.86	-5.63	Moderately soluble	High	1	0.55	3
Fisetin	286.24	21	16	0	1	6	4	76.01	111.13	1.97	-3.35	Soluble	High	0	0.55	0
Garcinioic acid	426.59	31	6	0.52	10	4	2	129.69	66.76	7.27	-6.55	Poorly soluble	Low	1	0.56	3
Garcinianin	556.47	41	28	0.07	3	11	7	145.63	198.12	4.68	-6.55	Poorly soluble	Low	3	0.17	2
Kolaviron	588.52	43	24	0.16	4	12	7	148.84	203.44	3.97	-6.14	Poorly soluble	Low	3	0.17	2
Garcinal GB1	558.49	41	24	0.13	3	11	7	142.35	194.21	4	-6.06	Poorly soluble	Low	3	0.17	2
Garcinal GB2	574.49	42	24	0.13	3	12	8	144.37	214.44	3.11	-5.59	Moderately soluble	Low	3	0.17	1
δ-tocotrienol	396.61	29	6	0.56	9	2	1	127.92	29.46	8.58	-7.26	Poorly soluble	Low	1	0.55	3

Physicochemical properties: Molecular weight (MW), Heavy atom (HA), Aromatic heavy atoms (AH), Fraction Csp3 (FC), Rotatable bonds (RB), H-bond acceptors (HBA), H-bond donors (HBD), Molar Refractivity (MR), Total polar surface area (TPSA), Lipophilicity: XLOGP3, Water Solubility: ESOL Log S, ESOL Class, Phramacokinetics: GI absorption, Druglikeness: Lipinski violations, Bioavailability Score, Medicinal Chemistry: Leadlikeness Violation

CONCLUSION

This study has showed those components *garcinia kola* (bitter kola) are potential therapeutics Ebola virus glycoprotein entry during infestation of human. As we have tried to show, the more a chemical entity becomes interesting and promising, the more should be known on its molecular properties. Therefore, we suggest further computational and experimental exploration of the targets of *garcinia kola* that were disclosed in this study, in relevance to Ebola virus disease.

REFERENCES

[1] N Sullivan; Z Yang; GJ Nabel. *J Virol*. **2003**, 77(18), 9733-9737.
[2] EI Ohimain. *IJTDH*. **2015**, 10(4), 1-17.
[3] SR Jun; MR Leuze; I Nookaew; EC Uberbacher; M Land; Q Zhang; V Wanchai; J Chai; M Nielsen; T Trolle; O Lund. *FEMS Microbiol Rev*. **2015**, fuv031.
[4] TW Geisbert; AC Lee; M Robbins; JB Geisbert; AN Honko; V Sood; JC Johnson; S De Jong; I Tavakoli; A Judge; LE Hensley. *Lancet*. **2010**, 375(9729), 1896-905.
[5] CI Buba; SE Okhale; I Muazzam; G Kola. *IJP*. **2016**, 3(2), 67-81.
[6] KY Anchang; M Garba; FT Manjong; T Yvette. *Am J Clin Exp Med*. **2015,** 3(1-1), 1-6.
[7] IC Uzochukwu; OO Olubiyi; FC Ezebuo; IC Obinwa; EE Ajaegbu; PM Eze; CE Orji; MC Onuoha; II Ejiofor; MN Ikegbunam. *J Pharm Res.* **2016**, 1(1), 000105.
[8] A Bell; K Lewandowski; R Myers; D Wooldridge; E Aarons; A Simpson; R Vipond; S Gharbia; M Zambon. *Euro Surveill.* **2015**, 20(20), 21131.
[9] A Basu; B Li; DM Mills; RG Panchal; SC Cardinale; MM Butler; NP Peet; H Majgier-Baranowska; JD Williams; I Patel; DT Moir. *J Virol*. **2011**, 85(7), 3106-3119.
[10] K Chandran; NJ Sullivan; U Felbor; SP Whelan; JM Cunningham. *Science*. **2005**, 308(5728), 1643-1645.
[11] SS Ou-Yang; J Lu; X Kong; Z Liang; C Luo; H Jiang. *Acta Pharmacologica Sinica*. **2012**, 33, 1131-1140.
[12] RD Finn; J Clements; SR Eddy. *Nucleic Acids Res*. **2011**, 39, W29-W37.
[13] MF Adegboye; DA Akinpelu; AI Okoh. *Afr J Biotechnol*. **2008**, 7(21), 3934-3938.
[14] D Gfeller; O Michielin; V Zoete. *Bioinformatics.* **2013**, 29, 3073-3079.
[15] H Fatoki; D Sanni; O Adeoyo; B Faleye. *J Nat Prod Plant Resour*. **2016**, 6(6), 21-25.

[16] DP Gamblin; EM Scanlan; BG Davis. *Chem Rev.* **2009**, 109, 131-163.
[17] G Mameli; D Cossu; E Cocco; J Frau; MG Marrosu; M Niegowskaa; LA Sechi. *J Neuroimmunol.* **2015**, 280, 66-68.
[18] A De Brogniez; AB Bouzar; JR Jacques; JP Cosse; N Gillet; I Callebaut; M Reichert ; L Willems. *J Virol*. **2015**, 89(17), 8945-8956.
[19] L Larsson; B Bjerregaard; JF Talts. *Histochem Cell Biol.* **2008**, 129, 551-561.
[20] S Rom; Y Persidsky. *J Neuroimmune Pharmacol.* **2013**, 8(3), 608-620.
[21] K Palczewski; T Kumasaka; T Hori; CA Behnke; H Motoshima; BA Fox; I Le Trong; DC Teller; T Okada; RE Stenkamp; M Yamamoto; M Miyano. *Science*. **2000**, 289, 739-745.
[22] D Van Der Poorten; M Shahidi; E Tay; J Sesha; K Tran; D McLeod; JS Milliken; V Ho; LW Hebbard ; MW Douglas; J George. *PLoS One*. **2010**, 5(9), e12841.
[23] CM Costantino; A Gupta; AW Yewdall; BM Dale; LA Devi; BK Chen. *PloS One*. **2012**, 7(3), e33961.
[24] K Joronen; R Ala-aho; ML Majuri; H Alenius; VM Ka¨ha¨ri; E Vuorio. *Ann Rheum Dis.* **2004**, 63, 656-664.
[25] K Kassegne; W Hu; DM Ojcius; D Sun; Y Ge; J Zhao; XF Yang; L Li; J Yan. *J Infect Dis*. **2013**, jit659.
[26] BS Weeks; ME Klotman; E Holloway; WG Stetler-Stevenson; HK Kleinman; PE Klotman. *Aids Res Hum Retrov*. **1993**, 9(6), 513-518.
[27] GI Shapiro. *J Clin Oncol*. **2006**, 24(11), 1170-1187.
[28] LM Schang. *J Antimicrob Chemother*. **2002**, 50, 779-792
[29] D Wang; C de la Fuente; L Deng; L Wang; I Zilberman; C Eadie; M Healey; D Stein; T Denny; LE Harrison; L Meijer. *J Virol*. **2001**, 75(16), 7266-7279.
[30] MM Iwu; AR Duncan; CO Okunji. Alexandria VA, ASHS Press, **1999**, 457-462.
[31] CA Lipinski; F Lombardo; BW Dominy; PJ Feeney. *Adv Drug Deliv Rev*. **2001**, 46, 3-26.
[32] TH Keller; A Pichota; Z Zheng Yin. *Curr Opin Chem Biol*. **2006,** 10, 357-361
[33] D Lavillette; M Marin; A Ruggieri; F Mallet; FL Cosset; D Kabat. *J Virol*. **2002**, 76(13), 6442-6452.