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Promising Antimicrobial Peptides

Ebola Virus Disease in sub-Saharan

Highlights

History, Contemporary Epidemiology

Minimal Physical Activity in Adolescents

Discovering Thoughts, Inventing Future

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Ebola Virus Disease in sub-Saharan Africa: History, Contemporary Epidemiology and Recommendations

By Olusola Bamidele Ojo

Abstract- Between 2017 and 7 March 2025, Ebola virus disease occasioned eight outbreaks killing 2,471 people out of 3,892 cases in sub-Saharan Africa. Given the pandemic capacities of the largest Ebola epidemics that occurred in West Africa from 2014-2016 and the socio-economic catastrophe wrought by the COVID-19 pandemic, all hands must be on deck to mitigate recurrent Ebola outbreaks. Hence, this piece explores the historical trajectory of Ebola virus disease and its epidemiological attributes in the contemporary era. We deployed a narrative review of relevant articles on the Ebola virus disease from its inception in 1976 to 2025. Between 2001 and 2025, 26 outbreaks of EVD occurred in sub-Saharan Africa, with an average case fatality rate of 43 percent. We discovered that 46 percent of the epidemics occurred in DRC Congo, and Zaire Ebola strains were responsible for 73 percent of outbreaks.

Keywords: *ebola virus, ebola virus disease, sub-Saharan africa, case fatality ratio, epidemic, outbreak.*

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Ebola Virus Disease in sub-Saharan Africa: History, Contemporary Epidemiology and Recommendations

Olusola Bamidele Ojo

Abstract- Between 2017 and 7 March 2025, Ebola virus disease occasioned eight outbreaks killing 2,471 people out of 3,892 cases in sub-Saharan Africa. Given the pandemic capacities of the largest Ebola epidemics that occurred in West Africa from 2014-2016 and the socio-economic catastrophe wrought by the COVID-19 pandemic, all hands must be on deck to mitigate recurrent Ebola outbreaks. Hence, this piece explores the historical trajectory of Ebola virus disease and its epidemiological attributes in the contemporary era. We deployed a narrative review of relevant articles on the Ebola virus disease from its inception in 1976 to 2025. Between 2001 and 2025, 26 outbreaks of EVD occurred in sub-Saharan Africa, with an average case fatality rate of 43 percent. We discovered that 46 percent of the epidemics occurred in DRC Congo, and Zaire Ebola strains were responsible for 73 percent of outbreaks. Hence, we maintain that the recurrent rate of Ebola virus disease epidemics in contemporary sub-Saharan Africa is mainly due to rapid urbanization, political instabilities, and weakened health systems. Therefore, this work underscores the need for continuing community education and engagement regarding Ebola, strengthening of disease surveillance, and a clarion call for peace in war-troubled regions of sub-Saharan Africa.

Keywords: ebola virus, ebola virus disease, sub-Saharan africa, case fatality ratio, epidemic, outbreak.

I. Introduction

There is a recurrence of high-risk pathogenout breaks in the contemporary era. Filoviral diseases including Ebola virus diseases (EVD) have occasioned different epidemics with catastrophic mortalities in 21st century sub-Saharan Africa. Given the devastating economic and social consequences of the COVID-19 pandemic, efforts should be geared towards mitigating another cataclysmic pandemic. In 2022, the World Health Organization marshaled an action plan against high-risk pathogens capable of triggering the next pandemic. These contagious organisms included viral hemorrhagic viruses including Ebola virus and Marburg virus.[1] Ebola virus disease (EVD) and Marburg virus disease (MVD) have extremely high fatality rates ranging up to 90 percent inpast and recent epidemics. They belong to the family, filoviridae composed of three genera Cuevavirus, Marburgvirus, and Ebolavirus. Six species have been identified in the genus Ebolavirus, they are Zaire, Bundibugyo, Sudan, Tai Forest, Reston, and Bombali.[2] The latter two strains have not been attributed to any Ebola infections.

The repeated outbreaks of EVD in contemporary sub-Saharan Africa and the likelihood of escalation to worldwide pandemics spurred our interest in this review. For example, Uganda has witnessed five outbreaks of Sudan virus disease (SVD), a family of EVD since 2000, with the largest outbreak in 2022 claiming 55 lives out of 164 cases.[3] As of 20 February 2025, one death has been recorded out of nine cases of SVD in Uganda.[3] The largest epidemic of EVD specifically ravaged Liberia, Guinea, and Sierra Leone between 2014 and 2016, claiming 11, 308 lives out of 28, 600 cases.[4] Between 2017 and 2022, sub-Saharan Africa has witnessed seven outbreaks of EVD with five of them occurring in Democratic Republic of Congo and one occurring in Guinea and another one in Uganda. Within this period, there were 2412 reported deaths out of 3714 Ebola sufferers.[4] Given the increasing rate of road interconnectivity, transportation, and urbanization across sub-Saharan Africa, the risk of escalation of filoviral epidemics to pandemics has never been greater, hence the continuing advocacy for efficient disease preparedness, community engagement and continuing vaccine research against filoviruses.

The Ebola virus genome is a negative-sense single-stranded RNA. It is composed of a viral envelope, matrix, and nucleocapsid components. It encrypts seven structural proteins: nucleoprotein (NP), polymerase cofactor (VP35, VP40, GP), transcription activator (VP30, VP24), and RNA-dependent RNA polymerase.[5] Ebola virus belongs to the family of filoviridae and comprises of six identified species. They are Zaire, Bundibugyo, Sudan, Reston and Tai Forest, and Bombali. Zaire, Bundibugyo, and Sudan ebolaviruses have been linked to huge epidemics in sub-Saharan Africa. The virus occasioning the catastrophic 2014 West African epidemics belongs to the Zaire species.[6] Fruit bats of the family, *Pteropodidae* are the documented vector and reservoir of the virus. The first cases of EVD were reported in 1976 in Nzara, Sudan, and in Yambuku in Zaire, now Democratic Republic of Congo. The latter occurred around Ebola River where the contagion derived its appellation[6]. Due to its high fatalityrates, Ebola virus is listed as an organism included in WHO

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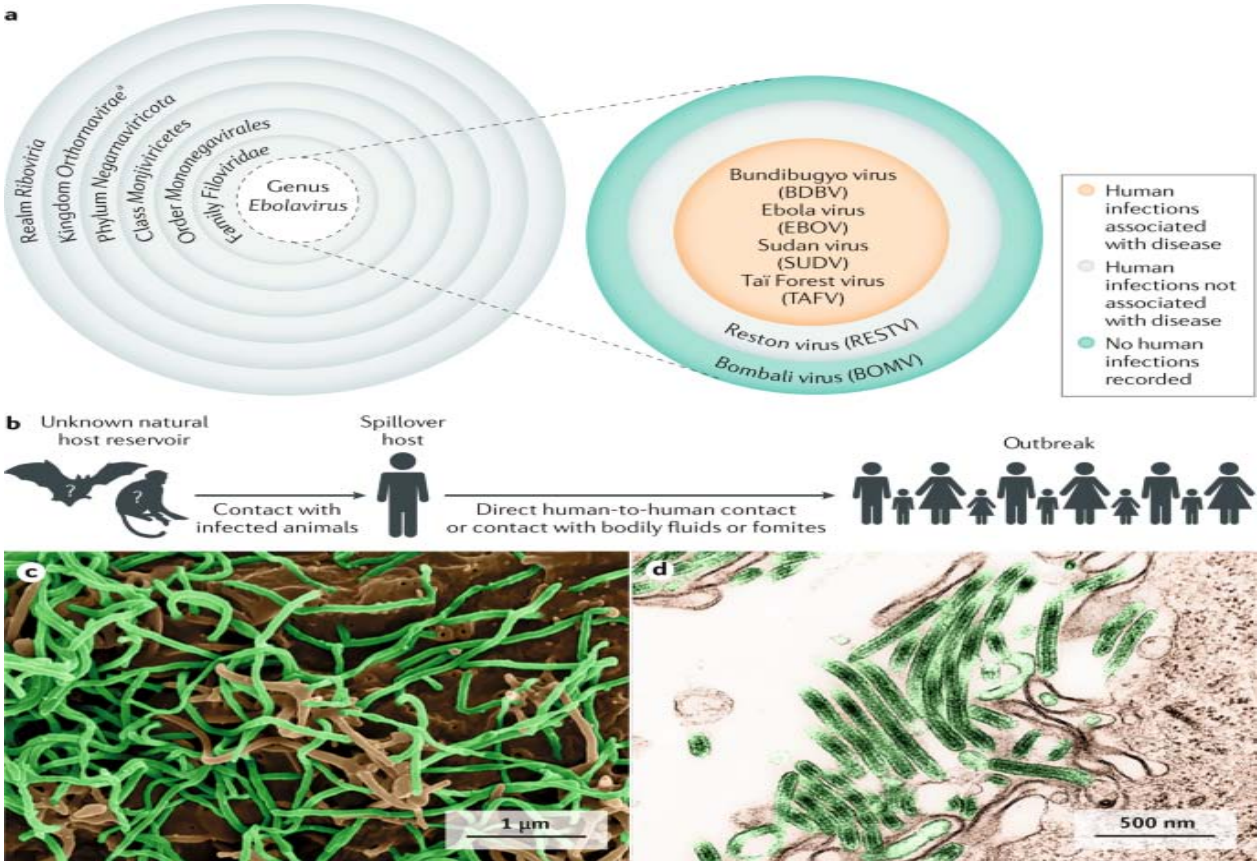
Risk Group 4 Pathogen requiring biosafety level 4-equivalent containment.

There is no paucity of research on the transmission of the Ebola virus as well as the clinical manifestations, diagnosis, and management of EVD. [7-10] Most scholars document initial zoonotic transmission from Ebola virus reservoirs via contact with them and their droppings or through their consumption as foods. Such reservoirs include fruits bats, chimpanzees, monkeys, and antelopes. Subsequently, after a spillover, human transmission assumes the dominant dispersal mechanism via close contact with sufferers, blood, sweats, clothing, and even seminal fluids. Moreover, contact with infected bodies could transmit EVD. Therefore, hygienic and safe burial practices are recommended for Ebola dead bodies to mitigate the dispersal of the high-risk pathogen. Regarding its clinical features, the hallmark of EVD is hemorrhagic fever. The incubation period is between two to 21 days. It is characterized by an initial phase of flu-like manifestations like fever, chills and rigors, myalgia, and weakness.[6] Aftera few days, patients usually present with gastrointestinal features such as vomiting, diarrhea, and abdominal pain. The most common symptoms are fever, extreme weakness, vomiting, and maculopapular rash around the face, neck, and arms occurring between five and 7 days of the onset of symptoms.[8] After the flu-phase, hemorrhagic manifestations occur in EVD leading to conjunctival hemorrhage, petechiae, and ecchymosis. Other non-common features include cough, dyspnea, and localized chest, abdominal, muscle, and joint pains.[11] Some patients may recover at this stage while others progress into severe EVD. Massive bleeding usually occurs in severe disease, particularly in the gastrointestinal tract.[12] Such patients are prone to massive fluid loss, electrolyte imbalance, shock, and multi-organ failure leading to a higher risk of mortalities.[13]



https://www.nature.com/articles/s41572-020-0147-3/figures/

Figure 1: Epidemiology of Ebola Virus Disease

Taxonomy of the genus *Ebolavirus*. Thus far, five ebolaviruses have been associated with human infections, and four of them have been identified as pathogens. *b)* The natural reservoir host(s) of Ebola virus (EBOV) has (have) yet to be identified. Multiple data indicate a direct or indirect role of bats in EBOV ecology, but to date, EBOV has not been isolated. *c)* Scanning electron microscopic (SEM) image of EBOV particles (green) budding from grivet cells. *d)* Transmission electron microscopic (TEM) image of EBOV particles (green) budding from grivet cells.

Adapted from Jacob, S.T., Crozier, I., Fischer, W.A. *et al.* Ebola virus disease. *Nat Rev Dis Primers 6,* 13 (2020). https://doi.org/10.1038/s41572-020-0147-3

Some scholars dwell on the need for prompt diagnosis of EVD to mitigate the wider spread of the outbreaks, especially in some sub-Saharan settings with weakened health systems. Given that the initial phase of EVD is indistinguishable from other prevailing ailments like malaria, typhoid, yellow fever, and dengue. Therefore, adequate case definition and a high index of clinical suspicion, especially in high-risk areas remain an effective modality of initial empirical diagnosis and outbreak containment.[14] In sub-Saharan Africa and elsewhere, laboratory confirmation of EVD is essential amidst myriads of high-risk pathogens, which could cause hemorrhagic fever. To confirm infection, Antigen-capture enzyme-linked immunosorbent assay (ELISA) testing, IgM ELISA, polymerase chain reaction (PCR), and virus isolation tests are deployed as diagnostic tests.[8,14] Due to its highly virulent nature, EVD's laboratory diagnosis should be performed in a well-equipped laboratory with up to bio safety level 4 bio-contaminant facilities for viral culturing. Prompt case identification, early fluid care, and hospitalization improve the chances of survival in EVD patients. Regarding the prevention of EVD, there are two recommended vaccines. ERVEBO was licensed by the European Medicines Agency and the Food and Drug Administration in 2019 and is indicated for use in persons above12 months. It has a shelf life of three years. ERVEBO*, rVSV-ZEBOV* was developed against Zaire type Ebola virus. It made massive ring vaccinations of contacts and health workers possible during the 2016 epidemic in Guinea, and the 2018-19 outbreaks in the Democratic Republic of Congo .[14, 15] The vaccine has been approved in Burundi, Central African Republic, Côte d'Ivoire, Democratic Republic of the Congo (DRC), Ghana, Guinea, Republic of the Congo, Rwanda, Sierra Leone, Uganda, and Zambia .[16] The second vaccine , the chimpanzee adenovirus serotype 3 vectored vaccine (ChAd3-EBO-Z), boosted with the MVA vaccines, Zabdeno/Mvabea is recommended for preventive vaccination in areas at lower risk for Ebola or areas neighboring an epidemic because the full regimen requires two doses administered 56 days apart.[16]. However, there are concerns regarding the long-term side effects and immunogenicity of the vaccines. Besides, the vaccine is not effective against other strains, hence community engagement and surveillance systems, early case identification and management, and prompt disease preparedness remain the effective modalities of mitigating EVD outbreaks in sub-Saharan Africa.[17-20]

Despite the ravaging impacts of recurrent EVD epidemics in sub-Saharan Africa, there is dearth of a research highlighting the current epidemiological features of EVD. Some researchers have focused on the epidemiological attributes of EVD from global perspectives without much emphasis on contemporary sub-Saharan situations.[8,9&14] Besides, only a handful of studies have attempted to calculate a case fatality ratio specifically for contemporary sub-Saharan Africa. For instance, a recent review deploying global data computed a grand case fatality ratio of EVD without a detailed recourse to contemporary sub-|Saharan African realities.[21] A focus of this caliber is significant. It would help to reinforce the enormity of EVD in the region to continually advocate for community engagement, disease preparedness, and strengthening of health systems to redress recurrent high-risk pathogen outbreaks. Given the frequency of EVD outbreaks in sub-Saharan Africa, the importance of delineating the contemporary epidemiological attributes of EVD could not be overemphasized in the context of preventing the next pandemic. Hence, we set out to highlight the epidemiological characteristics of recent EVD outbreaks including the computation of the case fatality ratio of this highly virulent pathogen in sub-Saharan Africa.

II. Methodology

We deployed mainly anarrative review and a measure of rapid systematic review for this study. We synthesize and analyze relevant existing studies on Ebola through a descriptive approach to arrive at laudable research conclusions. We searched PubMed, Google Scholar, and Web of Science for publications with the terms "Ebola", "Ebola virus", "Ebola transmission", "Ebola epidemiology", "Ebola virus disease", "Ebola pathogenesis", "Ebola diagnosis", and "Ebola treatment" in various combinations without any language limitations. The search covered the period from 1976, the year of the discovery of EVD, until January 2025. Review articles were cited when appropriate and additional sources were derived from selected journals via snowballing method. Research published in Google Scholar published since the 2014–16 West African outbreak was given particular attention. Research focusing extensively on other hemorrhagic fever was excluded from this review article. We excluded research articles on Reston and Bombali Ebolavirus because they do not cause human infections. We provide an overview of Ebola by focusing on updating the reader on recent advances and controversies. Information from sources other than peer-reviewed journals has been evaluated but was not considered to add indispensable new details, so is not covered in this review article.

a) Historical Epidemiology of Ebola Virus Disease

Filoviruses have probably existed for over 10 million years, however Marburg virus and Ebola virus departed from a common progenitor over 10, 000 years ago.[22,23] In August 1967, the first reported cases of Marburg virus disease occurred in Marburg when

laboratory workers were exposed to African green monkeys (*Cercopithecus a ethiops*) imported from Uganda.[24] Laboratories in Belgrade and Frankfurt that also receiveda consignment of green African monkeys reported sick staff after their exposures to the mammals. Consequently, nine deaths were recorded out of 37 cases.[25] The first cases of EVD were reported nine years later in the Democratic Republic of Sudan. On 27 June1976, a man residing in a rural area but working in a factory in a township, Nzara, developed fever, headache, and chest pain.[26] Due to the severity and persistence of his symptoms after four days, he was admitted for acute care in a hospital, after which he developed bleeding from the nose and mouth, and bloody diarrhea on the second day at the hospital. He subsequently died on July 6 after infecting his co-workers via close contacts. The heightened human-human dispersal led to a devastating outbreak of EVD that killed 151 people out of 284 victims.[25]

The Democratic Republic of Congo (formerly Zaire) encountered the next outbreak of EVD between September and October1976. The clinical manifestations of the index patient revealed other epidemiological features of EVD different from the previous cases in Sudan. The patient presented with malarial symptoms and was duly treated with intramuscular chloroquine. There was an initial resolution of his clinical signs and symptoms. Nevertheless, he developed a set of new clinical features within five days along with other patients who were admitted with him at Yambuku Mission Hospital (YMC) around the Ebola River.[27] They developed a severe sore throat, macula-papular rash, and varied gastrointestinal manifestations such as nausea, dysphagia, abdominal pain, and gastrointestinal hemorrhage. Despite the limited diagnostic protocols at the time, some patients developed non-icteric hepatitis, acute pancreatitis, and disseminated intravascular coagulopathy (DIC).[27] Hence, they were incorporated into the usual clinical manifestations of EVD. By the end of the epidemic in late October 1976,280 patients have died out of 380 identified cases.[25,27] Among the dead included women who attended ante-natal clinics and took injections at YMC. This gives credence to the theory that YMC staff used non-sterilized needles and syringes among patients, thereby facilitating the exchange of body fluids which promoted the rapid dispersal of EVD, and hence fatalities.

The *Sudan ebolavirus* virus and *Zaire ebolavirus* were isolated and ascribed correspondingly to the EBV epidemics in Sudan and Congo DRC. However, in 1989, the third strain, *Restonebolavirus* was isolated in cynomologus monkeys (*Macaca fascicularis*) imported from the Philippine Islands to Reston, Virginia, where they were intended for research purposes.[28] The virus has not been linked to any human infections and the swine has been recognized as its natural host.[29] In 1994, *Tai forest ebolavirus,* another species of ebolavirus was isolated in West Africa. An ethnologist conducting an autopsy on a dead chimpanzee at Parc National de parc in Tai, Cote D' Ivoire contracted EVD. She survived after spending two weeks in the hospital. In 2007, in Uganda, the fifth species, *Bundibugyo ebolavirus* triggered an outbreak killing 30 persons out of 116 infected patients. Between 1976 and 2013, there were 24 outbreaks of EVD, with most epidemics linked to Zaire ebolavirus and Sudan ebolavirus. [25] From the largest outbreak between 2014-2016 in West Africa to 2025, there have been eight outbreaks till 7 March 2025.

b) Epidemiology of Ebola Virus Disease

This study concentrates on the epidemiological features of EVD in sub-Saharan Africa. We focus on the Ebola virus outbreaks from 2001 to 2025 for our contemporary era's analysis. However, before we delve into this review, we will show a detailed table of the epidemiology of EVD in the past era, from 1976-2000. It would ensure appropriate comparison with the epidemiological attributes of EVD in the 21st century. Table 1 shows the epidemiological features of EVD from 1976to 2000.

Table 1: Epidemiological Features of EVD between 1976 and 2000 in Sub-Saharan Africa

Year of Outbreak	Country of Affectation	Ebola virus Species	Cases	Mortality	Case Fatality Ratio (CFR)
1976	Sudan	Sudan virus	284	151	53.2
1976	Congo DRC	Zaire virus	318	280	88.1
1977	Congo DRC	Zaire virus	1	1	100
1979	Sudan	Sudan virus	34	22	64.7
1994	Gabon	Zaire virus	51	31	60.8
1994	Cote D' Ivoire	Tai Forest virus	1	0	0.0
1995	Congo DRC	Zaire virus	315	254	80.6
1996	Republic of South Africa	Zaire virus	2	1	50.0
1996	Gabon	Zaire virus	91	66	72.5
2000	Uganda	Sudan virus	425	224	52.7
Total			1522	1030	67.8

Adapted and Modified from Izudi J, Bajunirwe F. Case fatality rate for Ebola disease, 1976–2022: A meta-analysis of global data. Elservier Journal of Infection and Public Health. 2024; 17: 25–34.

Table (1) displays the main epidemiological features of EVD outbreaks in sub-Saharan Africa. We could decipher that EVD outbreaks occur predominantly in Congo DRC, Sudan, and Gabon. The western and south African region were outliers regarding the prevalence of EVD epidemics. The Sudan and Zaire ebolavirus were responsible for most epidemics of EVD between 1976 and 2000. In all, there were ten outbreaks of EVD from 1976 to 2000, which resulted in 1030 fatalities out of 1522 patients. The case fatality ratio(CFR)spanned from 50 to 100, and the average CFR was 67.8. The Zaire ebolavirus had the highest case fatality ratio ranging from 50 to 100 percent compared to the Sudan ebolavirus with a CFR of 52.7 to 64.7 percent. Congo DRC had the highest CFR of 100 during the 1977 epidemic while the Republic of South Africa witnessed the lowest CFR in the 1996 outbreak. All the afflicted countries had a zoonotic spread of Ebola virus before human-human contacts predominated as the main transmission route. Table (2) displays the current EVD's epidemiological attributes between 2001 and 2025.

Table 2: Epidemiological Features of EVD between 2001 and 2025 in Sub-Saharan Africa

Year of Outbreak	Country of Affectation	Ebola virus Species	Cases	Mortality	Case Fatality Ratio (CFR)
2001	Gabon	Zaire virus	65	53	81.5
2001	Congo DRC	Zaire virus	59	44	74.6
2003	Congo DRC	Zaire virus	178	157	88.2
2004	Sudan	Sudan virus	17	7	41.2
2005	Congo DRC	Zaire virus	12	10	83.3
2007	Congo DRC	Zaire virus	264	187	70.8
2007	Uganda	Bundibugyo virus	131	42	32.1
2008	Congo DRC	Zaire virus	32	15	46.9
2011	Uganda	Sudan virus	1	1	100
2012	Uganda	Sudan virus	17	7	41.2
2012	Congo DRC	Bundibugyo virus	36	13	36.1
2014	Congo DRC	Zaire virus	69	49	71.0
2014	Senegal	Zaire virus	1	0	0.0
2014	Guinea	Zaire virus	3814	2544	66.7
2014	Liberia	Zaire virus	10678	4810	45.0
2014	Sierria Leone	Zaire virus	14124	3956	28.0
2014	Mali	Zaire virus	8	6	75.0
2014	Nigeria	Zaire virus	20	8	40.0
2017	Congo DRC	Zaire virus	8	4	50.0
2018	Congo DRC	Zaire virus	3524	2320	65.8
2020	Congo DRC	Zaire virus	130	55	42.3
2021	Guinea	Zaire virus	23	12	52.5
2021	Congo DRC	Zaire virus	23	15	65.2
2022	Congo DRC	Zaire virus	6	6	100
2022	Uganda	Sudan virus	164	55	33.5
2025	Uganda	Sudan virus	14	4	28.6 (As of 7 March 2025)
Total			33, 418	14,380	43.0

Adapted and Modified from Izudi J, Bajunirwe F. Case fatality rate for Ebola disease, 1976–2022: A meta-analysis of global data. Elservier Journal of Infection and Public Health. 2024; 17: 25–34and Schnirring, Lisa. Second Cluster recorded in Uganda's Ebola Sudan Outbreak. Centre for Infectious Diseases and Research Policy. 7 March 2025. https://www.cidrap.umn.edu/ebola/second-cluster-reported-ugandas-ebola-sudan-outbreak[30]

Table (2) highlights the epidemiological features of EVD in the contemporary era, 2001-2025 in sub–Saharan African context. Likewise in the previous EVD era between 1976 and2000, DRC Congo, Uganda and Gabon witnessed the lion's share of EVD epidemics in sub-Saharan Africa.DRC Congo particularly witnessed EVD epidemics, 12 times out of 26 between 2001 and 2025 (46 percent). Apart from the 2014-2016 large EVD epidemics in West African clime, central and southern Africa were particularly afflicted with recurrent EVD outbreaks. Zaire ebolavirus and Sudan ebolavirus are the predominant causes of EVD epidemics in sub-Sahara Africa. Zaire virus was responsible for EVD on

19 occasions out of 26 epidemics encountered between 2001 and 2025 (73 percent). Contrastingly to the previous era, Zaire ebola virus had a casefatality ratio of 28 to 100 percent, which compares with the case fatality ratio of the Sudan ebolavirus ranging from 28.6 to 100 percent.

As shown in Table (1), EVD outbreaks particularly occurred in the central African belt of DRC Congo and Uganda. West African countries such as Guinea, Liberia, Mali, Sierra Leone, Senegal, and Nigeria encountered the main incursion of Ebola epidemics only from 2014 to 2016, and they remained the largest outbreaks of EVD claimingover 11,300 lives out of over 28,600 cases.[21] The CFR during the West African outbreaks ranged from 28 to 75 percent while the overall case fatality ratio between 2001 and 2025 spanned from 28 to 100 percent. From 2017 to 7 March 2025, Table (2) shows that sub-Saharan Africa witnessed 3,892 cases including 2,471 fatalities with a CFR of 64 percent. Table (2) highlights 26 epidemics of EVD resulting in a total of 14,380 deaths out of 33,418 cases with an average CFR of 43.0 between 2001 and 2025. It is in sharp contrast to ten EVD epidemics occasioning 1030 mortalities out of 1522 sufferers and an average CFR of 67.8 from 1967 and 2000.

III. Discussions

Congo DRC has witnessed the lion's share of EVD outbreaks since its first epidemics in 1976. From our analysis, 46 percent of EVD epidemics occurred in DRC Congo between 2001 and 2025. The reasons are not far-fetched. Congo DRC formerly Zaire, especially the eastern area has been bedeviled with a long history of wars, political instabilities, and concomitant social displacements.[31] It has the highest rate of Internally Displaced Persons (IDPs) globally.[32] This has continuously occasioned populations' encroachment into the sanctuaries of wild games and bats. Consequently, they are exposed to known reservoirs of ebolavirus. The weakened health system, frequent migration of people from troubled areas, and poor health infrastructures have predisposed Congo DRC to frequent outbreaks of filoviral diseases including MVD and EVD. On the regional front, the risk of another EVD outbreak is even greater soon because of the present socio-political unrest. As of 26 February 2025, the M23 faction has captured Goma and Bukavu in eastern Congo DRC with the possibilities of heightened hostilities and its attendant humanitarian crisis spreading around central Africa.[33]

Regarding other surrounding countries, Uganda and Sudan as well as most sub-Saharan states are blessed with lush and thick forests that harbor fruit bats, which are known as the major dispersal of fruits and enhancer of forestation through their extensive migration.[33] It is noteworthy that the principally hot and humid climate of most sub-Saharan states favors the breeding and migration of fruit bats.[34] Their migratory impacts to nearby countries with weakened health systems and poor surveillance, especially in predominantly rural settings enhance recurrent EVD epidemics. Concerning the 2014-16 Ebola epidemics in West Africa, the consumption of wild animals, cultural practices regarding dead bodies and mistrust of Western medicine, and poor surveillance and disease preparedness facilitated the swift dispersal of EVD causing huge mortalities in Liberia, Guinea, and Sierra Leone.[35]

Regarding the causative strains of EBV, Zaire virus and Sudan virus are the predominant Ebola virus species causing over 90 percent of outbreaks between 2001 and 2025. Zaire ebola virus is regarded as the primary cause of EBV. It has the highest mortality rates among the six strains of ebolavirus due to its high virulence and transmissibility rate among humans after initial zoonotic transmission.[36] However, this piece reveals comparable case fatalities of Sudan's ebola virus in the contemporary era. This might be due to increasing urbanization and exposure of humans to other virulent strains of ebolavirus from wild games and bats.

In this review, we discovered a case fatality rate of 28 to 100 percent and an average of 43.0 percent CFR between 2001 and 2025. This high fatality rate is consistent with W.H.O data that stipulates a CFR range of 25 to 90 percent, and an average CFR of 50 percent from past outbreaks depending on response and peculiarities.[2] Our average value of 43.0 CFR is more consistent with W.H.O data and a similar reviews that calculated an average CFR of 43.8 percent for all EVD outbreaks since 1976.[37,38] This is because we collated data and computed the CFR from past EVD epidemics like W.H.O and previous reviews on EVD, hence the correlation of epidemiological data. However, the average CFR of EVD between 2001 and 2025, 43.0 percent, is comparatively lower than 67.8 percent obtained from 1976 to 2000. It might be due to marginal improvement in disease preparedness, clinical suspicion, laboratory testing, and community awareness and engagement. For instance, in the Ebola outbreak in Uganda, in 2022, 55 mortalities were recorded out of 164 and a lower CFR rate of 38.6 percent was reported. The index cases were promptly identified, contacts were traced, and the victims had hygienic burial to mitigate escalation to wider epidemics. The 2025 Ebola outbreak in Uganda was even better managed and effectively contained so far. Within days of announcement of Sudan ebola virus outbreak, the Ugandan Ministry of Health had launched a trial of candidate Ebola vaccine and vaccinated up to 264 contacts.[30] The distribution of about 2000 doses of antiviral drugs effective in stemming the viral loads in EVD patients is ongoing in Uganda.

IV. Conclusions and Recommendations

Given the high virulent and transmissibility rate of EVD, its capability of transcending regional boundaries to escalate to a devastating pandemic like COVID-19 remains plausible. The devastating mortalities occasioned by the Zaire ebolavirus in West Africa from 2014-16 is a testament to this reality. Between 2017 and 7 March 2025, there were eight ebola epidemics in sub-Saharan Africa. They killed 2,471 people out of 3,892 cases with a CFR of 64 percent. The recurrent rate of EVD outbreaks is a grave public health issue in the post-modern era. Hence the world needs to mitigate the pandemics occasioned by high-risk pathogens such as ebolavirus. In this light, this work chronicled the historical trajectory of EVD, computed and compared key epidemiological values in sub-Saharan Africa between 1976 and 2000 to and 2001-2025. We discovered there were ten EVD epidemics from 1976 to 2000, in contrast to 26 EVD outbreaks encountered between 2001 and 2025 in sub- Saharan Africa. DRC Congo witnessed the lion's share of EVD outbreaks since1976 due to its recurrent political turmoil, massive socio-cultural displacements, weakened healthcare in frastructures. There was a preponderance of Zaire ebolavirus in the causation of EVD from 1976 to 2025. However, its dominance as a cause of EVD became more pronounced in the present era. It accounted for 73 percent of outbreaks between 2001 and 2025. We found an average CFR of 43.0 percent for EVD outbreaks between 2001 and 2005 as opposed to a higher CFR of 67.8 percent from 1976 to 2000. The reasons alluded for this difference are marginal improvement in disease preparedness, surveillance, case testing and vaccinations as well as community education and promotion in contemporary settings.

To mitigate recurrent EVD epidemics in sub-Saharan Africa, there should be heightened community education about common reservoirs, early symptoms and general management of EVD. This is imperative given the indistinguishable nature of early EBV from common tropical diseases such as malaria, typhoid, gastroenteritis, and yellow fever. Some evidence proves transmission via care giving and burial preparations in cultural contexts of some sub-Saharan African countries.[39] Hence, there is a continuing need for community education regarding hygienic burials and prevention of undue exposure to Ebola dead bodies to prevent escalations of infections. Local populations and tourists should be abreast of transmission reservoirs such as fruit bats and wild animals, and efforts should be made to minimize contact with them and their discharges. Miners and tourists should be equipped with hand gloves, face masks, and protective clothing when they visit mines and caves.

Even though fruit bats have been recognized as the putative reservoirs of the Ebola virus, it is astounding to highlight that the virus has never been isolated in them.[40,41] Therefore, we do not completely understand how fruits bats transmit it to other animals and humans. To comprehend these dynamics, we must intensify our studies on fruit bats, especially of the order *Chiroptera* that serve as the intermediate hosts of ebolavirus. Bat surveillance should be strengthened to understand the migration, breeding, and transmission mechanism of *Chiroptera* among themselves and between chimpanzees, monkeys, wild games and humans. This would further enhance our knowledge concerning the epidemiology of EVD to curtail its dispersal to humans and prevent its recurrent outbreaks in contemporary sub-Saharan Africa.

The predominantly rural settings of most sub-Saharan countries are characterized by overwhelming poverty and ignorance of dwellers, and poor access to health care. Besides, there is a level of mistrust in government health institutions due to inadequate manpower and laboratory capacities. To adequately manage the contagion, a quick and correct laboratory diagnosis is imperative. The early infections are detected through serological assays detecting immunoglobulin M via enzyme-linked immunosorbent assay (ELISA). The confirmation of diagnosis of EVD is mainly premised on detecting viral RNA in blood samples using reverse transcription-polymerase chain reaction (RT-PCR).[42] Many sub-Saharan climes, especially the rural and remote areas lack these laboratory capacities in their healthcare system. Therefore, the need for improvement of health funding and strengthening of disease surveillance mechanisms in most economies could not be overstated. It behooves African Union as well as the United Nations to find lasting solutions to recurrent wars to curb social and health crises in the DRC Congo and the surrounding countries. This would go a long way in stemming recurrent EVD outbreaks occasioned by social dislocations of people with concomitant exposure to reservoirs of ebola virus.

There is no definitive treatment for EBV except supportive management. However, the WHO has approved the deployment of two monoclonal antibodies for treating EBV, namely mAb114 (Ansuvimab; Ebanga) and REGN-EB3 (Inmazeb).[2] They have been shown to reduce the production of anti-Ebola virus antibodies in survivors of the disease, which could predispose to reinfection or reactivation.[43] Regarding the prevention of EVD and reduction of frequent outbreaks, ERVEBO and Zabdeno/Mvabea are two recognized vaccines against Zaire ebola virus. However, they are only effective against Zaire ebola virus limiting their use for EVD caused by other strains. Other research aimed at discovering vaccines for other strains is ongoing. However, there are fears regarding the suitability and safety of the existing Ebola vaccines on the long term. In the future, we foresee the discovery of vaccines for

othere bolavirus species and their deployment in vulnerable areas in sub-Saharan Africa. Apart from vaccines, community education, early diagnosis, and commencement of prompt management of EVD cases are effective in reducing its fatalities and curbing the swift dispersal of ebolavirus. Then, early hospitalization, early fluid and electrolyte replacement, oxygen care, blood transfusion, and other supportive treatment remain the mainstay of management in the present era and the nearest future. They remain viable and effective modalities of reducing mortalities due to EVD and mitigating its escalation to a cataclysmic pandemic.

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Five Plants with Promising Antimicrobial Peptides

By Hend N. Essawaf, Hadeer N. Abuwarda, Abdelraouf A. Elmanama & Fadel A. Sharif

Abstract- Antimicrobial peptides (AMPs) are bioactive molecules known for their strong antimicrobial properties against a variety of microorganisms. The increasing prevalence of antibiotic-resistant infections, coupled with a scarcity of newly developed antibiotics, has intensified the search for novel sources of AMPs. Plant-derived AMPs present several benefits compared to conventional antibiotics. Consequently, this research focused on screening various plant species to identify new antimicrobial peptides. Water-soluble proteins were extracted from the dried plant materials using an aqueous extraction buffer. The resulting extracts were evaluated for antibacterial activity, with the minimum inhibitory concentration (MIC) being determined, followed by separation through thin layer chromatography and subsequent agar overlay bioautography. Protein extracts from five different plants exhibited antibacterial properties against both Gram-positive and Gram-negative bacteria, with inhibition zones measuring between 11 and 22 mm.

Keywords: *antimicrobial peptides, plant extract, antibiotic resistance, gram-positive, gram-negative bacteria.*

GJMR-C Classification: *NLMC: QK861*

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Five Plants with Promising Antimicrobial Peptides

Hend N. Essawaf [α], Hadeer N. Abuwarda [σ], Abdelraouf A. Elmanama [ρ] & Fadel A. Sharif [ω]

Abstract- Antimicrobial peptides (AMPs) are bioactive molecules known for their strong antimicrobial properties against a variety of microorganisms. The increasing prevalence of antibiotic-resistant infections, coupled with a scarcity of newly developed antibiotics, has intensified the search for novel sources of AMPs. Plant-derived AMPs present several benefits compared to conventional antibiotics. Consequently, this research focused on screening various plant species to identify new antimicrobial peptides. Water-soluble proteins were extracted from the dried plant materials using an aqueous extraction buffer. The resulting extracts were evaluated for antibacterial activity, with the minimum inhibitory concentration (MIC) being determined, followed by separation through thin layer chromatography and subsequent agar overlay bioautography. Protein extracts from five different plants exhibited antibacterial properties against both Gram-positive and Gram-negative bacteria, with inhibition zones measuring between 11 and 22 mm. The extract from *Raphanus sativus* demonstrated the most extensive antibacterial activity, with inhibition zones ranging from 16 to 19 mm against *Staphylococcus aureus* ATCC 12493, *Streptococcus epidermidis* ATCC 12228, *Escherichia coli* NCTC 13846, and *Acinetobacter baumannii* ATCC 19606. The extract of *Vitis labrusca* showed inhibition zones of 18 mm and 11 mm against *S. aureus* and *S. epidermidis*, respectively, while *Ricinus communis* exhibited inhibition zones of 15 mm and 22 mm against the same bacteria. *Hibiscus sabdariffa* extract displayed a smaller inhibition zone of 12 mm against *Pseudomonas aeruginosa* ATCC 27853. Concentrations ranging from 0.45 to 1.97 mg/ml of the protein extracts from *V.labrusca, R. communis, R. sativus*, and *Prunus dulcis* effectively inhibited the growth of *S. aureus*, while concentrations between 0.45 and 4.06 mg/ml were effective against *S. epidermidis*. Additionally, *R. sativus* inhibited the growth of *E. coli* and *A.baumannii* at concentrations of 0.53 and 1.07 mg/ml, respectively. *H. sabdariffa* inhibited the growth of *P. aeruginosa* at a concentration of 0.45 mg/ml. Subsequent examination through TLC bioautography targeted the inhibitory substances present in *V. labrusca, R. communis, R. sativus*, and *H. sabdariffa*. This research revealed that specific protein extracts derived from five different plant species demonstrated antibacterial properties against particular bacterial strains. Notably, the protein extracts from *R. sativus, R. communis*, and *H. sabdariffa* exhibited broad-spectrum antibacterial activity and were recognized as having a significant likelihood of containing antimicrobial peptides (AMPs). Nevertheless, additional investigation is necessary to pinpoint the exact antibacterial components that contribute to the observed effects.

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Keywords: antimicrobial peptides, plant extract, antibiotic resistance, gram-positive, gram-negative bacteria.

I. Introduction

Every year, around 700,000 patients succumb to antimicrobial resistance (AMR) globally. Projections indicate that this mortality rate could rise to 10 million by the year 2050 (O'Neill, 2014).

The emergence of superbugs, which are pathogenic bacteria resistant to the majority or entirety of available antibiotics, presents a significant challenge. The World Health Organization has cautioned that these multidrug-resistant pathogens could potentially revert global health to conditions reminiscent of the pre-antibiotic era (WHO, 2017).

There is a pressing necessity to identify novel alternatives to address the issue of antimicrobial resistance. Potential solutions include bacteriophages, antibodies, probiotics, lysins, and antimicrobial peptides (AMPs), which have shown promise in tackling this critical challenge (Ghosh, Sarkar, Issa, & Haldar, 2019).

AMPs are short oligopeptides, comprising up to 50 amino acids, that are present in all living organisms. Plant-derived AMPs represent a valuable natural alternative to synthetic antibiotics for applications in human healthcare and agriculture, particularly for protection and healing (Almeida et al., 2020; Tang, Prodhan, Biswas, Le, & Sekaran, 2018).

These peptides exhibit a wide range of activities, including antibacterial, antifungal, antiviral, and even anticancer properties (R. E. W. Hancock, 2001; Kamysz, Okrój, & Łukasiak, 2003). Their mechanisms of action are varied and closely linked to their structural characteristics; AMPs interact electrostatically with bacterial cell walls, leading to a disruption of membrane integrity (Tzong-Hsien, Kristopher, & Marie-Isabel, 2016). Additionally, these peptides possess the ability to penetrate biological membranes, allowing them to exert effects intracellularly (Cardoso et al., 2019). Beyond their antimicrobial functions, AMPs also play a significant role in immunomodulation (Haney & Hancock, 2013).

The broad activity spectrum, straightforward synthesis, and specific mechanisms of action associated with antimicrobial peptides (AMPs) significantly mitigate the risk of bacterial resistance. This makes them attractive candidates for the development of new therapeutic agents to address the pressing issue

of bacterial resistance (Baltzer & Brown, 2011). There is a critical need to identify new AMPs that can either replace or improve the effectiveness of current antibiotics. Moreover, AMPs can be easily obtained from natural sources, such as plants, for applications in human healthcare, agriculture, and the food industry.

This research focused on screening a variety of plant species to identify novel antimicrobial peptides. We achieved this by extracting protein and peptide fractions from the plants and evaluating their antimicrobial efficacy against several standard bacterial strains. The study involved determining the minimum inhibitory concentration of the extracts exhibiting antibacterial properties, isolating the plant protein mixture into distinct proteins and peptide fractions, and localizing the active compounds present in the extracts with antibacterial activity.

II. Materials and Methods

a) Plant Material

Seeds and leaves of various plants screened for the presence of antimicrobial peptides (AMPs) are listed in Table 1.

Table 1: List of Various Plants Screened for the Presence of Antimicrobial Peptides

No.	Binomial Name	Family	Part tested	Protein Concentration (µg/ml)
1.	*Punica granatum*	*Lythraceae**	seeds	261
2.	*Vitis labrusca 'Fragola'*	*Vitaceae**	seeds	4064
3.	*Annona*	*Annonaceae**	seeds	4285
4.	*Ricinus communis*	*Euphorbiaceae**	seeds	1797
5.	*Ocimum basilicum*	*Lamiaceae**	seeds	4561
6.	*Foeniculum vulgare*	*Apiaceae**	Grounded seeds	2557
7.	*Cucumis melo flexuosus*	*Cucurbitaceae**	seeds	5806
8.	*Ammi visnaga*	*Apiaceae**	seeds	1037
9.	*Brassica rapa subsp. Rapa*	*Brassicaceae**	seeds	1659
10.	*Matricaria recutita*	*Asteraceae**	Leaves, flowers	2351
11.	*Raphanus sativus*	*Brassicaceae**	seeds	2156
12.	*Coriandrum sativum*	*Apiaceae**	Leaves	2557
13.	*Lepidium sativum*	Brassicaceae*	Leaves	414
14.	*Thymus vulgaris*	*Lamiaceae**	Leaves	3253
15.	*Olea europaea*	*Oleaceae**	Leaves	4936
16.	*Psidium guajava*	*Myrtaceae**	Leaves	2323
17.	*Zingiber officinale*	*Zingiberaceae**	roots	2653
18.	*Pimpinella anisum*	*Apiaceae**	Leaves	3285
19.	*Rosmarinus officinalis*	*Lamiaceae**	Leaves	1543
20.	*Trigonella foenum-graecum*	*Fabaceae**	Leaves	4147
21.	*Nigella sativa*	*Ranunculaceae **	seeds	4117
22.	*Rumex obtusifolius L*	*Polygonaceae**	seeds	1707
23.	*Salvia hispanica*	*Lamiaceae**	seeds	1102
24.	*Cuminum cyminum*	*Apaiaceae**	Grounded seeds	1382
25.	*Cinnamomum zeylanicum*	*Lauraceae**	Cinnamon sticks	2599
26.	*Prunus dulcis*	*Rosaceae**	seeds	1974
27.	*Hibiscus sabdariffa*	*Malvaceae**	flowers	1828
28.	*Brassica oleracea var. capitata f. rubra*	*Brassicaeae**	Leaves	4212

**Source: ("USDA, NRCS. 2022. The PLANTS Database,").*

b) Bacteria, Growth Media and Reagents

The isolated antimicrobial peptides (AMPs) were evaluated for their efficacy against various bacterial strains from the ATCC collection, which included Gram-positive species such as *Staphylococcus aureus* ATCC 12493, *Streptococcus epidermidis* ATCC 12228, and *Bacillus subtilis* ATCC 6633, as well as Gram-negative species including *Escherichia coli* NCTC 13846,

Klebsiella pneumoniae ATCC 2021, *Pseudomonas aeruginosa* ATCC 27853, and *Acinetobacter baumannii* ATCC 19606.

Muller Hinton Agar (MHA), Nutrient Agar (NA), Nutrient Broth (NB), Brain Heart Infusion Broth (BHIB), 50 mM phosphate buffer pH 7, 2 mM EDTA, Glycerol, 50 mM NaCl, distilled water (D.W), tap water, 0.1% 2,3,5 triphenyl tetrazolium chloride (TTC), n-butanol, acetone, acetic acid, 5 % ammonia, ninhydrin, methylthiazol tetrazolium (MTT).

c) Total Water-Soluble Proteins Extraction

The plant material underwent a washing process with tap water followed by distilled water, after which it was dried under sunlight. For the extraction of water-soluble proteins, the milled plant material was combined with a cold extraction buffer at a ratio of 1:10 (w/v), consisting of 50 mM phosphate buffer at pH 7, 2 mM EDTA, 5% glycerol, and 50 mM NaCl. Subsequently, the mixture was agitated on a shaker for two hours at a temperature of 4°C, followed by centrifugation at 12,000 rpm for 20 minutes at the same temperature. The clear solution was then filtered through sterile gauze and stored at -20°C (Aliahmadi, Roghanian, Emtiazi, & Ghassempour, 2011).

d) Total Protein Concentration Estimation

The concentration of the extracted water-soluble proteins was estimated using a nano-drop spectrophotometer.

e) Testing Extracts on Bacteria by Agar Well Diffusion Assay

Muller Hinton agar (MHA) plates were inoculated using a cotton swab that had been moistened with a McFarland standardized test organism. Using a sterile pipette blue tip, holes measuring 6 to 8 mm in diameter were created and subsequently filled with 50 μL of the plant extract. The petri dishes were then incubated at 37°C for a duration of 24 hours. The diameters of the zones of growth inhibition were measured. The inoculum was prepared in a sterile saline solution, with its turbidity adjusted to the 0.5 McFarland standard (10^8 CFU/mL). A 3% potassium iodide (KI) solution was utilized as a positive control (Abed, 2015; Nigussie, Davey, Legesse, Fekadu, & Makonnen, 2021).

f) Determination of Minimum Inhibitory Concentration (MIC) by Microtiter Broth Dilution Method

In a 96-well microtiter plate, 100 µL of plant extract was introduced into the first well, while 50 µL of BHIB media was allocated to each of the remaining wells. Subsequently, 50 µL was extracted from the first well and transferred to the second well, continuing this process until the last well to achieve serial dilution for each extract. A volume of 50 µL of bacterial suspension, calibrated to the 0.5 McFarland standard, was added to each well, with the exception of the last well designated as the "negative control." The plates were then covered and incubated at 37°C for 24 hours. Following this incubation, 20 µL of 0.1% 2,3,5 triphenyl tetrazolium chloride (TTC) was introduced into each well, and the plates were re-incubated for an additional 15 minutes. The plates were subsequently examined, and the final dilution that exhibited antibacterial activity was identified by noting the last well that did not display red coloration, as the presence of red color indicates bacterial growth (Abou-Elkhair, Fadda, & Abu-Mohsen, 2010).

g) Protein and Peptide Separation by Thin Layer Chromatography (TLC)

This technique was derived from the work of Jaskiewicz et al. (2016), utilizing Silica gel 60 RP-18 F254S (Merck) thin-layer chromatography (TLC) plates as the stationary phase. A volume of 5 µL from each plant extract was applied to the TLC plate. The separation process employed a mobile phase composed of n-butanol, acetone, acetic acid, 5% ammonia, and distilled water in a ratio of 4.5:1.5:1:1:2 (v/v/v/v/v) within a sealed chamber. The separation was allowed to proceed until the solvent front reached the end line, at which point the plates were removed and air-dried to facilitate the evaporation of the eluents. Subsequently, the dried plates were treated with a 2% (w/v) ninhydrin solution in a mixture of acetone and glacial acetic acid (25:1, v/v). Following this, the plates were left to dry for several minutes at ambient temperature before being heated in an oven at 80°C until the peptide zones became visible (Gwarda, Tomczyszyn, Misicka, & Dzido, 2013; Sharma, Abid, & Sajgotra, 2017). For subsequent antibacterial assays, the TLC plates were sterilized under ultraviolet light for 15 minutes. The identification of specific compounds was constrained by the lack of available reference standards.

h) TLC-Agar-Overlay Bioautography

Developed TLC plates were placed within a sterile Petri dish. A suspension of representative bacterial strains, measuring one milliliter, was combined with 10 mL of melted Mueller-Hinton agar, which was then poured as a thin layer over the developed TLC plate. Following the solidification of the medium, the TLC plate was incubated for 24 hours at a temperature of 35 ± 2°C. Subsequently, the TLC-bioautography plates were sprayed with an aqueous solution of methylthiazol tetrazolium (MTT) at a concentration of 2.5 mg/mL (Sigma, USA). Clear zones of inhibition were noted against a purple background (Valle, Puzon, Cabrera, & Rivera, 2016).

III. Results

a) Total Protein Concentration Estimation

The plant protein extracts examined in this study exhibited a range of protein concentrations between 261 and 5806 μg/ml. Among these, the protein extract from

Punica granatum displayed the lowest protein content, while the extract from *Cucumis melo flexuosus* demonstrated the highest protein concentration. Detailed protein concentrations for each plant extract can be found in Table (1).

b) *Testing Plants Protein Extracts on Bacteria by Agar Well Diffusion Assay*

The protein extracts derived from five different plant species exhibited varying levels of antibacterial activity against a range of both Gram-positive and Gram-negative bacteria, with inhibition zones measuring between 11 and 22 mm. Extracts from *Ricinus communis* and *Vitis labrusca* demonstrated antibacterial effects against the Gram-positive bacteria *S. aureus* and *S. epidermidis*. The extract from *Raphanus sativus* displayed a more extensive antibacterial spectrum, affecting both Gram-positive (*S. aureus* and *S. epidermidis*) and Gram-negative bacteria (*E. coli* and *A. baumannii*). Notably, none of the protein extracts exhibited antibacterial activity against *B. subtilis* or *K. pneumoniae*. Antibacterial effects were recorded against *S.aureus* for the extracts of *R. sativus, R. communis, V. labrusca,* and *Prunus dulcis*, with inhibition zones measuring 16, 15, 18, and 14 mm, respectively (Figure 1).

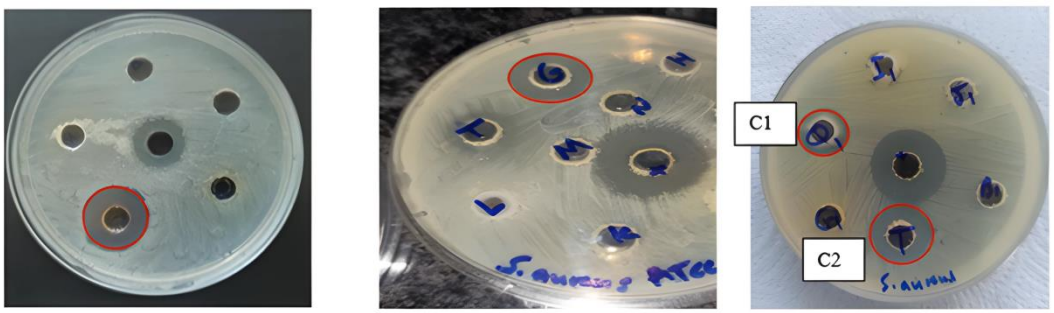


Figure 1: Antibacterial activity of plant protein extracts against *S. aureus*(A) *Vitis labrusca*, (B) *Ricinus communis*, (C1) *Prunus dulcis* and (C2) *Raphanus sativus*

Figure 2 illustrates that the extracts of *R. sativus*, *R. communis*, and *V. labrusca* exhibit antibacterial properties against *S. epidermidis*, resulting in zones of inhibition measuring 20 mm, 22 mm, and 11 mm, respectively.

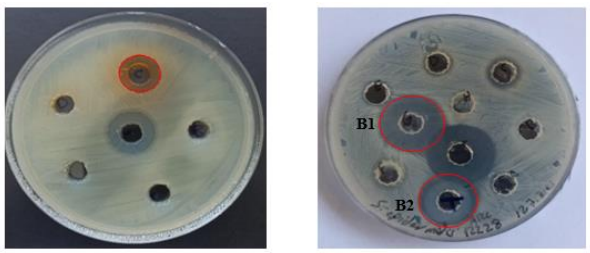


Figure 2: Antibacterial activity of plant protein extracts against *S. epidermidis*.(A) *Vitis labrusca*, (B1*) Ricinus communis*, (B2) *Prunus dulcis*

R. sativus has demonstrated antibacterial properties against *E. coli*, exhibiting an inhibition zone of 19 mm, and against *A. baumannii*, with an inhibition zone of 16 mm, as illustrated in Figure 3.

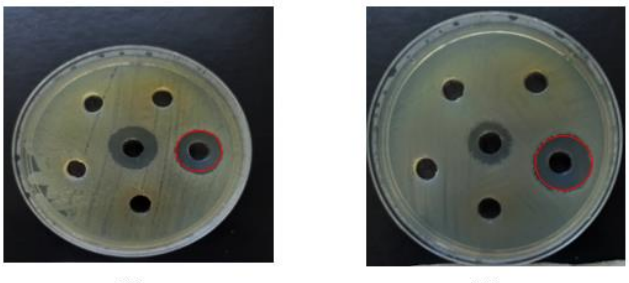


Figure 3: Antibacterial activity of *R. sativus* protein extract. (A) *A. baumannii* and (B) *E. coli*

The growth of *P. aeruginosa* was suppressed by the protein extract of *H. sabdariffa*, resulting in a modest zone of inhibition measuring 12 mm, as illustrated in Figure 4.

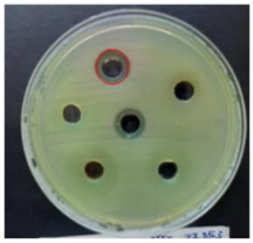


Figure 4: Antibacterial Activity of *Hibiscus Sabdariffa*protein Extract Against *P. Aeruginosa*.

c) *Determination of Minimum Inhibitory Concentration (MIC) by Microtiter Broth Dilution Method*

The minimum concentration that resulted in the inhibition of bacterial growth by plant protein extracts was determined through the observation of color changes in the wells of a 96 microtiter plate, utilizing the micro broth dilution assay alongside TTC as a growth indicator. The protein extracts from *V. labrusca, R. communis, R. sativus*, and *P. dulcis* exhibited growth inhibition against *S. aureus* at concentrations of 2.03, 0.45, 0.54, and 1.97 mg/ml, respectively. Conversely, the protein extracts from *V. labrusca, R. communis*, and *R. sativus* demonstrated inhibitory effects on the growth of *S. epidermidis* at concentrations of 4.06, 0.45, and 0.54 mg/ml. Additionally, the growth of *A. baumannii* and *E. coli* was inhibited at concentrations of 1.07 mg/ml and 0.53 mg/ml for *R. sativus*, respectively. Furthermore, the growth of *P. aeruginosa* was inhibited by a concentration of 0.45 mg/ml of *H. sabdariffa* protein extract.

d) *Protein and peptide separation by Thin Layer Chromatography (TLC)*

Antibacterial activity from plant protein extracts was isolated using the thin-layer chromatography (TLC) technique, and the resulting fractions were analyzed with ninhydrin spray. The separation of extracts from *V. labrusca* (Fig. 5-A), *P. dulcis* (Fig. 5-D), and *P. guajava* (Fig. 5-E) yielded two distinct bands. In contrast, the separation of *R. communis* resulted in a continuous line featuring two distinct bands, one positioned at the beginning and the other at the end of the migration line (Fig. 5-B). The separation of *Raphanus sativus* displayed two intertwined bands (Fig. 5-C). Additionally, the separation of *H. sabdariffa* resulted in three distinct bands.

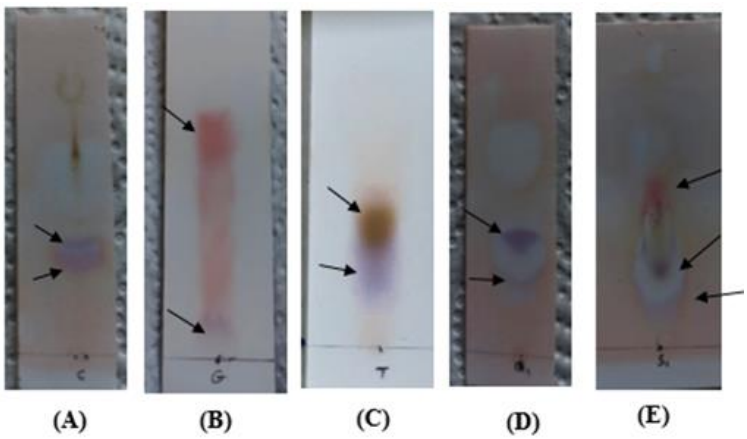


Figure 5: TLC plates of plant protein extracts visualized after coloring with ninhydrin solution. (A) *Vitis labrusca*, (B) *Ricinus communis*, (C) *Raphanus sativus*, (D) *Prunus dulcis*, and (E) *Psidium guajava.* Arrows point to separated fractions

e) *TLC-Agar-Overlay Bioautography*

The antibacterial activity of the compounds separated on TLC was further evaluated using thin-layer chromatography (TLC) agar overlay bioautography. The antibacterial properties of *V. labrusca, R. communis, R. sativus, Prunus dulcis, Psidium guajava,* and *H. sabdariffa* were evidenced by the presence of clear zones of inhibition against the respective bacteria on a purple background. Notably, *V. labrusca, R. communis,* and *R. sativus* exhibited inhibitory effects against both *S. aureus* and *S.epidermidis*. Additionally, *R. sativus* demonstrated inhibitory activity against *E. coli* and *A. baumannii*, while *H. sabdariffa* showed effectiveness against *P. aeruginosa*. Conversely, *Prunus dulcis* did not

produce any zones of inhibition against *S. aureus.* The antibacterial effects of *V. labrusca* (Fig. 6-A) and *R. sativus* (Fig. 6-C) against *S.aureus* are illustrated by clear zones near the origin line, with the absence of stained bands on the corresponding TLC plates indicating that the inhibitory compounds are non-protein in nature. Two stained compounds on the TLC plates of *R. communis* (Fig. 6-B) resulted in two zones of inhibition, one located near the origin line (R_f = 0.06) and the other positioned in the middle of the TLC plate (R_f = 0.73).

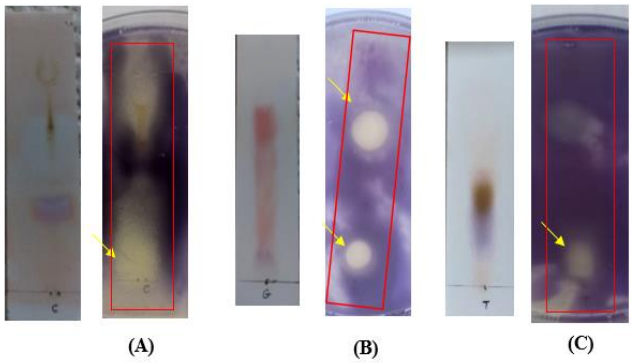


Figure 6: TLC-agar-overlay bioautography of seed extract of (A) *V. labrusca*, (B) *R.communis*, and (C) *R. sativus* against *S. aureus*. Growth inhibition zones are indicated by arrows.

Comparable outcomes were observed in agar overlay bioautography using S. epidermidis, where V. labrusca (Fig. 7-A) and R. sativus (Fig. 7-C) exhibited minor inhibitory zones near the origin line. In contrast, two stained compounds on the R. communis TLC plate (Fig. 7-B) revealed two distinct zones: one located close to the origin line (R_f = 0.06) and the other positioned in the middle of the TLC plate (R_f = 0.73).

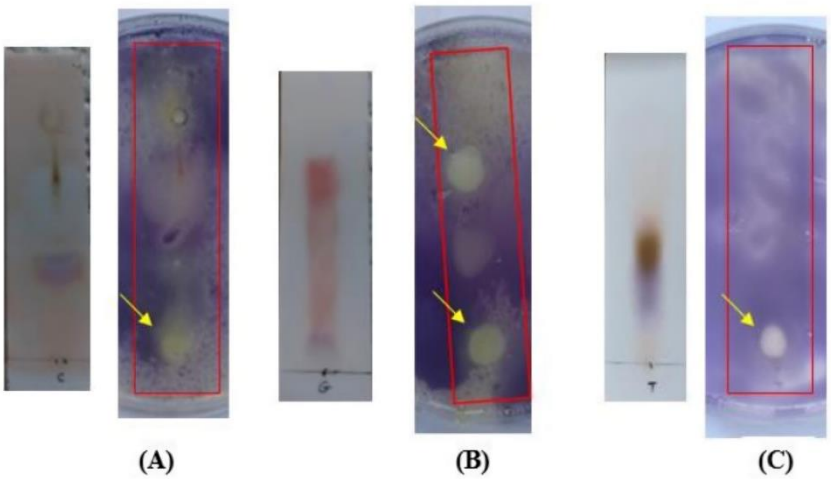
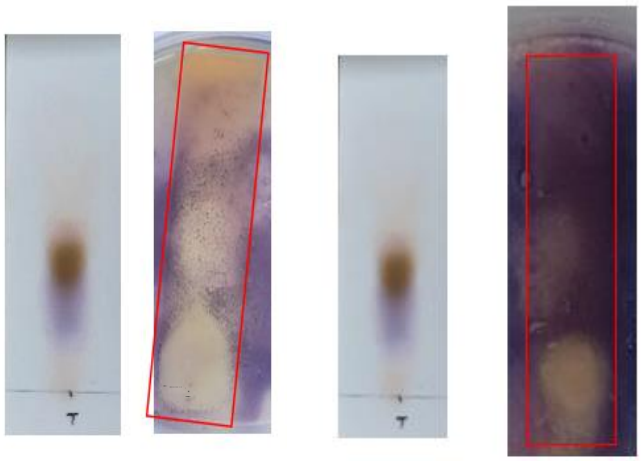


Figure 7: TLC-agar-overlay bioautography of seed extract of (A) *V. labrusca*, (B) *R. communis,* and(C) *R. sativus* against *S. epidermidis*

Non-stained fraction on the line of origin of *R. sativus* TLC plate exhibited a large inhibitory zone against *E. coli* (Fig. 8-A) and *A. baumannii* (Fig. 8-B).



(A) (B)

Figure 8: TLC-agar-overlay bioautography of *R. sativus* seeds extract against (A) *E. coli* NCTC 13846 and (B) *A. baumannii* ATCC 19606.

Figure 9 illustrates that the TLC plate of *H. sabdariffa* exhibited three inhibitory zones against *P. aeruginosa*. The first zone had an R_f value of 0.11, the second zone had an R_f value of 0.46, and the third, which was a non-stained compound, generated a zone close to the end line.

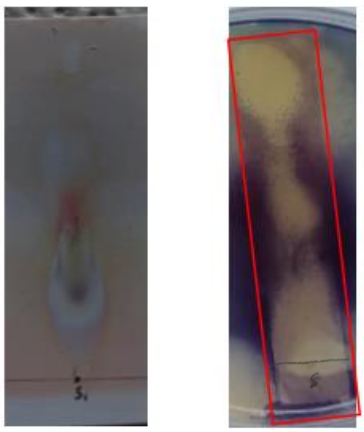


Figure 9: TLC-agar-overlay bioautography of *H. sabdariffa flowers* extract against *P. aeruginosa*

IV. Discussion

The rise in antimicrobial resistance to existing antibiotics is a major global health issue, limiting treatment options. This problem is worsened by the scarcity of newly developed antibiotics. Research shows that antibiotic resistance has increased worldwide over the past twenty years, mainly due to overprescription and misuse of antibiotics. Finding new antimicrobial agents is crucial, yet the development of new antibiotics has slowed down, stressing the need for innovative solutions (Klein et al., 2018; O'Neill, 2016).

Plants have been used for medicinal purposes for a long time and are important in discovering new antimicrobial agents. Many plants contain natural compounds that fight infections. Research emphasizes the value of plants as a source of new antimicrobial compounds due to their diverse secondary metabolites and cost-effectiveness (Cowan, 1999b; Newman & Cragg, 2012; Newman & Cragg, 2020; Atanasov et al., 2015). The extraction of antimicrobial compounds from plants has advantages over synthetic methods, such as lower toxicity risks and unique mechanisms of action. Therefore, exploring plants for new antimicrobial compounds can lead to effective treatments for drug-resistant infections (Anand et al., 2022; Akinyemi, Oladapo, Okwara, Ibe, & Fasure, 2005; Djeussi et al., 2013; Jubair, Rajagopal, Chinnappan, Abdullah, & Fatima, 2021).

Plants contain a diverse range of antimicrobial peptides that can be extracted and studied for their potential therapeutic applications (Broekaert et al., 1997;

Lei et al., 2019; Tam, Wang, Wong, & Tan, 2015). Several studies found that antimicrobial peptides were effective against drug-resistant bacteria, suggesting their potential as a therapeutic option for these infections (Chung & Khanum, 2017; S.-C. Park, Park, & Hahm, 2011).

This research was designed to evaluate various plant species in order to identify novel antimicrobial peptides through the extraction of protein and peptide fractions. The antimicrobial activity of these extracts was tested against a range of standard bacterial strains, and the peptide nature of the isolated substances was confirmed following their separation and subsequent activity testing. This study builds upon previous investigations that have highlighted the potential of peptides derived from plants as a source of new antibiotics (da Silva et al., 2012; Gully et al., 2019; Lage et al., 2018; Ljoljić Bilić et al., 2022).

During the study, twenty-eight plant seeds, leaves, or flowers were collected from local markets. A phosphate buffer was used to extract water-soluble proteins and peptides, followed by purification through TLC chromatography (Melnikova, Mineev, Finkina, Arseniev, & Ovchinnikova, 2016; Osborn et al., 1995; Taveira et al., 2014). The total protein concentration of the extracts was measured using a NanoDrop instrument, which has been documented in various studies (Jafari, Khavari Nejad, Vaziri, and Siadat 2017; Najib 2017; Desjardins and Conklin 2010).

a) *Antibacterial Activity of Plant Protein Extracts Against Several Standard Bacteria*

As part of this study, *Ricinus communis* seeds protein extract has been studied for its activity against several Gram-positive and Gram-negative standard bacteria. The findings of this study indicate that the protein extract from *R. communis* demonstrates antibacterial activity against *S. aureus* and *S. epidermidis*. The observed antibacterial properties of the protein extract may be linked to the presence of lectins, such as ricin, which are recognized for their antibacterial effects (Al-Mamun et al., 2016), as well as the presence of antimicrobial peptides (Al-Mamun et al., 2016; Boldbaatar, Gunasekera, El-Seedi, & Göransson, 2015).

Numerous studies have evaluated the antimicrobial properties of *R. communis* seed protein extract against various bacteria. For example, research conducted by Al-Mamun et al. (2016) assessed the antibacterial efficacy of the protein extract against *E. coli, P. aeruginosa,* and *S. aureus* through agar well diffusion and MIC assays, yielding MIC values of 250, 125, and 62.5 µg/ml, respectively. Additionally, a study by Afzal, Bakhsh, Ahmad, Manzoor, and Liaquat (2011) utilized the disc diffusion method alongside MIC assays to explore the antibacterial effects of *R. communis*seed protein extract against *S. aureus*, revealing a MIC value of 15.29 mg/ml. Conversely, research by Patil and Bhise (2015) examined the antimicrobial activity of *R.communis* seed aqueous extract against several bacteria, including *S. aureus* and *P.aeruginosa*, using the agar well diffusion method, and found significant antibacterial activity solely against *P. aeruginosa*. Furthermore, a study by Abd-Ulgadir, Suliman, Zakria, and Hassan (2015) concluded that the seed protein extract exhibited no notable antimicrobial activity against a variety of Gram-negative and Gram-positive bacteria.

The variations noted in the results of different studies could be attributed to the diverse methodologies used in the protein extraction from seeds (Ben Brahim et al. 2022; Djeussi et al. 2013). Additionally, differences in bacterial strains and the specific experimental culture conditions applied may also contribute to these discrepancies (Ben Brahim et al. 2022). Furthermore, the exact mechanism through which the protein extract functions has not been sufficiently clarified (Worbs et al. 2011).

This study demonstrates that the extract derived from *Raphanus sativus* seeds exhibited antibacterial properties *S. aureus*, *S. epidermidis*, *E. coli*, and *A. baumannii*, with concentrations ranging from 0.537 to 2.15 mg/ml. The antibacterial effects observed may be linked to the presence of compounds such as alkaloids, flavonoids, and saponins, which are likely to be extracted using an aqueous cold extraction method (Ahmad, Hasan, Chishti, & Ahmad, 2012).

Two investigations conducted by Ahmad et al. (2012) and Khamees (2017) assessed the antimicrobial properties of *R. sativus* seed extract against various bacterial strains, including *S. aureus, K. pneumoniae*, *P. aeruginosa*, and *E. coli*. The findings from both studies indicated that the *R. sativus* seed extract demonstrated considerable antibacterial efficacy against all bacterial strains examined. Additionally, both studies revealed the presence of various bioactive compounds, such as alkaloids, flavonoids, and saponins, within the extract, which are believed to play a role in its antibacterial effects. In a related study by Jadoun, Yazbak, Rushrush, Rudy, and Azaizeh (2016), the antibacterial activity *of R. sativus* seed extract was tested against several standard bacterial strains, including *S. aureus, Escherichia coli*, and *K. pneumoniae*, with minimum inhibitory concentrations ranging from 0.5 to 1 mg/mL. The study also identified a novel sulfur compound that contributes to the antibacterial activity. Collectively, these studies indicate that *R. sativus* extract holds promise as a natural antibacterial agent, with potential applications in food preservation and various other industries.

A study conducted by Törün, Çoban, Biyik, and Barişik (2017) examined the antimicrobial properties of methanolic extracts from *R. sativus* against ten distinct pathogenic microorganisms, such as *S. aureus, E. coli*, and *K. pneumoniae*. Utilizing the Disc diffusion method,

the findings revealed that the extract exhibited antibacterial activity solely against *S. aureus*.

In the current study, the growth of *S. aureus* was found to be inhibited by 2.03 mg/ml of *Vitis labrusca* seeds protein extract. Additionally, the extract was also observed to inhibit the growth of *S. epidermidis* at a concentration of 4.06 mg/ml. Few studies have investigated the antibacterial properties of *V. labrusca* seed extracts. Junior et al. (2021) found that the extracts presented some inhibitory effect against *E. coli* (IAL 2064) and *S. aureus*.

In this investigation, the findings indicate that the protein extract derived from *Prunusdulcis* seeds exhibits a minimal antibacterial effect against *S. aureus*. Nevertheless, the extract failed to show any antibacterial properties against the other bacteria that were examined. Conversely, research conducted by Hifza (2018) on the antimicrobial characteristics of fatty acids extracted from *P. dulcis* seeds revealed a moderate inhibitory effect against three bacterial strains, including *S. aureus*, *B. subtilis*, and *E.coli*, which aligns partially with the results of the current study.

Additional research has explored the antibacterial properties of *P. dulcis* seed extract, suggesting that the presence of various bioactive compounds, such as phenolic compounds and flavonoids, may play a role in their antibacterial efficacy (Dhingra, Kar, Sharma, & Bhasin, 2017; Shelly, Shikha, & Narayan, 2015). Consequently, while the protein extract from *P. dulcis* seeds appears to have limited antibacterial effects against *S. aureus*, it is plausible that other compounds within the seeds possess more potent antibacterial properties, warranting further investigation to assess their potential applications.

The findings of this research indicate that *Hibiscus sabdariffa* effectively inhibited the growth of *P. aeruginosa*. Numerous studies have investigated the antibacterial properties of *H. sabdariffa* calyces extract. In 2011, a study found that the methanolic extract showed antibacterial activity against various bacteria (Elmanama et al., 2011). In 2022, Khalil et al. found that the *H. sabdariffa* extract had bactericidal effects against multidrug-resistant *P. aeruginosa* from burn wound exudates. Al-Hashimi's 2012 study found significant inhibition zones for *E. coli* and *S. aureus*. Vargas-Sánchez et al.'s 2018 study found moderate to high antibacterial activity against various Gram-positive and Gram-negative bacteria.

b) Thin-layer chromatography (TLC) and TLC agar overlay bioautography for plant protein extracts

During the course of this research, the use of TLC chromatography with agar overlay bioautography to investigate *Ricinus communis* produced noteworthy findings, demonstrating two separate zones of inhibition on the agar plate against *S. aureus* and *S. epidermidis*. One zone corresponded to a fraction with an Rf value of 0.06, while the other was linked to a fraction with an Rf value of 0.73. These zones of inhibition may be attributed to the presence of lectins, such as ricin, known for their antibacterial properties, as well as antimicrobial peptides, as indicated by Al-Mamun et al. (2016). Further investigations are necessary to isolate and characterize the chemical nature of each fraction. In a separate study conducted by Lekganyane (2015), TLC chromatography was employed to separate phytochemical compounds from various leaf extracts, including that of *R. communis*. The resulting fractions were subjected to bioautography to evaluate their antimicrobial efficacy against different bacterial strains. The study revealed that a fraction with an Rf value of 0.77 demonstrated significant antimicrobial activity against *S. aureus*.

The antibacterial activity of *V. labrusca* seed extract was assessed using TLC plates and a TLC agar overlay bioautography assay. The results showed two stained fractions but no antibacterial effects against *S. aureus* and *S. epidermidis*. A zone of inhibition was noted near the sample application line, suggesting that non-proteinaceous compounds may be responsible for the antibacterial effects. Previous studies identified bioactive polyphenols like anthocyanins, flavonoids, and resveratrol in *V. labrusca*, which are known for their antioxidant, anti-inflammatory, and antimicrobial properties (Xia, Deng, Guo, & Li, 2010; Yu & Ahmedna, 2013). These compounds likely contribute to the antibacterial activity observed in the extract.

In the context of this study, the thin-layer chromatography (TLC) analysis of *Raphanus sativus* seed extract revealed two overlapping bands, neither of which demonstrated any antibacterial properties. Nevertheless, a notable zone of inhibition was detected adjacent to the sample application line on the TLC plate when tested against *S. aureus, S. epidermidis, E. coli,* and *A. baumannii*. The findings suggest that the antibacterial effects of *R. sativus* extract are unlikely to be linked to the proteins or peptides present, as evidenced by the lack of antibacterial activity in the two protein bands observed. Instead, the inhibition zone implies that other constituents within the extract may be responsible for the antibacterial effects. Research has pointed to sulfur compounds, particularly isothiocyanates, as significant contributors to the antibacterial properties of *R. sativus* extract, with sulforaphane being a prominent isothiocyanate known for its efficacy against various bacterial strains (Gutiérrez & Perez, 2004; Jadoun et al., 2016; Lim, Han, & Kim, 2016). The presence of these sulfur compounds may elucidate the antibacterial activity observed in *R. sativus* extract, underscoring its potential as a natural source of antimicrobial agents

In this research, the antibacterial properties of the extract derived from *Prunus dulcis* seeds were assessed. Interestingly, although the agar well diffusion

method indicated minimal antibacterial effects against *S. aureus*, the agar overlay bioautography did not reveal any significant antibacterial activity from the extract. This discrepancy may suggest that the concentration of antibacterial compounds present in the extract was insufficient to be detected by the agar overlay bioautography technique (Bieleski & Turner, 1966).

In the course of this investigation, the protein extract from the calyces of *Hibiscussabdariffa* was subjected to thin-layer chromatography (TLC), which successfully identified three distinct stained bands on the chromatographic plate. Subsequent evaluation of the *H. sabdariffa* extract against *P. aeruginosa* through agar overlay bioautography demonstrated the presence of several large zones of inhibition, a stark contrast to the smaller zone noted in the agar well diffusion assay. This discrepancy in findings may be attributed to the potential antagonistic interactions among the extract's components during the agar well diffusion assay; however, following separation via TLC, these compounds likely ceased to interact, thereby allowing for individual antimicrobial activity and resulting in the observed larger inhibition zones in the agar overlay bioautography. The phenomenon of antagonism among constituents within a single plant extract has also been documented in research conducted by Vaou et al. (2022) regarding artemisinins, which are antimalarial compounds derived from *Artemisia annua*. Additionally, another plausible reason for the variation in outcomes between the agar well diffusion assay and the agar overlay bioautography could be that the TLC process eliminated certain interfering substances that were present in the original extract, as noted by Bieleski and Turner (1966).

The identification of several zones of inhibition against *P. aeruginosa* indicates that the plant extract is likely composed of various bioactive compounds with antibacterial properties. Notably, only one of these inhibition zones was linked to the protein fraction, while the remaining two zones were located close to the sample application line and the edge of the TLC plate. This observation implies that a limited aspect of the antibacterial effect may be due to the presence of antimicrobial peptides. Furthermore, there is a lack of prior research that has successfully isolated or evaluated antimicrobial peptides derived from the extract of *H. sabdariffa* calyx.

In the present work, the antibacterial properties of several plant extracts, including *Vitis labrusca, Ricinus communis, Raphanus sativus, Prunus dulcis,* and *Hibiscus sabdariffa*, were examined through various methodologies such as agar well diffusion assay, micro broth dilution, bioautography, and thin-layer chromatography (TLC). The findings indicated that the antibacterial effects of these extracts could not be exclusively linked to the presence of proteins or peptides; rather, other phytochemicals, including polyphenols, flavonoids, tannins, and alkaloids, may play a significant role in their efficacy. The specific active compounds were not identified, which points to the necessity for additional investigations employing advanced techniques like mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy. The study also highlighted the intricate nature of plant extracts and the difficulties associated with pinpointing their active constituents. Future research endeavors may focus on the isolation and characterization of individual compounds, as well as exploring their mechanisms of action against bacterial strains. Overall, this investigation offers valuable insights into the potential of plant extracts as antibacterial agents and emphasizes the critical need for continued research in this field.

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Tolerance to Minimal Physical Activity in Adolescents with Systemic Lupus Erythematosus

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Abstract- In modern medicine, the issue of rheumatic diseases remains one of the most significant and socio-economically impactful problems due to the high rates of disability and loss of working capacity among these patients. The main cause of decreased quality of life and mortality in patients with systemic lupus erythematosus (SLE) is cardiovascular pathology, leading to the development of heart failure. An early symptom of myocardial dysfunction is the patient's loss of ability to perform routine physical activities in daily life. The aim of the study was to assess tolerance to minimal physical exertion in adolescents with systemic lupus erythematosus. A total of 46 adolescents with SLE were examined. They underwent the Six-Minute Walk Test (6MWT) with measurement of several parameters at rest and during the first minute of recovery following exertion.

Keywords: *systemic lupus erythematosus, six-minute walk test, adolescents.*

GJMR-C Classification: *NLMC: WD 365*

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Tolerance to Minimal Physical Activity in Adolescents with Systemic Lupus Erythematosus

Tetiana Holovko

Abstract- In modern medicine, the issue of rheumatic diseases remains one of the most significant and socio-economically impactful problems due to the high rates of disability and loss of working capacity among these patients. The main cause of decreased quality of life and mortality in patients with systemic lupus erythematosus (SLE) is cardiovascular pathology, leading to the development of heart failure. An early symptom of myocardial dysfunction is the patient's loss of ability to perform routine physical activities in daily life. The aim of the study was to assess tolerance to minimal physical exertion in adolescents with systemic lupus erythematosus. A total of 46 adolescents with SLE were examined. They underwent the Six-Minute Walk Test (6MWT) with measurement of several parameters at rest and during the first minute of recovery following exertion.

Results: Patients with SLE covered a shorter distance during the 6-minute period. Both before and after the Six-Minute Walk Test, elevated heart rate (HR) and respiratory rate (RR) values were recorded. This may indicate strain on the adaptive capacities of the cardiovascular and respiratory systems against the background of sympathetic activation of the autonomic nervous system, which likely leads to impaired autonomic regulation of these systems and disruption of myocardial perfusion.

Keywords: systemic lupus erythematosus, six-minute walk test, adolescents.

I. Introduction

The issue of rheumatic diseases in modern medicine is being studied as one of the most significant medical and socio-economic challenges [1, 2, 3]. One of the most prevalent diseases within this group is systemic lupus erythematosus (SLE). Typically manifesting in childhood or early adulthood, the disease persists throughout life and exhibits continuous progression. Among the primary causes of reduced quality of life and mortality in these patients are cardiovascular system (CVS) pathologies, leading to the development of heart failure (HF) [4, 5, 6]. According to the functional classification of HF by the New York Heart Association (NYHA), the key diagnostic indicators of subclinical HF are the patient's subjective symptoms, such as dyspnea during physical exertion and an inability to perform daily physical activities [7, 8].

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In adolescent patients and young adults, such complaints are generally uncommon and are most often identified retrospectively during active questioning by the physician [8]. Therefore, for the diagnosis of early cardiovascular system (CVS) impairments in patients with various somatic diseases during this age period, the assessment of tolerance to minimal physical exertion through the use of different exercise tests becomes particularly important [9, 10, 11].

Recently, the six-minute walk test (6MWT) conducted in a corridor setting has gained widespread use for addressing these diagnostic challenges. This test is simple to perform, does not require expensive equipment, and can be conducted in any environment that is safe for both the patient and the investigator [11, 12].

II. Objective of the Study

To assess the tolerance to minimal physical exertion in adolescents with systemic lupus erythematosus.

a) Data Collection

The study group comprised 46 pediatric patients diagnosed with systemic lupus erythematosus (5 males and 41 females). The mean age of the patients was 13.89 ± 0.39 years.The control group consisted of 36 practically healthy adolescents (5 males and 31 females) with a mean age of 14.53 ± 0.38 years ($p_t < 0.1$).

The children in the main group underwent a comprehensive health assessment at the Cardiorheumatology Department, while the control group was examined at the Pediatric Department of the State Institution "Institute for the Protection of Children's and Adolescents' Health of the National Academy of Medical Sciences of Ukraine" during the period from 2017 to 2022. The healthy children had no history of inflammatory diseases, joint lesions, or congenital malformations.

The study was conducted in accordance with the principles of the Declaration of Helsinki on human rights (1948), as well as ethical and moral-legal requirements of the Statute of the Ukrainian Association of Bioethics, the standards of Good Clinical Practice (GCP, 1992), the Council of Europe Convention on

Human Rights and Biomedicine (1997), and considering the requirements of the Law of Ukraine "On Medicinal Products" (1996, Articles 7, 8, 12), the principles of Good Laboratory Practice (GLP, 1998), and the ICH GCP guidelines (2008) [13, 14, 15].

The study protocol and the use of human biological materials were approved by the Ethics Committee of the State Institution "Institute for the Protection of Children's and Adolescents' Health of the National Academy of Medical Sciences of Ukraine" (Kharkiv, Ukraine), and written informed consent was obtained in accordance with the Declaration of Helsinki.

Clinical diagnoses were established in accordance with the International Classification of Diseases, 10th Revision (ICD-10). For the diagnosis of systemic lupus erythematosus (SLE), the guidelines of the Ministry of Health of Ukraine dated January 31, 2017, No. 00446 "Systemic Lupus Erythematosus" were followed, as well as the classification criteria of the European League Against Rheumatism (EULAR) and the American College of Rheumatology (ACR), published in 2019. According to these criteria, a diagnosis of SLE was made in the presence of positive antinuclear antibodies (ANA) along with clinical manifestations from the proposed domains totaling 10 or more points [16, 17].

To assess disease activity in patients with SLE, the Systemic Lupus Erythematosus Disease Activity Index 2000 (SLEDAI-2K) was calculated. This index includes 24 parameters (16 clinical and 8 laboratory), each assigned a score ranging from 1 to 8 points. The maximum possible total score is 105. Only those manifestations present within the 10 days prior to evaluation were considered, regardless of the severity of the symptoms or any improvement or deterioration observed at the time of examination.Based on the SLEDAI score, the following degrees of disease activity were defined: 0 points – no activity (grade 0); 1–5 points – low activity (grade 1); 6–10 points – moderate activity (grade 2); 11–19 points – high activity (grade 3); and more than 20 points – very high activity (grade 4) [16].

b) Research Methods

To assess tolerance to minimal physical exertion in adolescents with systemic lupus erythematosus (SLE), a six-minute walk test (6MWT) was performed. The test was conducted in the morning hours in the corridor of the cardiology and cardiorheumatology department, with a corridor length of 58.6 meters. Each patient completed the test once. Prior to the test, the children rested in a seated position for three minutes. No dietary restrictions or harmful habits (such as smoking, alcohol consumption, or drug use) were reported among the participants. The test was performed at an individually maximal self-paced speed, avoiding the onset of pain, dyspnea, muscle fatigue or heaviness in the legs, dizziness, or weakness, allowing the patient to cover the maximum distance possible within six minutes. The distance covered (6-minute walk distance, 6MWD) was measured in meters.

The clinical status of each patient was monitored before and after the test by recording heart rate (HR), respiratory rate (RR), oxygen saturation (SpO_2) using pulse oximetry, and arterial blood pressure (BP). Upon completion of the test, during the first minute of the recovery phase, in addition to the aforementioned parameters, the percentage increase in heart rate (%HR increase) and respiratory rate (%RR increase) in response to minimal physical exertion was calculated. Heart rate was measured in a seated position over 15 seconds using a pulse oximeter, followed by counting respiratory rate for 15 seconds using a stopwatch. Blood pressure was also measured in a seated position using a cuff placed on the left upper limb by the Korotkoff method with a Microlife AG1-20 device [11, 12].

c) Statistical Analysis

The statistical analysis of the obtained data was performed using the SPSS 17 software package (license 4a180844250981ae3dae-s / nSPSS17). The arithmetic mean and its standard error were calculated, and the upper and lower quartiles were determined for all parameters. The measured values were compared to the corresponding values obtained from adolescents in the control group. Differences between means were assessed using parametric methods (Student's *t*-test, Fisher's angular transformation test) in cases where the distribution of values was normal. When the data did not meet the criteria for normal distribution, non-parametric methods (Wilcoxon–Mann–Whitney test) were employed.

Correlation analysis was conducted using Pearson's pairwise correlation to evaluate relationships between disease duration, disease activity, percentage increase in heart rate (%HR increase), percentage increase in respiratory rate (%RR increase), and the distance covered during the six-minute walk test (6MWD). The strength of the correlation was interpreted according to Chaddock's scale: a value of 0.10–0.29 indicated a weak correlation, 0.30–0.49 a moderate correlation, 0.50–0.69 a substantial correlation, 0.70–0.89 a strong correlation, 0.90–0.99 a very strong correlation, and 1.00 indicated a functional (perfect) correlation. Differences were considered statistically significant at $p < 0.05$.

III. Results

In terms of physical development, patients with SLE demonstrated the following differences compared to their healthy peers: they were of shorter stature (1.55 ± 0.02 m vs. 1.67 ± 0.02 m, $p_t < 0.001$), whereas body weight did not significantly differ between the two groups (51.44 ± 2.24 kg vs. 54.38 ± 2.14 kg, $p_t < 0.1$).

However, the body mass index (BMI) of patients with SLE was significantly higher (21.01 ± 0.59 kg/m^2 vs. 19.21 ± 0.45 kg/m^2, $p_t < 0.01$).

The majority of the patients were female ($p_x < 0.001$). There were no significant differences between boys and girls with SLE in terms of age or physical development parameters ($p_t < 0.3$). Therefore, analysis of six-minute walk test (6MWT) outcomes by sex was deemed unnecessary.

The mean age of SLE onset across the group was 10.87 ± 0.54 years. The disease onset occurred before the age of 5 years in 2 patients (4.35 ± 3.01%), between the ages of 5 and 10 years in 14 patients (30.43 ± 6.78%), and after the age of 10 years in 30 patients (65.22 ± 7.02%; $p < 0.05$ compared to the groups with disease onset before the age of 5 years and between 5 and 10 years).

At the time of examination, the mean disease duration was 3.63 ± 0.41 years (40.41 ± 4.30 months). Disease duration between 1 and 3 years was observed in 26 patients (56.52 ± 7.37%), while 20 patients (43.48 ± 7.31%) had a disease duration of more than 3 years.

The six-minute walk test (6MWT) was performed in 30 patients, including 3 boys (10%) and 27 girls (90%). The remaining patients did not undergo the 6MWT due to various reasons: 3 patients had arthralgia associated with complications of aseptic necrosis of the femoral head, 9 patients were in a severe condition due to the underlying disease, and 4 patients refused to participate in the test.

Before the test, 4 patients with SLE (13%) reported complaints: three patients experienced joint pain and one patient reported fatigue; however, all of them agreed to complete the test. Upon pre-test assessment, baseline values of heart rate (HR), respiratory rate (RR), and diastolic blood pressure (DBP) were within normal limits but were significantly higher compared to healthy controls ($p_t < 0.01$, $p_t < 0.01$, $p_t < 0.05$, respectively) (Table 1).

Table 1: Six-minute walk test indicators in patients with systemic lupus erythematosus(M±m)

Indicators	Patients with SLE, n = 30		Control group, n = 36	
	Before 6MWT	After 6MWT	Before 6MWT	After 6MWT
Complaints, M ± m, %	13,33 ± 6,21	36,67 ± 8,80	0	0
HR, bit/min	83,87±2,65**	112,47±3,04***	74,17 ± 2,15	99,78 ± 2,23
RR, breath/min	20,80± 0,64**	26,90±1,09***	18,75 ±0,41	21,33±0,38
SpO_2, %	98,33 ± 0,16*	98,70± 0,11*	97,39±0,49	97,72±0,49
SBP, mmHg	112,20±2,79	118,23±2,59	110,06 ± 2,44	116,67 ± 2,89
DBP, mmHg	71,00±1,86*	72,40± 1,61***	66,94 ± 1,36	65,72 ± 1,42
% increaseinHR	-	35,62±3,03	-	36,97 ± 3,63
% increasein RR	-	30,32±4,97***	-	14,36±1,36
6MWD, m	-	483,68±9,73**	-	519,29 ± 8,56

*Note: p < 0.05, ** p < 0.01, *** p < 0.001 – significance of differences between patient group and control group*

After the Six-Minute Walk Test (6MWT), complaints were noted in 11 patients (36.67%). Among them, the predominant complaints were shortness of breath (in 6 patients), arthralgia in 4 children, and fatigue persisted in 1 girl. In response to minimal exertion, heart rate (HR), respiratory rate (RR), and diastolic blood pressure (DBP) increased and remained significantly higher compared to the control group (p < 0.001). The percentage increase in heart rate was comparable to the control group, whereas the percentage increase in respiratory rate was significantly higher than in healthy adolescents. The distance covered in 6 minutes by patients with systemic lupus erythematosus (SLE) was shorter (p < 0.01) (Table 1).

When analyzed by age groups, significant differences were observed in blood pressure values, which is physiological. No differences were found in the distance covered (Table 2).

Table 2: Six-minute walk test indicators in patients with Systemic Lupus Erythematosusconsidering patient age (M±m)

Indicators	Patients aged 10 – 13 years, n = 11		Patients aged 14 – 18 years, n = 19	
	Before 6MWT	After 6MWT	Before 6MWT	After 6MWT
HR, bit/min	87,28±4,97	117,73±5,47	81,89± 3,06	109,42±3:53
RR, breath/min	20,91±1,25	27,45± 1,95	20,74± 0,74	26,58±1,35
SpO_2, %	98,36± 0,36	98,91± 0,09	98,32± 0,15	98,58± 0,16
SBP, mmHg	103,27±5,03***	108,09±4,55***	117,37±2,76	124,11±2,31
DBP, mmHg	65,72±3:53**	67,82±2,49**	74,05± 1,83	75,05± 1,88
% increaseinHR	-	36,26 ±3,99	-	35,25±4,26
% increasein RR	-	31,47±5,08	-	29,66±7,38
6MWD, m	-	476,79±18,48	-	487,66±11,34

*Note: ** $p_t < 0,01$, *** $p_t < 0,001$– significance of differences between patient groups of diferent age*

Patients with a disease duration of less than 3 years had significantly higher heart rate (HR) values both before ($p < 0.01$) and after ($p < 0.01$) the test, but the percentage increase in HR in response to minimal physical exertion was similar. The distance covered was also not different between patients with varying disease durations (Table 3).

Table 3: Six-minute walk test indicators in patients with Systemic Lupus Erythematosusconsidering disease duration(M±m)

Indicators	Patients with disease duration up to 3 years, n = 12		Patients with disease duration of more than 3 years, n = 18	
	Before 6MWT	After 6MWT	Before 6MWT	After 6MWT
HR, bit/min	92,42 ± 4,09**	121,33 ± 4,18**	78,17 ± 2,83	106,56 ± 3,68
RR, breath/min	20,33 ± 0,95	26,42 ± 1,40	21,11 ± 0,87	27,22 ± 1,60
SpO_2, %	98,33 ± 0,22	98,58 ± 0,19	98,33 ± 0,24	98,78 ± 0,13
SBP, mmHg	114,33 ± 3,04	120,83 ± 3,27	110,78 ± 4,22	116,50 ± 3,76
DBP, mmHg	74,58 ± 2,14*	75,25 ± 1,67	68,61 ± 2,64	70,50 ± 2,38
% increaseinHR	-	32,82 ± 4,42	-	37,49 ± 4,13
% increasein RR	-	30,60 ± 5,24	-	30,13 ± 7,64
6MWD, m	-	470,83 ± 15,58	-	492,24 ± 12,40

*Note: * $p_t < 0,05$, ** $p_t < 0,01$– significance of differences between patient groups with diferent disease duration*

Regarding disease activity, the majority of patients (43.48%) had a high degree of activity, 21.74% had very high activity, and 19.57% had moderate activity. Only 3 patients (6.52%) were in the inactive phase of the disease (Table 4).

The analysis of Six-Minute Walk Test (6MWT) indicators considering disease activity showed that baseline values in patients with systemic lupus erythematosus (SLE) did not differ, except for the respiratory rate, which was significantly lower in children with the third degree of disease activity ($p < 0.05$). After the test, the respiratory rate significantly differed in patients with the fourth degree of activity, and these patients also had the highest percentage increase in respiratory rate (Table 4). The distance covered in 6 minutes by patients with varying degrees of activity was almost the same.

Table 4: Six-minute walk test indicators in patients with Systemic Lupus Erythematosusconsidering disease activity(M±m)

Indicators	Patients with grade 1-2 activity, n = 9		Patients with grade 3 activity, n = 13		Patientswithgrade4 activity, n = 8	
	Before 6MWT	After 6MWT	Before 6MWT	After 6MWT	Before 6MWT	After 6MWT
HR, bit/min	84,33±4,62	114,22±5,33	82,23±4,73	109,77±5,19	86,00±4,29	114,88±5,37
RR, breath/min	22,00±1,15	25,78± 1,18 £	19,23± 0,74 £ *	25,46±1,83	22,00±1,46	30,50±2,26 *¥
SpO_2, %	98,56± 0,24	98,67± 0,24	98,00± 0,30	98,69± 0,17	98,63± 0,18	98,75± 0,16
SBP, mmHg	115,56± 2,62	125,67 ±3,64	108,23±4,61	111,54±4,25 ££	114,88±6,81	120,75±4,17
DBP, mmHg	75,44±2,66	76,33± 1,86	67,77±3,19 £	69,31 ±2,91 £	71,25±3,24	73,00 ±2,75
% increaseinHR	-	36,88±5,59	-	35,62±5,47		34,19±4,26
% increasein RR	-	17,77±2,60	-	32,35±7,97		41,15±12,46*
6MWD, m	-	492,57± 19,86	-	474,55±13,23		488,51±21,02

*Note: £ $p < 0,05$, ££ $p < 0,01$ when comparing patients with activity grades 1–2 to patients with activity grade 3; * $p < 0,05$ when comparing patients with activity grades 1–2 to patients with activity grade 4; ¥ $p < 0,05$ when comparing patients with activity grade 3 to patients with activity grade 4;* $p < 0,05$ when comparing patients with activity grade 2 to patients with activity grade 3.*

Subsequently, using quartile distribution, two groups of children were identified based on the smallest and largest distances covered (less than 437.25 m and more than 524.70 m, respectively). The first group included 8 patients, 3 of whom were aged 10 to 13 years, with the rest being older. In terms of disease activity, 2 (25%) had first-degree activity, 2 (25%) had fourth-degree activity, and 4 (50%) had third-degree activity. Regarding disease duration, the patients were equally divided: 4 (50%) had a disease duration of less than 3 years, and 4 (50%) had more than 3 years. The second group consisted of 9 children, 4 (45%) of whom were aged 10 to 13 years, and 5 (55%) were older. In terms of disease activity, 1 (11%) had first-degree activity, 2 (22%) had second-degree activity, 2 (22%) had third-degree activity, and 4 (45%) had fourth-degree activity. Regarding disease duration, 3 (33%) patients had a disease duration of less than 3 years, while the remaining had more than 3 years.

Table 4: Six-minute walk test indicators in patients with Systemic Lupus Erythematosusin the lower quartile compared to patients in the upper quartile(M ± m)

Indicators	Patients in the lower quartile, n = 8		Patients in the upper quartile, n = 9	
	Before 6MWT	After 6MWT	Before 6MWT	After 6MWT
Complaints, M ± m, %	12,50 ± 12,50	37,50 ± 18,30	11,11 ± 11,11	22,22 ± 14,70
HR, bit/min	95,38±3,08 ***	116,50±4,37	78,89±3,31	111,22±5,17
RR, breath/min	21,50± 0,82	26,38±1,69	22,67±1,15	27,78±2,07
SpO_2, %	98,38± 0,26	98,63± 0,26	98,67± 0,17	98,56± 0,24
SBP, mmHg	106,88 ±5,55	110,63±5,76	105,44±4,84	118,78±5,43
DBP, mmHg	69,88 ±4,61	68,75±4,30	66,44 ±3,01	72,33±3,06
% increaseinHR	-	22,40±3,79**	-	41,16±3,84
% increasein RR	-	22,80±6,71	-	21,98±4,39
6MWD, m	-	413,19 ±7,71***	-	542,59±7,88

*Note: p < 0.05, ** p < 0.01, *** p < 0.001 – significance of differences between patient groups*

IV. Discussion

It has been established that in patients with rheumatic diseases, the leading cause of death is cardiovascular disease, including myocardial infarction, ischemic heart disease, strokes, and others [1, 18, 19]. These conditions primarily lead to the development of heart failure, which remains the leading cause of mortality. Cardiovascular mortality in patients with rheumatic diseases is 1.7 times higher than in the general population [20, 21].

It is known that the cardiovascular system in these patients is involved in the pathological process due to systemic autoimmune inflammation, which exerts a direct toxic effect on cardiomyocytes, the microcirculatory bed, vascular endothelium, and the extracellular matrix, accelerating myocardial remodeling and contributing to its ischemia [22, 23, 24, 25].

Heart failure at an early stage often has no clinical manifestations and frequently remains undetected. However, it is known to be characterized by a reduced tolerance to minimal, everyday physical exertion [7, 8]. Exercise tolerance is an integral indicator of the physiological capabilities of the body. It is significantly influenced by the state of the cardiovascular, respiratory, and musculoskeletal systems, as well as the overall health of the individual [11, 12].

For the early detection of the first signs of heart failure, the six-minute walk test is widely used. In adult patients, a correlation has been established between the distance covered and indicators of their quality of life, as well as with the functional class of heart failure. Additionally, in adults, the distance covered in 6 minutes serves as a strong predictor of mortality and disability in various cardiopulmonary diseases [26, 27]. In recent years, this test has increasingly been used in pediatrics with no changes in the protocol [11, 12].

In our study, patients with systemic lupus erythematosus covered a shorter distance compared to healthy children ($p < 0.01$). The analysis of this indicator was not dependent on patient age (Table 2), disease duration (Table 3), or the degree of activity of the pathological process (Table 4).

It is known that the results of physical stress tests often simultaneously reflect the functional capabilities of several systems, and sometimes the organism as a whole. This is because the function of any particular visceral system is under significant neurohormonal regulation. For example, the pulse response to physical exertion may reflect the functional status of the heart, vascular response, as well as the features of the autonomic regulation of the cardiovascular system [9, 11].

Patients with systemic lupus erythematosus had higher heart rate and respiratory rate both at rest and after exertion, as well as a greater percentage increase in RR (Table 1). Blood oxygen saturation, however, was not affected. These changes may indicate the impact of the disease on the overall state of the body due to long-

term subclinical inflammation. Against this background, the likely development of comorbid conditions such as hyperlipidemia and hypercoagulability may occur, which in turn contribute to the formation of atherosclerosis and atherothrombosis, ultimately leading to chronic heart failure [7].

It is known that in systemic lupus erythematosus, all organs and systems are involved in the pathological process. The longer the active inflammatory process persists, the more internal organs are affected. However, it is during the acute inflammatory phase that the highest likelihood of developing multiple organ failure exists [22, 25].

In the group of children under study, the highest HR levels, both before and after exertion, were observed in patients with a disease duration of less than three years ($p < 0.01$). This may suggest activation of neurohormonal regulatory systems, primarily the sympathoadrenal system, both in the context of the pronounced inflammatory process and due to the use of aggressive immunosuppressive therapy, which leads to increased strain on the cardiovascular system.

The respiratory rate at the first minute of recovery, as well as its percentage increase, were highest in patients with a very high degree of disease activity ($p < 0.05$), with preserved saturation according to pulse oximetry. The baseline heart rate values in patients who covered a shorter distance were significantly higher ($p < 0.001$), while the percentage increase in HR was greater in patients who covered a longer distance ($p < 0.01$).

Thus, when assessing tolerance to minimal physical exertion, patients with systemic lupus erythematosus covered a shorter distance in 6 minutes. Both before and after the six-minute walk test, higher heart rate and respiratory ratevalues were observed in these patients. This may indicate strain on the adaptive capacities of the cardiovascular and respiratory systems against the background of sympathetic activation of the autonomic nervous system, which likely leads to impaired autonomic regulation of these systems and disruption of myocardial perfusion.

V. Conclusions

1. Patients with systemic lupus erythematosus demonstrated decreased tolerance to minimal physical exertion, as evidenced by the shorter distance covered during the six-minute walk test ($p < 0.01$).
2. Adolescents with a disease duration of less than three years had the highest heart rate levels both before ($p < 0.01$) and after ($p < 0.01$) the test.
3. In cases of very high disease activity in SLE, the respiratory system's functionality is strained in response to exertion, as indicated by the higher respiratory rate ($p < 0.05$).
4. Reduced tolerance to minimal physical exertion, alongside elevated heart rate and respiratory rate levels in children with systemic lupus erythematosus, may reflect a deterioration in the functional state of the heart, vascular response, respiratory system strain, and the characteristics of autonomic regulation of these systems.

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Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template

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Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

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Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for writing a good quality Medical Research Paper

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of medical research then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

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- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

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Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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