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Dr. Hafiza Rizwana Kasur
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Design concept of cost effective LEO satellite system for Automatic Dependent Surveillance-Broadcast (ADS-B)

Ghulam Jaffer [1*], Rameez Ahmed Malik [2]

Abstract

The air traffic surveillance has been performed by radar over the past few decades. In recent days, it is gradually being replaced by technologically advanced Automatic Dependent Surveillance–Broadcast (ADS-B). ADS-B offers numerous advantages in terms of superior accuracy and range and low power consumption, etc. The coverage of terrestrial radar and ADS-B is confined to continental parts of the globe, leaving oceans and poles uncovered by instantaneous surveillance measures. Meanwhile available airspace has become congested due to rapidly growing air traffic. The paper presents the mission design of a satellite-based ADS-B system for air traffic surveillance over intercontinental trans-oceanic flight routes. Performance assessment of the designed constellation is based on the analysis of various parameters such as regional and global coverage and satellite availability. LEO satellite handover mechanism and link budget for the ADS-B system are also discussed. The results of parametric analysis indicate that the constellation provides adequate coverage in the simulated global and regional areas. The constellation is a feasible and cost effective solution for global air surveillance which can supplement/replace the terrestrial ADS-B and radar systems.

Keywords: Radar; ADS-B; Air traffic; Surveillance, Simulation, Link budget

1. Introduction

Air surveillance is a fundamental requirement of civil and military aviation industries. In past few decades, the tracking is based on numerous technologies, i.e. Primary Surveillance Radar (PSR), Secondary Surveillance Radar (SSR) and Automatic Dependent Surveillance-Broadcast (ADS-B). ADS-B

Figure 1 Global ADS-B coverage, adopted from (Maps of World, World air routes, 2022)

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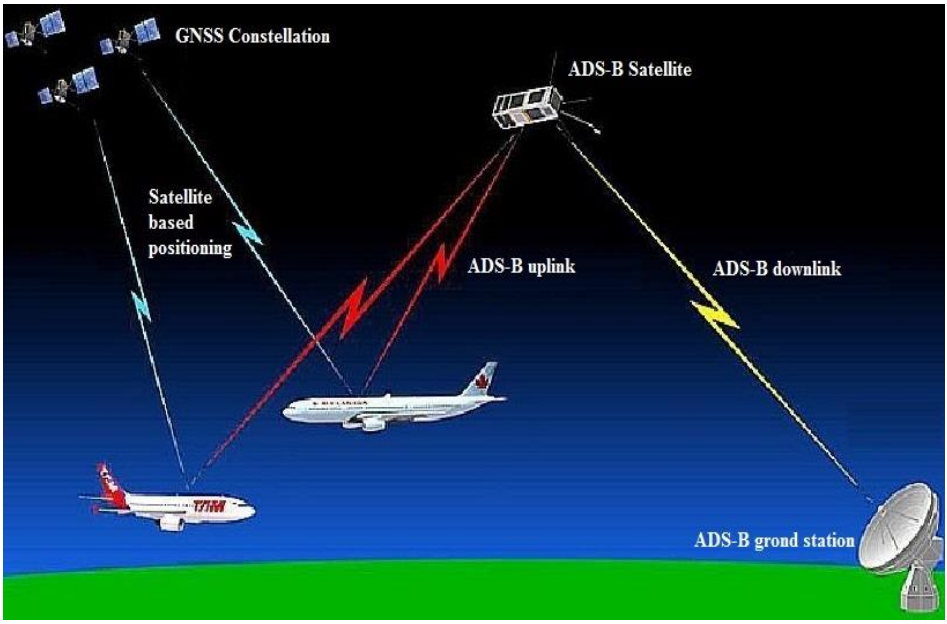

Figure 2 Illustration of the concept of space-based ADS-B system, adopted from (Space based ADS-B, 2018).

is comparatively a new technology which is quickly being adopted as an aviation standard around the globe past few years. The technology has several benefits over radar, such as cost effective and simple ground architecture, less data latency and data update rate of 1sec (Strohmeier, Schäfer, Lenders, & Martinovic, 2014) (Blomenhofer, Pawlitzki, Rosenthal, & Escudero, 2012). ADS-B equipped aircrafts drive their geo-coordinates and velocity via an on-board GNSS receiver. The geo-location information is combined with other parameters such as, aircraft identification, intent uncertainty level etc. The entire set of parameters is then transmitted over an Extended Squitter (ES) 1090 MHz SSR-Mode-S downlink signal, which is called ADS-B out. The ADS-B signal is received by neighbour air traffic for collision avoidance and by the ground station network (Ali, 2016), (Werner, Bredemeyer, & Delovski, 2014).

At this time, a terrestrial infrastructure provides ADS-B services to Air Traffic Control (ATC) authorities. This ground based infrastructure is dependent on the constraint of "line of sight" of aircrafts within a defined range of the ground station (Nguyen & Dixon1d, 2015). Typically, a terrestrial ADS-B system is effective within 80 nautical miles which means that oceanic coverage is just confined to the coastal areas. This is due to the fact that installation of ground station in remote areas like poles and oceans is either technically or economically not viable. Consequently, air traffic cannot be monitored over these remote regions. Moreover, it is also costly to cover large land areas using poor infrastructure (Alminde, Christiansen, Kaas, Midtgaard, Bisgard, Jensen, Gosvig, Birklykke, Koch, & Le Moullec 2012). In case of an air crash in non-radar or ADS-B airspace, the rescue operation has to be performed without valid information about the crash site location. This makes the situation even more complex. A similar situation was witnessed during the incidents of Air France's flight 447 in 2009 and Malaysian MH370 in 2014. The accurate locations of these accidents were unknown and it took several days to figure out possible crash locations. The ground based global ADS-B coverage area is presented in Figure 1, which shows significant gaps over the

oceans which act as basic routes for inter-continental flights.

As a consequence of the terrestrial ADS-B constraints, the gap regions over the oceans can be covered with a space based ADS-B. The whole idea is to place a highly sensitive receiver on a satellite, capable of receiving ADS-B signals in space. Afterwards, the data would be relayed to ADS-B ground receiving stations. The whole concept of space based ADS-B is illustrated in Figure 2. The subject has already gained interest of the key stakeholders of the aviation industry and numerous projects have been initiated. Proba V, a mission of the European Space Agency, is a classical example of ongoing advancements in space based air traffic surveillance. It is primarily an Earth observation satellite with an added secondary experimental payload of ADS-B receiver. The satellite was launched in 2013 and is successfully receiving ADS-B signals without any moderation in the existing ADS-B infrastructure of the aircrafts. This mission has proved that the technology is feasible as a primary payload on small satellites (Delovski, Bredemeyer & Werner, 2016).

Based on orbital altitude, satellite-based communication networks can be classified into three main classes, Geostationary Orbit (GEO), Medium Earth Orbit (MEO), and Low Earth Orbit (LEO) satellite systems. GEO satellites have an altitude of nearly 36000 km above the equatorial plane, where the satellite's orbital motion is synchronized with the Earth's rotation. At GEO altitude a few satellites are required for global coverage. However, GEO satellites exhibit some major drawbacks for communication networks. The user terminals and the space segment are not power efficient, and the propagation delay is comparatively high in these systems. LEO systems, on the other hand, have prominent advantages, like efficient bandwidth utilization, low propagation delay, and are more power efficient as compared to GEO systems (Henderson & Katz 2000).

The aim of this study is to design a low cost LEO constellation that can provide competitive global ADS-B surveillance by utilizing the least number of satellites. Typically, LEO constellations are deployed within 400-2000 Km altitude with circular orbits. However, the orbital geometry and altitude of the constellation design varies greatly according to the space mission requirements. LEO constellations are widely used in the communication sector. There are various space missions that have been planned for numerous applications, e.g. LeoSat, Samsung and Telesat LEO etc. The total number of satellites of these planned missions may range between 70 and 4700. Iridium is also launching NEXT generation constellation of 66 satellites for global internet communication and it would also be used as ADS-B Link Augmentation System (ALAS) (Noschese, Porfili, & Di Girolamo, 2011), (Gupta, 2011).

2. Constellation Geometry

Satellite constellation is defined as a group of satellites, operating to achieve a common goal. The orbital geometry is calculated with a set of six keplerian orbital elements, including Inclination angle (i), Semi-major axis (a), Right Ascension of Ascending Node (RAAN), True anomaly, Eccentricity (e), and Argument of Perigee (ω) (Cakaj, 2014). The design parameters are thoroughly investigated as follows:

2.1. Constellation Altitude Selection

The orbital altitude is one of the key design parameters of a constellation. The RF communication a requirements and satellite coverage have direct relationship with the orbital altitude. If the orbital altitude is low, it would be less demanding in terms of the Equivalent Isotropic Radiated Power (EIRP) requirement of the system. Moreover, signal propagation losses would also be low but a higher number of satellites would be required for coverage. This setting would certainly

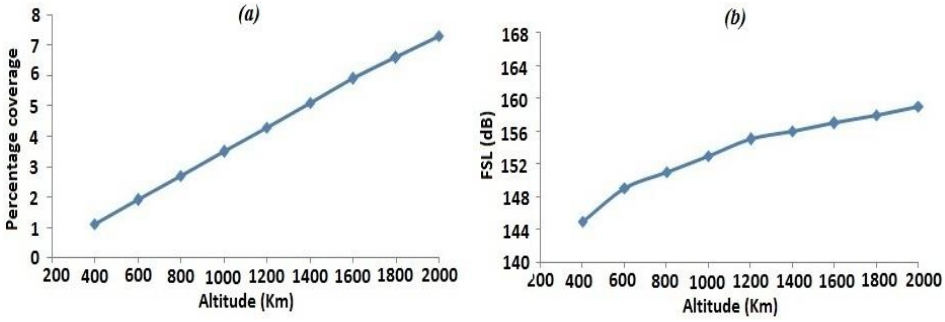

Figure 3 Variation in percentage coverage and FSL of LEO constellation with altitude (a) Variation in LEO satellite percentage coverage of the globe, (b) Variation of the FSL with the distance, considering f = 1090 MHz

escalate the entire space mission design cost. The selection process of the system's orbital altitude generally requires a balance between space mission deployment cost and quality of service (QoS) (Long, 2014). During the altitude finalization process, one must consider the fact that the EIRP requirement is satisfied, which means that the spacecraft has an adequate signal to noise ratio (SNR) and therefore it can be detected in space. The relationship between LEO satellite orbital altitude with the percentage coverage area and free space loss (FSL) is shown in Figure 3. Additionally, some space weather constraints (Earth's atmospheric drift and Van Allen belts) should also be taken into consideration in the altitude selection algorithm (Horne, Thorne, Shprits, Meredith, Glauert, Smith, Kanekal, Baker, Engebretson, Posch, 2005).

2.2. Constellation Orbital Geometry Selection

To determine optimum orbital geometry, the total number of orbital planes and satellites necessary for ADS-B coverage needs to be determined. The relative satellite-observer geometry is determined by two angles, i.e. elevation and azimuth angles. Azimuth is the angular direction of the satellite which is calculated on the horizontal plane in the clockwise direction from the geographic north. Its range is 0˚ to 360˚. The elevation is defined as the angle between the observer's horizontal plane and the line joining to satellite in a vertical plane. Its range is 0˚ to 90˚. The coverage of the satellite is generally represented by a circle on a surface of the globe and generally refers as footprint. We can communicate with the satellite within its footprint at a predefined minimum elevation angle. This minimum value of elevation angle is a dependent on system constraints. Ideally, big footprint is accomplished at minimum elevation. But, practically we may not be able to communicate with a spacecraft at too low elevations due to natural or man-made barriers. To overcome these barriers, the minimum elevation angle constraint for the LEO system ranges between 5˚-10˚ (Cakaj, Kamo, Lala, & Rakipi 2014). For satellite view geometry calculation, it is assumed the worst scenario in which the minimum elevation is considered 10˚.

The cross-sectional coverage area of a LEO satellite at the equatorial plane is depicted in Figure 4 (a). We have selected the equatorial circle because it is a great circle that has the maximum radius, shown in Figure 4 (b). All of the other latitudinal circles (parallels) of the sphere are small circles whose radius is lesser as compared to that of the equator. This implies that higher LEO planes and satellites are required to cover the equatorial plane. We can obtain the longitudinal range of a LEO satellite footprint by calculating the Earth's central angle (ϕ). Total LEO planes and satellites required for full ADS-B coverage of the equatorial plane can then be

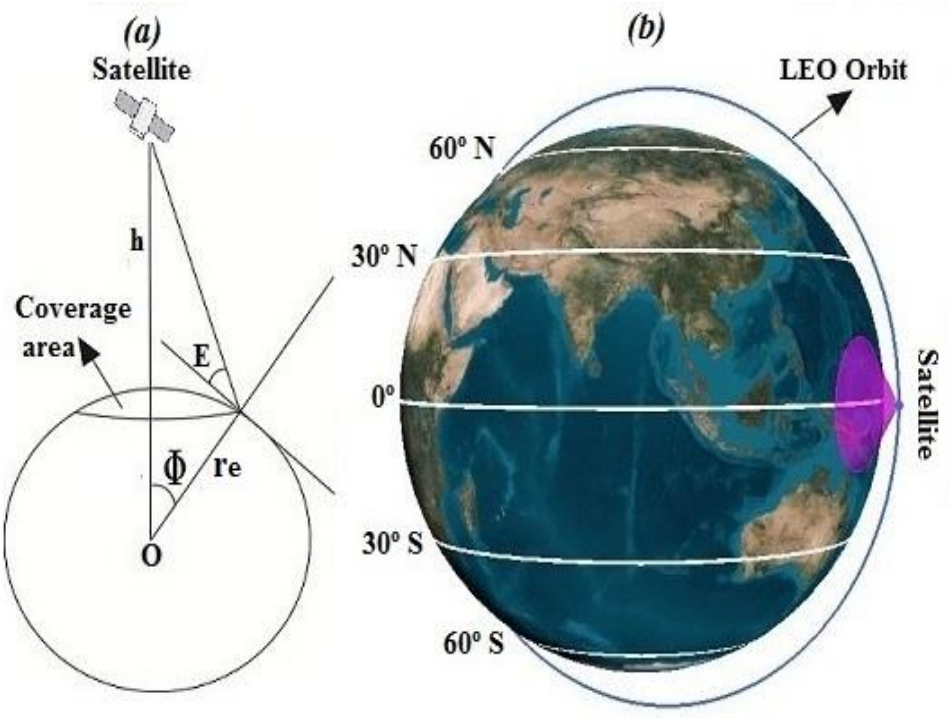

Figure 4 Illustration of the concepts of satellite view geometry: (a) LEO satellite cross-sectional coverage area of the equatorial plane, (b) 3-D visualization of LEO satellite foot print.

calculated. ϕ is obtained by the following equation:

$$\phi = [\cos^{-1}[\frac{\text{re}\cos E}{Re+h}]] - E \qquad (1)$$

In the above equation, r_e represents Earth's radius, *h* is the spacecraft's altitude and *E* is the elevation angle. By incorporating the values of *Re*, *h* and *E* in equation (1), ϕ comes out to be 19.90°. From Figure 4 (a), one LEO satellite covers 2ϕ or 39.8° of longitude of the equator, the quantity of orbital planes for global coverage is calculated as:

$$\text{Total number orbital planes} = 360°/4\phi = 5 \qquad (2)$$

As we have not covered the polar region due to the absence of major air traffic activity, so one orbital plane is reduced and a LEO constellation with four orbital planes is designed which is adequate to cover the airspace with dense traffic (59º N to 58º S).The total in-orbit satellites per plane can be found with the following equation:

$$\text{Satellites per plane} = 360°/2\phi = 9 \qquad (3)$$

Overall, a delta walker constellation of 36 equidistant satellites, separated by 40º of true anomalies in 4 orbital planes at 60º inclination is required for full coverage of the globe within the latitudinal range of 59ºN - 58ºS. A walker constellation has several orbits at the same inclination that are rotated about the poles, i.e. have different RAAN. The surface coverage area of LEO satellite can also be calculated using the Earth's central angle as follows:

$$\text{Percentage Coverage} = \frac{\text{Surface coverage area of satellite}}{\text{Surface area of the Earth}} \times 100 \qquad (4)$$

$$\text{Percentage Coverage} = \frac{2\pi Re^2 (1-\cos\phi)}{4\pi Re^2} \times 100 \qquad (5)$$

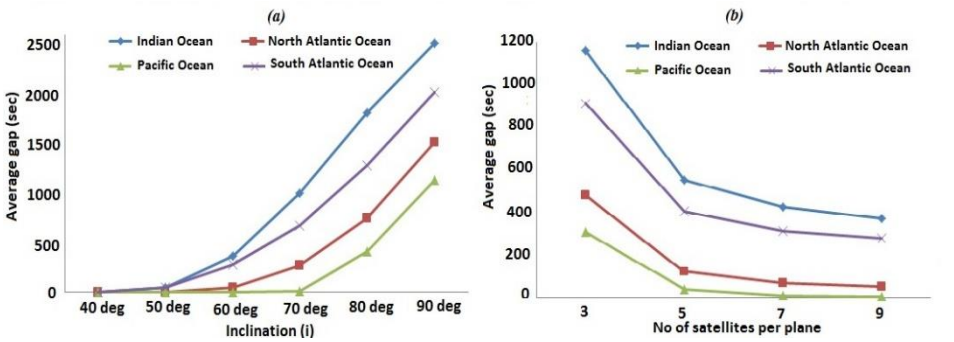

Figure 5 Geometry optimization results (a) Variation in average gap duration of oceanic regions with inclination angle considering satellites per plane = 9, (b) variation in average gap duration of oceanic regions with satellites per plane considering i=60˚.

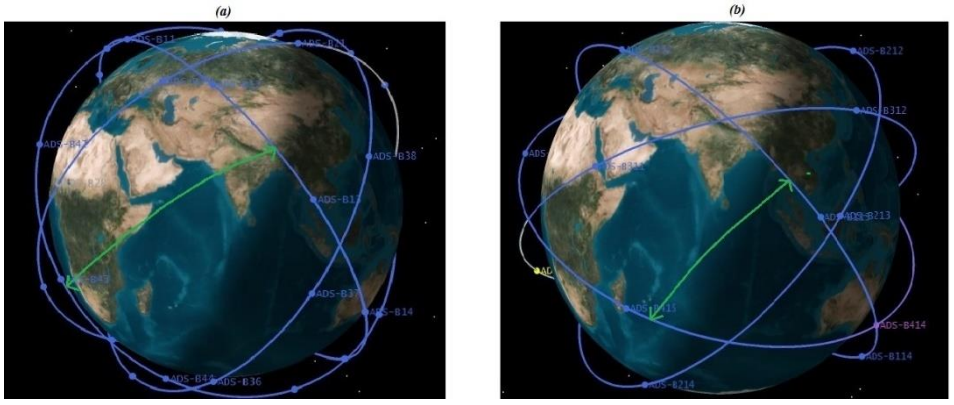

Figure 6 3-D visualization of constellation models, (a) Theoretical delta walker constellation of 36 satellites, (b) Proposed constellation of 20 satellites.

The footprint a LEO satellite orbiting the Earth at 850 Km altitude is computed as 15261970Km^2 with 2206 Km of coverage radius. Incorporating this value in equation (5), the percentage surface area covered by one LEO satellite is calculated as 2.98% of the entire globe.

Regardless of the overall advantageous effect of this LEO tracking system, launching 36 satellites for global air traffic surveillance would be costly, particularly when considering the maintenance and replacement of satellites at the end of life. The total number of satellites must be balanced against the overall cost of the space mission. As a fundamental requirement, space based ADS-B system should ensure surveillance over the North Atlantic, South Atlantic, Pacific and Indian Oceans. These oceanic regions have the highest amount of un-tracked air traffic by the terrestrial systems (Radar or ADS-B). Tracking these regions with satellites in specific orbits can greatly diminish the constellation deployment cost. Thus, in order to lower the manufacture and launch cost of the ADS-B mission constellation size is systematically condensed using an optimization approach. A series of tests is conducted in which the geometric parameters of the constellation are optimized to analyze the effect on coverage of the trans-oceanic flight route regions. The geographical spread and significance of the simulated oceanic regions is discussed in detail in the section 3.

According to the fundamentals of orbital mechanics, the keplerian elements that influence the coverage include semi-major axis, inclination and eccentricity. As LEO orbits are circular, their eccentricity

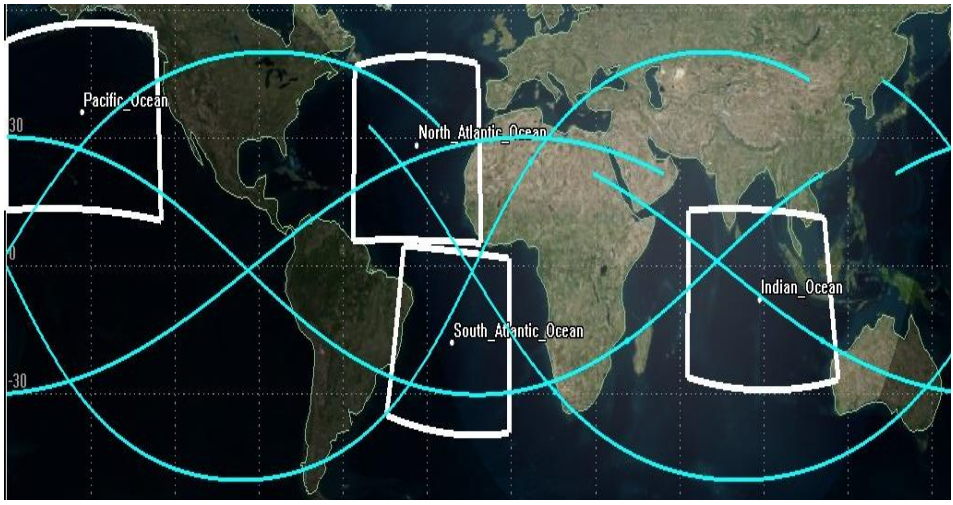

Figure7 ADS-B constellation ground tracks and study areas.

is very close to zero so the orbital eccentricity is fixed. There is a direct relation between semi-major and orbital altitude and therefore it is already being fixed based on ADS-B link requirements as explained in Section 2. The true anomaly does not directly affect the coverage of the LEO system when satellites are evenly spread in each of the orbital planes. Therefore, the orbital inclination and number of spacecrafts per plane are optimized to find the most suitable combination of these parameters. The results of the optimization process are shown in Figure 5, which shows the variation in average gap duration in selected oceanic regions against inclination and spacecrafts per plane.

The inclination optimization demonstrates that the average gap duration of all the oceanic regions is below 500 sec and increases with the inclination, e.g. from 0˚ to 90˚. The gap duration escalates as the inclination rises above 50˚. Similarly, the number of satellites per plane also has a major impact on the gap duration. The duration of a gap for each of the oceanic regions is less than 600 sec and sharply fluctuates by decreasing the satellites per plane to 3. These results suggest that the constellation with $i \leq 60^{\circ}$ and 5 satellites per plane is an optimum choice to achieve maximum surveillance and reduced gap duration over the trans-oceanic flight route regions. Once a, e, i, and the number of satellites per plane are

Table 1 Orbital configuration of ADS-B constellation.

Orbital parameter	**Unit**	**Orbit 1**	**Orbit 2**	**Orbit 3**	**Orbit 4**
Orbit type	NA	Prograde	Retrograde	Prograde	Retrograde
Inclination (deg)	Deg	50	-50	30	-30
Altitude (Km)	Km	850	850	850	850
Eccentricity (e)	N/A	0	0	0	0
Argument of Perigee (deg)	Deg	Undefined	Undefined	Undefined	Undefined
RAAN (deg)	Deg	0	0	90	90
No of Satellites	NA	5	5	5	5

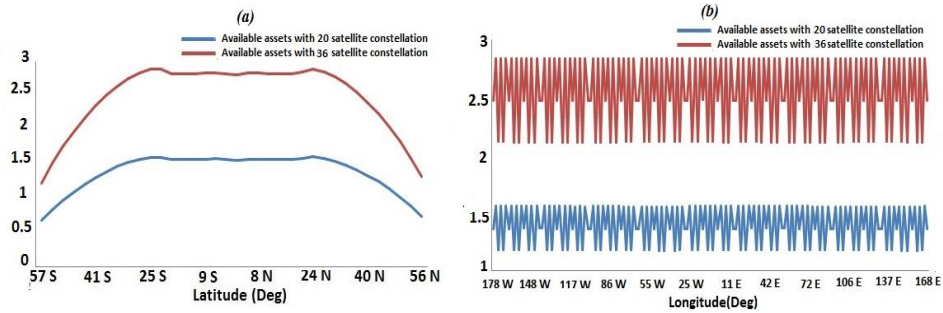

Figure 8 Spatial variation in asset availability of ADS-B constellation at 5˚ mask angle for the entire globe, (a) Latitudinal variation, (b) Longitudinal variation.

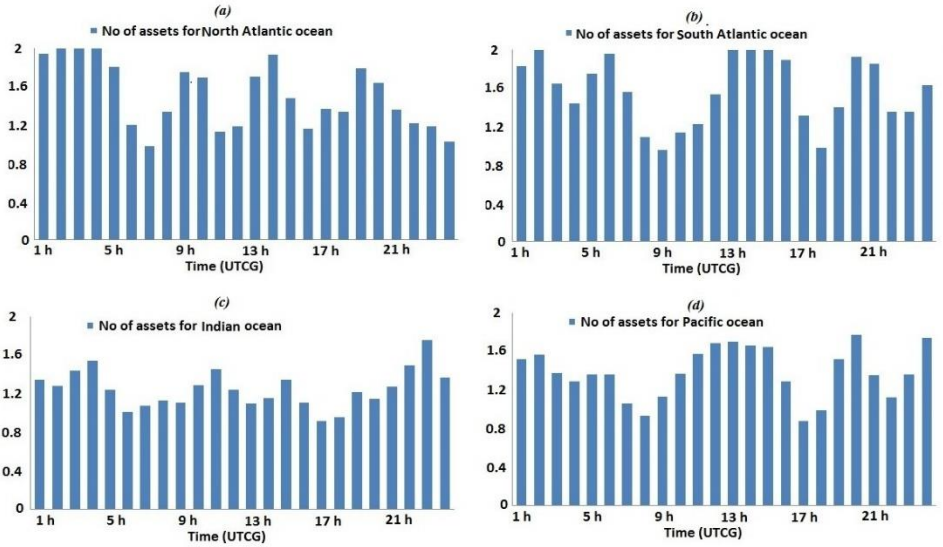

Figure 9 Temporal variation in assets availability for simulated oceanic regions, (a) North Atlantic Ocean, (b) South Atlantic Ocean, (c) Indian Ocean, (d) Pacific Ocean.

finalized, we can select the final geometry of the constellation.

The proposed constellation for global ADS-B surveillance is an inclined constellation of 20 satellites in two prograde (50º and 30º) and two retrograde (-50º and -30º) orbits at 850 Km altitude. Each orbital plane is equipped with five equally spaced satellites with true anomaly separation of 72º from each other. The orbital plane separation (RAAN) is 90°, starting from 0º for the first plane. The orbital velocity of the satellites at 850 km altitude is 7.42 km/s with a moment period of 102 min. The geometric arrangements of the theoretical delta walker constellation and the proposed constellation are given in Figure 6. As can be seen, the inter-planar spacing of the proposed constellation is small as compared to that of the delta walker constellation. This results in lowering the design and launch cost without compromising the space mission performance. The geometric arrangement of the proposed constellation is depicted in Table 1.

3. Simulation Analysis

ADS-B based in-flight tracking ability of the proposed system is evaluated in real-time dynamic simulator (AGI, Engineering Tools, 2022). The simulation parameters include altitude, orbital elements of the designed LEO system of

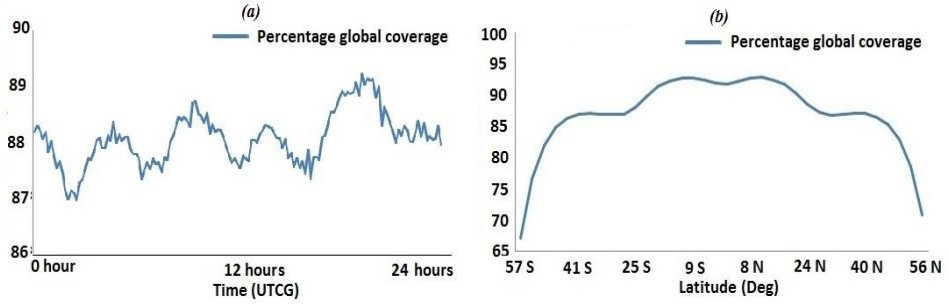

Figure 10 Temporal and spatial variation in percentage coverage area of the globe within latitudinal range of 59˚ N to 58˚ S.

20 satellites and regional and global coverage regions. Numerous testing parameters, such as coverage, satellite visibility, access time and time average gap are analyzed in a constrained setting. ADS-B link is analyzed separately using a test case trans-oceanic flight. The theoretical delta walker constellation is also simulated for a comparative analysis of the performance evaluation parameters.

The surveillance ability of the constellation is analyzed on the global and regional scales. Global surveillance of the constellation is assessed on a latitude bound global coverage definition between 59°N – 58°S. On the other hand, regional surveillance is assessed by simulating intercontinental flight route regions over the Atlantic, Indian and Pacific Oceans, shown in Figure 7. These oceanic regions are deprived of ground based radar or ADS-B surveillance and connect all major cities of Stockholm, Kuwait, Karachi, San Francisco, Los Angeles, New York, Stockholm, Zurich, Paris, London, Copenhagen, Vienna, Sydney, Tokyo, Nagoya and Osaka (Maps of World, World air routes, 2002). The analysis time of the simulation is one day (24 h), and the start time is 00:00:00 UTC, October 1, 2022. The parametric calculations are performed on 3° lat/long grid for both global and regional simulation analysis.

3.1. Constellation Visibility Analysis

To analyze ADS-B constellation visibility, the total number of available assets in various parts of the globe has been computed. The satellite visibility of the designed constellation is also compared with that of the theoretical constellation of 36 satellites to see if the visibility constraint is satisfied and to analyse the difference between the two scenarios. Firstly, to determine constellation visibility on global scale, we have computed system visibility for the simulated latitude bound region between 59°N - 58°S. The statistical results are shown in Figure 8, which suggest that 1-2 satellites are visible in the area of interest. A moderate variation can be observed in asset availability against the latitude. Satellite availability is slightly higher at low latitudes because of the optimum geometry of the constellation that allows better asset availability in dense air traffic regions, e.g. the equatorial, subtropical and mid latitude regions.

Secondly, satellite availability for the simulated oceanic regions has been computed which is graphically represented in Figure 9.The graphs are plotted by selecting 5˚ mask angle visibility constraint and data sampling interval of one hour. It can clearly be seen that at least one satellite is overhead for all the oceanic regions, which fulfills the basic space mission requirement of efficient air surveillance over trans-oceanic flight regions. Furthermore, different scenarios are simulated to evaluate the outcome of varying numbers of satellites in the constellation. The orbital geometry is preserved while spacecraft number is varied between 6 and 9 per plane. As estimated, a higher number of satellites per plane increases the coverage and reduces

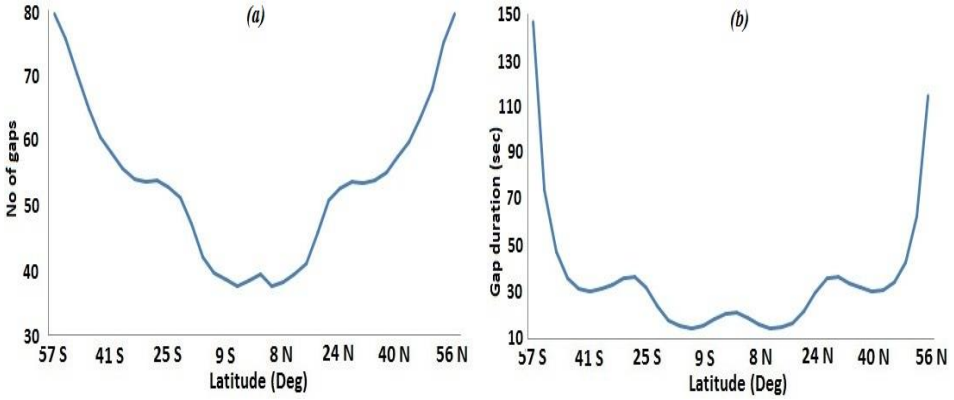

Figure 11 Spatial variation in coverage gaps throughout the simulation time of 24 hours, (a) Total number of gaps, (b) Gap duration.

gaps in the trans-oceanic flight regions. More satellites per orbit also increase the probability of one satellite accessibility for a particular region and decreases the revisit time.

3.2. Constellation Coverage Analysis

As specified previously, the constellation is designed to primarily cover the trans-oceanic intercontinental flight routes; therefore, a coverage analysis is carried out to determine the constellation's total coverage area on the entire globe. In simulations, the satellite footprint is expressed as a fraction (percentage) of the Earth's area. We also analyzed the spatial and temporal variation in the percentage coverage. Percentage coverage is mathematically expressed by the following equation:

$$\text{Percentage Coverage} = \frac{\text{Total access time to a satellite}}{\text{Total analysis time}} \times 100 \quad (6)$$

The simulation results of the coverage analysis are shown in Figure 10. The constellation of 20 satellites efficiently covers up to 88 % region of the Earth between 59° N - 58° S in overall simulation time with a moderate temporal variation in percentage coverage. This temporal variation is caused by the combined effect of Earth's rotation and orbital motion of the constellation. Likewise, there is a considerable latitudinal variation in the percentage coverage area, illustrated in Figure 10 (b). The coverage in the low latitude regions is higher as compared to the high latitude regions. This is due to higher satellite availability in the equatorial, tropical and mid latitude regions that provide a consistent ADS-B coverage in the busiest air traffic routes.

3.3. Constellation Gap Analysis

Theoretically, a walker constellation of 36 satellites is required for 100% ADS-B coverage within the latitudinal range of 59°N - 58°S. As such, to minimize space mission cost, number of spacecrafts per orbit is reduced, which has resulted in coverage gaps. A gap is a duration in which no satellite is accessible to a particular region on the Earth meaning e.g. that the aircrafts cannot establish a communication link with a satellite during this interval. The coverage analysis of the constellation suggests that 12 % of the total airspace remains uncovered from real time surveillance measures. To inspect the geographical distribution of the gap regions, the total number of gaps and time average gap has been computed. The results of the coverage gap analysis are presented in Figure 11.

The total number of gaps lies between 40 and 80 for different regions of the globe in a simulation time of 24 hours. The duration of each gap greatly depends on

Table 2 ADS-B link budget analysis

Link Budget Parameter	**Symbol**	**Value**	**Unit**
Carrier frequency	f_c	1090	MHz
Transmitter power	P_t	26.98	dBW
Transmitter gain	G_t	1	dB
Line loss	L_t	-2	dB
Maximum geometric distance (E = 0˚)	d_{max}	3291	Km
Minimum geometric distance (E = 90˚)	d_{min}	850	Km
Free space loss at d_{max}	FSL_{max}	-163.5	dB
Free space loss at d_{min}	FSL_{min}	-151.8	dB
Receiver antenna gain	G_r	11.2	dB
Atmospheric loss	L_{atm}	-2.5	dB
Polarization loss	L_{pol}	-6	dB
Minimum received power at d_{max}	$P_{r\,(min)}$	-134.8	dBW
Minimum received power at d_{min}	$P_{r\,(max)}$	-123.12	dBW
System noise temperature	T_{eq}	290	K
Carrier-to-Noise Spectral Power Density Ratio at $d_{max}$and d_{min}	C/N_0	69.1, 80.8	dB.Hz

the geographic location of the gap region. The gap duration is higher in sub polar regions and decreases towards the equator. The time average gap is less than 50 sec in mid latitude, tropical and equatorial parts of the globe. This is mainly due to the higher percentage of coverage provided by the constellation at low latitudes. The results of the coverage gap analysis indicate a consistent surveillance in dense air traffic routes over the oceans as a result of small gap intervals.

4. Communication Analysis

The ADS-B 1090 MHz ES signal is 120 µs long, comprising of 8 µs preamble and 112 µs data block. The signal is transmitted frequently within 0.4 to 0.6 seconds. The main purpose of this periodic randomization is to prevent the aircraft from obscuring each other's communication. As we know in a communication system, the signal is transmitted from the transmitter travels through the medium and is received by the receiver. Link budget is the quantitative representation of all the gains and losses of the signal propagation (Francis, Vincent, Noël, Tremblay, Desjardins, Cushley, & Wallace, 2011). The link has to be examined to evaluate the power of the transmitted ADS-B signal at a distance of several hundred kilometers. We must consider the fact that the detection and processing of weak signals at an altitude of about 850 Km cannot be compared with that of the terrestrial ADS-B system due to limited link margins. Thus, the link budget is calculated based on an assumption that the transmitter has a high gain antenna and the on-board receiver is of higher sensitivity as compared to the terrestrial receiver.

Table 3 Results of performance evaluation parameters of flight 447

Parameter	**Unit**	**Value**
Total simulation time	sec	43680.12
Coverage time	sec	35023.7
Total coverage	%	81.1
Average available assets		1.3
Total accesses		87
Average access duration	sec	669.4
No of gaps		31
Time average gap	sec	62.5
Approximate maximum slant range between Flight 447 and satellite	Km	3290
Minimum received power at d_{max}	dBW	-135
Carrier to noise spectral power density at d_{max}	dB.Hz	84.9

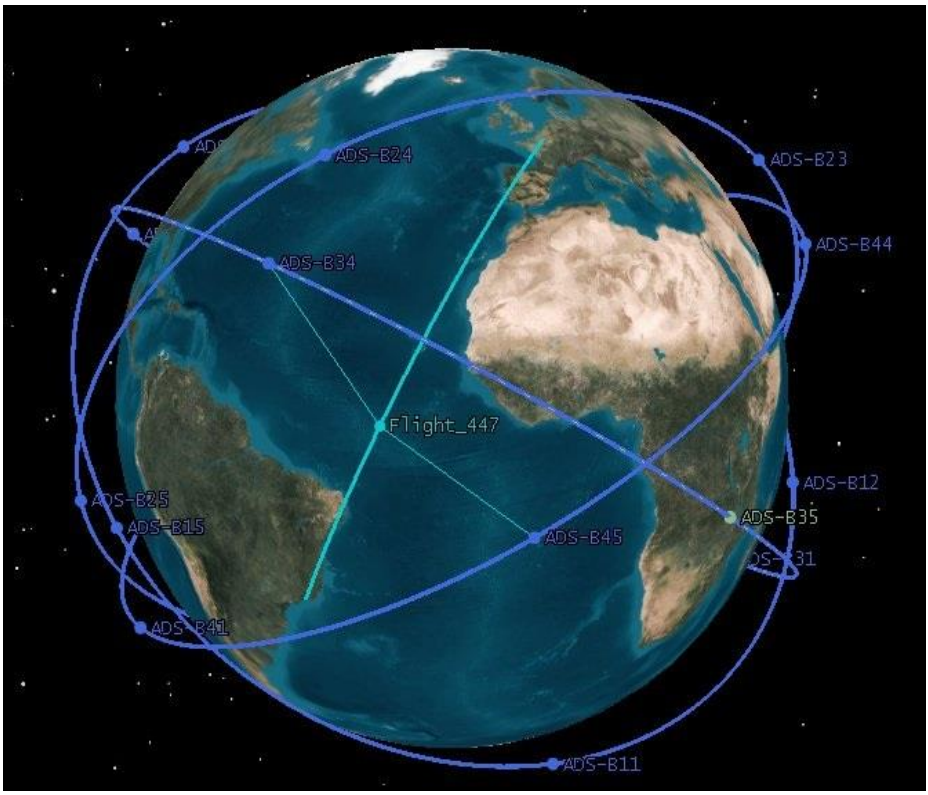

Figure 12 Access between Air France flight 447 and the satellites at the last known position.

The aviation standards for ADS-B allow aircraft to transmit signal at a transmitting power of 75W to 500 W, depending upon the aircraft type. Such a variation in transmitter power greatly affects the Minimum Detectable Signal (MDS) for a given ADS-B receiver (Betz, 2015). For the purpose of link analysis, we considered 1090 ES ADS-B omni-directional transmitter with board ADS-B receiver is considered the same as of Proba V. It is a planer and right hand polarized

antenna having 11.2 dBi gain. This link budget analysis is based on the reference

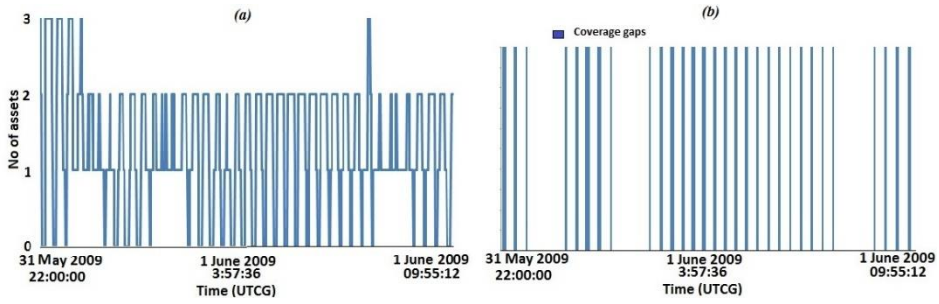

Figure 13 (a)Total number of available assets for flight 447 in overall simulation time, (b) Total number of coverage gaps for flight 447 in overall simulation time.

data and can be adjusted in the design process. The link budget is presented in the Table 2 (Betz, 2015), (Zhang, Wu, Cheng, & Zhu 2018).

5. Case Study

On 1 June 2009, Air France flight 447 crashed in the middle of the South Atlantic Ocean while going from Brazil to France. At 22:29:00 UTC on 31 May 2009, the flight took off from Rio de Janeiro-Galena airport. It had to arrive at Paris-Charles de Gaulle airport at 10:03:00 UTC but it was not that case. The known last communication of Brazilian ATC with flight 447 was made 3 hours and 6 minutes after. At 01:49 UTC, the airliner left Brazilian Atlantic radar surveillance and went through the airspace with no radar surveillance. It was expected to reach the Senegalese airspace at about 02:20 UTC, but got disappeared within the airspace of no radar surveillance. It was a modern airliner and was equipped with Aircraft Communication Addressing and Reporting System (ACARS).This system has the capability to transmit data messages by means of Very High Frequency (VHF) after every 10 minutes (Stone, Keller, Kratzke, & Strumpfer, 2011). Its final known geographical coordinates of the aircraft were 2°59′N lat 30°35′W long at about 02:10:34 UTC.

In this section, Air France flight 447 is used as a case study to demonstrate the tracking efficacy of this novel low cost space mission. The flight is simulated in a simulator to analyze the coverage, total gaps, gap duration and available assets to the aircraft. The simulation time is exactly the time of flight 447 from 30 May 2009 22:20 UTC to 1 June 2009 10:10UTC. The simulation results of coverage analysis parameters of flight 447 are illustrated in Table III. The ADS-B constellation of 20 satellites provides 80.1% coverage to the aircraft in overall simulation time with 87 accesses and 31 gaps. The average access time for flight 447 is 669.4 sec and the time average gap is 62 sec. The total number of available assets and gaps for the aircraft throughout the flight path is graphically presented in Figure 13. The aircraft entered the region of no radar coverage at 01:49 UTC and its last known position was reported at 02:10 UTC. We examined aircraft accesses in non-radar airspace, particularly at the last known position (2°59′N 30°35′W). The results from the simulation confirm that 2 satellites are in access with the aircraft at the last known position at the time when the simulation analysis of flight 447 reveals that the model LEO constellation of 20 satellites would have provided consistent coverage to flight 447 and the possible crash site location could precisely be Figured out with the space based ADS-B system provided the transponder of the aircraft remained operational.

6. Conclusion

In this paper, a low cost and technically feasible LEO tracking system has been proposed for global air traffic surveillance over the intercontinental trans-oceanic flight regions. The LEO system is designed according to the purpose and its

performance is evaluated by analyzing percentage global and regional coverage, satellite visibility and coverage gaps using dynamic simulations. The results of the parametric study recommend that the designed ADS-B satellite system provides up to 88% coverage in the simulated oceanic regions. We also analyzed the spatial and temporal variation in the performance evaluation parameters which has given some valuable results. The satellite visibility and coverage in lower latitude regions is higher due to the optimum constellation geometry selected by the optimization process.

The communication analysis was also carried out which involves ADS-B link design and analysis of the LEO satellite antenna handoff mechanism. The simulation analysis verifies the theoretical calculations of the link budget and suggests that the MDS of on-board ADS-B receiver should be greater than 104.8 dBm to receive and interpret weak ADS-B signals.

Although, Iridium has planned to launch a walker system of 66 LEO satellites which will assure global coverage without any gap region. But, the designed constellation with 20 satellites provides a consistent ADS-B coverage over intercontinental air traffic routes over the Atlantic, Pacific and Indian Oceans. This proves the proposed ADS-B system to be a cost effective alternative as compared to the Iridium Next. The primary payload of the constellation is the on-board ADS-B receiver, but it can be used for oceanic disaster management and ship navigation as well.

7. Acknowledgement

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8. Conflict of Interest

The authors declare no conflict of interest.

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Effect of Carrot-Beet Based Beverages to Modulate Hypertension

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Abstract

The objective of this study was to assess the effect of carrot and beetroot-based beverages to modulate hypertension in hypertensive subjects. Two different combinations of carrot-beetroot juice were prepared consisting of Beverage-I (carrot juice 20% and beetroot juice 80%) and Beverage-II (carrot juice 40% and beetroot juice 60%). Antihypertensive influence of beverages was assessed through physiological markers i.e., pulse rate, body temperature, systolic and diastolic blood pressure. Serum lipid profile including triglycerides, cholesterol, high density lipoprotein and low-density lipoprotein was also assessed at start and termination of the experimental trial. Results obtained at the end of the study revealed that Beverage-I influentially reduced the pulse rate, systolic and diastolic pressure, triglycerides, and LDL levels however, in case of total cholesterol, a more pronounced effect was observed in subjects receiving Beverage II. From the outcomes, it was concluded that both beverages showed modulation of hypertension and hence, these beverages can be considered for routine dietary therapy to address hypertension.

Keywords: Antioxidant, Carrot-beet Beverages, Hypertension, Lipid Profile, Dietary Nitrates

1. Introduction

Food is the elementary need of individuals for their survival, growth, and maintenance. According to health experts, dieticians and nutritionists, a healthy diet is now an important issue for all individuals. A healthy diet has more impact on the prevention and treatment of a number of diseases (Engelhard, Gazer, & Paran, 2006; Kim et al., 2011). In developing countries, metabolic disorders consequent to inadequate intake of micronutrients and unhealthy food consumption are the major concerns of this era (Iahtisham-Ul-Haq & Butt, 2015). To elaborate the importance of various food components in disease prevention and cure, the term "functional food" has been established. Functional foods are natural or industrially processed foods when regularly consumed as part of a diverse diet, provide health-boosting effects beyond basic nutritional needs (Granato et al., 2020). The foods that are derived from plants are often rich in phytonutrients and play a significant role in disease control alongside health maintenance. The popularity of functional foods has

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increased among consumers because of increased awareness about the importance of healthy diet in sustaining healthy living. Regular consumption of fruits and vegetables is associated with low incidence of lifestyle related degenerative ailments (Engelhard et al., 2006; Kim et al., 2011). Studies have revealed that the chronic diseases like diabetes, obesity, hypertension and chronic heart disease can be prevented by the regular intake of fruit, vegetables, and dietary fibers (Iahtisham-Ul-Haq et al., 2020).

Hypertension is a common, insidious and deadly disease often diagnosed incidentally at normal screening or routine clinical visit (Alexander, Ostfeld, Allen, & Williams, 2017). Globally, 31.1% of adults (1.39 billion individuals) are suffering from hypertension resulting in 9.4 million deaths annually (Gedamu & Sisay, 2021). In developing countries this burden is raising drastically because a number of hypertensive people remain undiagnosed, inadequately managed or untreated leading to increased burden of cardiovascular diseases (Undavalli & Mp, 2018). Although medications are available to treat hypertension, several studies have shown that lifestyle modifications *i.e.* physical activity, smoking avoidance and healthy dietary habits can help in preventing and/or treating hypertension (Duman, 2013). Increased intake of foods from plant sources rich in sterols, polyphenols and flavonoids have been found to be associated with lower risk of hypertension and cardiovascular diseases (Alexander et al., 2017; Duman, 2013). Therapeutic diets can sufficiently lower the cholesterol in plasma (Imran et al., 2020). Oxidative stress and inflammation have been observed as the major factors associated with initiation and progression of hypertension and linked anomalies (Pouvreau, Dayre, Butkowski, De Jong, & Jelinek, 2018). In this context, the natural antioxidants derived from plants have been studied for their oxidative stress mediated malfunction against free radical scavenging potential (Kujawska et al., 2009). Moreover, the harms of aging process and allied problems may be prevented by the consumption of natural antioxidant rich diet (Ravichandran et al., 2013; Vulić et al., 2014).

Carrot (*Daucus carota*) belongs to the family "Umbelliferae". Carrots are popular in Asian and western region of the world. The bioactive component abundantly found in carrots is known as "Beta-carotene" which is an orange-red pigment with sufficient nutraceutical potential. Moreover, carrots are also considered as rich source of fiber (Buijsse, Feskens, Kwape, Kok, & Kromhout, 2008). Besides, Alpha-carotene and Lutein present in carrots acts as potent antioxidant (Griep, Verschuren, Kromhout, Ocké, & Geleijnse, 2011). Vitamins, sugar, and minerals are very important for a healthy living and fresh plant-based beverages contain plenty of vitamins and nutrients. It has been proven that *Daucus Carota* extract enriched diet reduced the low-density lipoprotein, plasma triglycerides level, hypertension, and total dietary fat. The extract also showed substantial hypolipidemic activity and obstructs the production of free radicals (Singh & Hathan, 2014).

Red beetroot (*Beta vulgaris* L.) belonging to family "Chenopodiaceae" has number of variations and show yellow to red hue (Iahtisham-Ul-Haq, Butt, Randhawa, & Shahid, 2019a; Singh & Hathan, 2014). The active components in red beetroot are betalains with high biological value. Betalains are nitrogenous compounds capable of delivering protective effects against different diseases (Iahtisham-Ul-Haq, Butt, Randhawa, & Shahid, 2019b). There are further two subgroups of betalains: the red/purple betacyanins which are associated with the shade of red beetroot and yellow/orange betaxanthins that relates to the color of yellow beetroot (Iahtisham-Ul-Haq, Butt, Randhawa, & Shahid, 2020). It has been demonstrated that consumption of dietary nitrate could play a vital role in preventing cardiovascular disease and hypertension in

healthy individual (Bondonno et al., 2015). Higher concentration of inorganic nitrate is available in red beetroot, typically ranging between 110 to 3670 mg nitrate kg^{-1} that may positively alleviate hypertension (Siervo, Lara, Ogbonmwan, & Mathers, 2013). It has been proven that nitrate ingestion in the form of nitrate salt or in vegetable product form like beetroot juice could be helpful in reducing blood pressure greatly as well as beetroot juice also have nitrate supplement which appeared as a potential nutritive agent to prevent and cure of hypertension and coronary artery diseases (Wylie et al., 2013).

The primary objective of this research was to explore the antihypertensive effect of carrot and beetroot juice in combination. Hence, carrot and beetroot are employed in the current study for development of carrot-beetroot based beverages and their bio-evaluation to modulate hypertension.

2. Materials and Methods

2.1 Development of Beverages

Garden-fresh red beetroot and carrots were obtained based on uniformity in color, size, and absence of any physical damage. Both vegetables were washed to remove any adhered material from their surfaces. After that, they were peeled off and cut into small sized dices. Red beetroot juice and carrot juice was obtained using a home-scale juicer. Two beverages with different proportions of carrot and beetroot juices were prepared and the final products were kept in refrigerator at temperature (4-6℃). These proportions were obtained based on sensorial acceptability of the beverages. The beverage containing 20% carrot juice and 80% beetroot juice was regarded as Beverage-I whilst the beverage containing 40% carrot juice and 60% beetroot juice was regarded as Beverage-II.

2.2 Experimental Design

To assess the therapeutic potential of carrot-beetroot based beverages, hypertensive patients were enrolled in a clinical experimental trial after taking their consent. The study was approved after meeting all the ethical standards by the "bioethics committee" of the University of Agriculture, Faisalabad after institutional screening. After taking prior written consent, 24 hypertensive subjects of age between 23-45 years were randomly divided into 3 experimental groups having 8 patients in each group and were prescribed a controlled diet plan to follow during the trial. The subjects in group G1 were provided 250 mL of Beverage-I whilst subjects in group G2 were given 250 mL of Beverage-II on alternative days in the morning for 60 days whereas G0 served as control.

2.3 Biological Assessment

The body temperature was checked *via* a thermometer and blood pressure was recorded by using digital sphygmomanometer. To test the effect of beverages on lipid profiles, blood samples were evaluated at initiation and termination of the trial for triglycerides, total cholesterol, high- and low-density lipoproteins using commercial kits (Ecoline™ Merck KGaA) (Imran et al., 2018).

2.4 Statistical Analysis

All the data collected were statistically analyzed using Statistix 8.1 statistical software (Tallahassee, Florida, USA). The Microsoft Excel v2016 was used for handling data and graphs preparation. Two-way ANOVA was used for checking statistical significance established at $P<0.05$. Tukey's Honest Significant Difference test was used for post-hoc comparison of means.

3. Results

The results showed that carrot-beet-based beverages significantly ($P<0.05$) affected the pulse rate of hypertensive patients. Figure 1 illustrates that Beverage I had reduced pulse rate of participants in G1 (64.38±4.17) more influentially up to 23% than Beverage II in G2 (76.25±5.18) which reduced the pulse rate up to 9% when compared with untreated patients of G0 (83.75±5.18) group. Whilst, in the case of body temperature, statistical analysis revealed no significant differences amongst the groups.

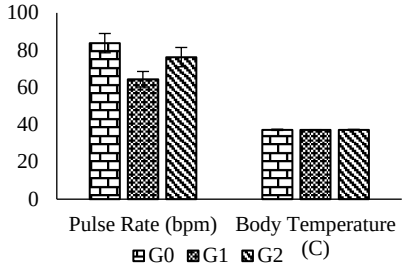

Figure 1 Effect of carrot-beet beverages on pulse rate and body temperature of hypertensive subjects [G0:control group, G1:group provided with 20% carrot juice and 80% beetroot juice as Beverage-I, G2: group provided with 40% carrot juice and 60% beetroot juice as Beverage-II]

Fig. 2 shows the average systolic and diastolic blood pressures of hypertensive patients. The statistical analysis depicted significant ($P<0.05$) variation in the average systolic as well as diastolic blood pressure of individuals enrolled in different groups. According to results obtained, treatment with Beverage I and Beverage II reduced systolic pressure in G1 and G2 up to 8.65 and 7.61%, respectively with trial progression as evident from the trend deduced from initial to final readings. Furthermore, in case of final values obtained at termination of experiment, G1 and G2 had lower systolic pressure as 118.75±6.41 and 121.25±8.35, respectively in comparison to G0 (130±9.26). For diastolic blood pressure, a reduction of 6.02 and 4.47% was recorded in Beverage I and Beverage II treated groups *i.e.,* G1 (78.75±8.35) and G2 (80±5.35), respectively than G0 (83.75±7.44).

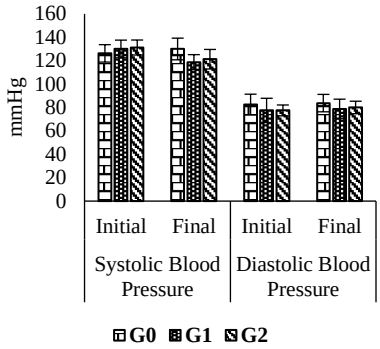

Figure 2 Effect of carrot-beet beverages on systolic and diastolic blood pressures [G0: control group, G1: group provided with 20% carrot juice and 80% beetroot juice as Beverage-I, G2: group provided with 40% carrot juice and 60% beetroot juice as Beverage-II]

The lipid profile of the hypertensive patients (Fig. 3) revealed that Beverage I and Beverage II caused significant ($P<0.05$) decline in cholesterol (A), triglycerides (B) and LDL (D) while increase in HDL (C) in G1 and G2, respectively than G0. Fig. 3 shows that at the end of experimental period, reduction in cholesterol, triglycerides and LDL was recorded in G1 up to 9.90, 10.44 and 14.22% while in G2 up to 13.62, 4.68 and 8.86%, respectively when compared to initial values. Contrary to this, an increase in HDL was observed by 11.29 and 7.40% in G1 and G2, respectively. Overall, carrot-beet based beverages significantly

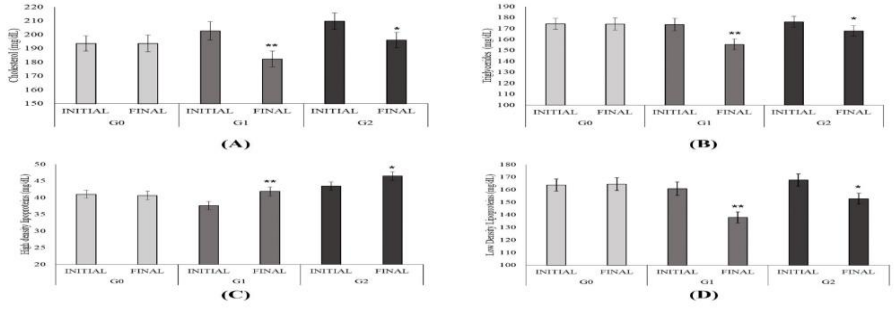

Figure 3 Effect of carrot-beet beverages on lipid profile of hypertensive subjects [G0: control group, G1: group provided with 20% carrot juice and 80% beetroot juice as Beverage-I, G2: group provided with 40% carrot juice and 60% beetroot juice as Beverage-II]

controlled the physiological markers related to hypertension.

4. Discussion

It is widely known that different foods are rich sources of various health promoting bioactive components, vitamins and minerals that support human health and bodily functions. Evidence is rising that higher consumption of functional foods lessens the chance of morbidity and mortality associated to lifestyle related disorders particularly cardiovascular disorders and hypertension. In this context, different combinations of carrot and beetroot beverages were examined for their antihypertensive effect in current experiment. Treatment with the beverages lowered pulse rate in hypertensive patients than the non-treated subjects. A possible reason behind could be the dilation of blood vessels in response to nitrates found in beetroots. Several previous studies have reported that beetroot juice does not influence the pulse rate significantly but a decreasing trend has been observed in people treated with beetroot juice than the placebo (Bender et al., 2018; Ormesher et al., 2018; Velmurugan et al., 2016). The significant decline in pulse rate recorded in current study might have occured due to combined effect of beetroot juice with carrot juice. Carrot juice is rich in potassium which is an important component of body fluids and helps in maintaining the heart rate (Aderinola & Abaire, 2019). Increased intake of potassium slows the rate of pacemaker depolarization during heartbeat and thus slows down the heart rate (Aziz, Li, & Tinker, 2018).

Regarding systolic and diastolic blood pressure, both beverages have shown the blood pressure reducing effect. The results are in line with those reported by Ormesher *et al.* (Ormesher et al.) who evaluated the hypotensive effects of nitrates from beetroot juice in hypertensive pregnant women. They found that beetroot juice significantly reduced the systolic blood pressure by 7% than baseline values after 24 hours of supplementation while for diastolic pressure they observed non-significant difference in baseline and after supplementation values. The dietary nitrates from beetroot juice are rapidly absorbed in saliva from blood *via* active transport which are then converted into nitrites by oral microflora (Kapil et al., 2013). These nitrites have been proven for their blood pressure-lowering effect in both hypertensive (Webb et al., 2008) and normotensive subjects (Ghosh et al., 2013). The other major component of the treatment beverages, carrot juice is full of antioxidants, phenolic acids and flavonoids and it has been reported that carrot juice reduces the oxidative stress that plays a key role in progression and pathogenesis of hypertension (Baradaran, Nasri, & Rafieian-Kopaei, 2014; da Silva Dias, 2014). A group of peers has recently explored the hypotensive potential of carrots and reported that carrot supplementation completely prevented the increase in systolic and diastolic blood pressure consequent to high fat diet consumption in mice (Soleti et al., 2021). They proposed that carrots showed the blood pressure lowering effect not only by reducing the reactive oxygen species but also increased the availability and production of nitric oxide which triggers the hypotensive influence (Craig et al., 2020).

Our results revealed that both beverages significantly reduced the total cholesterol, LDL, and triglyceride levels in hypertensive patients. These results agree with the findings of Asgary et al. (2016) who investigated hypolipidemic effects of raw beetroot juice and cooked beet on hypertensive patients and concluded that raw beetroot juice showed more prominent effect in lowering total cholesterol (10.5%), LDL (12%) and triglycerides (13.54%) than cooked beet. Current outcomes are in line with Rahimi et al. (2019) who reported that supplementation of red beetroot to atherosclerotic patients decreased the level of total cholesterol, triglycerides and LDL by 7, 6 and 12%, respectively in comparison to placebo

treatment. Besides, beetroot juice also increased the HDL content up to 4.42%. Furthermore, Holy and coworkers also observed reduction in serum levels of LDL, total cholesterol and triglycerides after beetroot juice supplementation to healthy objects however, they reported non-significant effect of beetroot juice on HDL concentration (NNIaBON, 2017).

Regarding the hypolipidemic effects of carrot juice these findings are supported by the outcomes of Żary-Sikorska, Fotschki, Fotschki, Wiczkowski, and Juśkiewicz (2019) who explored the impact of carrot-based preparations on lipid profile and antioxidant status of rats and observed that carrot preparations significantly decreased the triglycerides and total cholesterol in blood. They proposed the mechanism behind that could be the bioactive components especially anthocyanins in carrots affected the production of short chain fatty acids from gut microbiota and hence improves serum lipid profile.

Carotenoids are known to inhibit lipid peroxidation in arterial walls, influencing plaque stability, vasomotor function, platelet aggregation, and thrombosis (Asplund, 2002). Human studies suggest that elevated plasma β-carotene is associated with reductions in circulating cholesterol and the risk of myocardial infarction. Carotenoids in beetroot juice are cleaved by the 2 enzymes namely ß-carotene Oxygenase 1 (BCO1) and ß-carotene Oxygenase 2 (BCO2). Amongst them BCO1 is the only enzyme which is involved in production of vitamin A from provitamin A carotenoids (Amengual et al., 2013; Coronel, Pinos, & Amengual, 2019). Amengual et al. (2020) later explored that it is the activation of BCO1 enzyme, in response to carotenoids in carrot juice, which reduces the total cholesterol concentration in serum instead of ß-carotenes.

5. Conclusions

The research indicates the positive role of carrot and beetroot juice in controlling hypertension. The provision of carrot and red beetroot juice radically improved the lipid profile, pulse rate and systolic and diastolic blood pressure. Therefore, the consumption of carrot and beetroot juice may be beneficial for the maintenance of good cardiac health. Our results suggest the anti-hypertensive effect of nitrate-containing carrot and beetroot juice, but this study is conducted only by considering hypertensive patients. It is recommended that carrot and beetroot-based beverages should be incorporated as part of routine diet and used as nutritional therapy for hypertensive individuals. However, further research on synergistic or antagonistic effects of such beverages with other food components may be investigated for more personalized dietary regimens and nutrition therapies.

6. Conflict of Interest

The authors declare no coflict of interest.

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IL-17: New Insights into Advances from Diagnostics to Therapeutics of Inflammatory Diseases

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Abstract

Interleukin 17 (IL-17) is a pro-inflammatory cytokine having 6 related cytokines ranging from IL-17A to IL-17F. Over the past few years, the roles of IL-17 go beyond not merely activating inflammation but also the progression of autoimmune or inflammatory diseases like Psoriasis, Inflammatory bowel disease, and Crohn's disease. Evidence is mounting that IL-17 has significant roles in causing and developing various inflammatory diseases. Studies have shown that IL-17 is produced by a subset of T cells known as Th17 which involves the initiation and maintenance of many autoimmune or inflammatory diseases. And recently, several therapeutic approaches have been developed for targeting IL-17 including monoclonal antibiotics which block the activity of IL-17, and specific inhibitors molecules have improved in the treatment of these diseases. This review aims to highlight the general trends of these inhibitor molecules as a treatment against these diseases as well as the various diseases related to IL-17 inflammatory actions, and interactions with other cytokines that perform similar functions. It also highlights the development of more targeted therapies and works as an identification of new biomarkers for disease diagnosis. However, further research is needed to fully elucidate the role of IL-17 biology in these inflammatory diseases and to develop more effective treatments.

Keywords: Interleukin 17; Psoriasis; Rheumatoid arthritis; Crohn's disease; Quercetin

1. Introduction

IL-17 is a pro-inflammatory cytokine, consisting of many other members ranging from IL-17A to IL-17F (Tayefinasrabadi et al., 2020). Out Which IL-17A was the first to be discovered and its binding receptor; was later recognized as IL-17RA (Ge et al., 2020). Researchers then tested for IL-17A homologous genes and identified IL-17B to IL-17F. According to various studies, IL-17B is a key player in both cancer and inflammation. Six 20–30 kDa molecular weight isoforms of the secreted and glycosylated IL-17 family of proteins make up this family. The sequence homology between IL-17A and the remaining members of the IL-17 family ranges from 20 to 55%, with IL-17E having the lowest homology. Four cysteine residues in the C-terminus of IL-17 family proteins, which are structurally conserved, create intramolecular disulfide bridges

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(Kostareva et al., 2019). IL-17 was thought to be the cause of $CD4^+$ T helper 17 (Th17) cell production, but recently many studies have shown that IL-17 is produced by various kinds of immune cells; both adaptive and innate (Akitsu et al., 2018; Berry et al., 2022). IL-17 mostly arises from Th17 cells which produce the cytokines such as IL-17, IL-22, and IL-26 (Packi et al., 2022). Th17 lymphocytes can produce IL-17 by IL-23 maintaining the phenotype. In many cases, IL-17 production may also be the result of genetic polymorphism due to certain diseases (Bugaut et al., 2021). Even under stressed situations certain cells may produce IL-17, as in the incident of stroke astrocytes produce IL-17 (Brigas et al., 2021). IL-17 was initially cloned from a rodent-activated T cell hybridoma, where it was also discovered that it showed a strong variation in sequences of other known cytokines, of mammalian nature. This would later suggest a strong relation to evolutionary linkages (Brembilla et al., 2018; McGeachy et al., 2019). With its recent surge in modern technology, IL-17 is linked to numerous inflammatory diseases, and therefore, an elevated amount is an indicator or serves as an important biomarker. IL-17D possesses a unique C-terminal domain that mediates a unique receptor interaction (Liu et al., 2020). IL-17 communicates its function via IL-17RA and IL-17RC receptor subunits. Although IL-17RA is expressed everywhere, fibroblasts and endothelial cells have been identified to be the primary recipients of IL17A responses. Depending on the stimuli that cells are exposed to, their broad expression of IL17RA is dynamically altered. The IL-17A-IL-17F heterodimer both bind to this receptor complex (Molina et al., 2019). IL-17 has an essential role in tissue repairs, and although there is not much on its complete mechanisms. Every member of the family of cytokines functions as a homodimer or heterodimer and interacts with certain dimeric receptors. The cytokines also play vital roles in signaling pathways (Brevi et al., 2020). One of the key players in the host's fight against microbial diseases is IL-17. In both humans and mice, the IL-17 pathway controls antifungal immunity by increasing levels of proinflammatory cytokines, antimicrobial peptides, and neutrophil-recruiting chemokines, which prevent fungal overgrowth (Schinocca et al., 2021). IL-17A is by far the most examined member (Chen et al., 2018; Zhao et al., 2019). Hematopoietic cells such as Th17, CD8+ cytotoxic T cell (Tc17), T cell, natural killer cell, group 3 innate lymphoid cells (ILC3), and Th17 cell all generate IL-17A. It has been demonstrated that IL-17A has a role in the formation of tumors, neovascularization, and immunological inflammation (Chen et al., 2020; Furue et al., 2020; Shibabaw, 2020). The common SEFIR (SEF/IL-17R) cytoplasmic motif is activated when IL-17 cytokines bind to the corresponding IL-17 receptors. Additionally, the IL-17RA molecule functions as a subunit of the IL-17C, IL-17E, and IL-17F receptors. Early research employed animals lacking the IL-17RA (or IL-17R) gene on a mouse model for the absence of IL-17A function; however, IL-17RA loss also impairs the capacity to respond to IL-17C, IL-17E, and IL-17F (Matsuzaki et al., 2018).

2. IL-17 Signaling

The cytokine receptors (IL-17Rs) are no different from other pro-inflammatory cytokines, communications between their respective IL-17 prompt different response, expressions, cellular processes, and more. Two extracellular domains that resemble fibronectin III and an intracellular SEFIR domain are present on each IL-17R, coupled with two subunits. Out of which one is necessary for identifying different ligands. When IL-17 binds to the IL-17RA-IL-17RC complex, the ubiquitin ligase adaptor protein Act1 is generated. Act1 then binds tumor necrosis factor receptor-associated factor 6 (TRAF6) to activate the nuclear factor-B (NF-B) and mitogen-activated protein kinase (MAPK) pathways, as well as increase the expression of several proinflammatory cytokines and chemokines (Swaidani et al., 2019). Transcription of the genes encoding pro-inflammatory and neutrophil-mobilizing cytokines and chemokines is caused by IL-

17-Act1-mediated signaling (Herjan et al., 2018). Under chronic conditions, IL-17 activation leads to the stimulation of Lys48-linked polyubiquitination followed by degrading Act1. Testing the deficiency of Act1 on experimental mice, yielded a decrease in IL-25-dependent gene expression which resulted in the animal being more impervious to IL-25-mediated allergic airway inflammation (Swaidani et al., 2019). These IL-17 homologs are believed to constitute a distinctive ligand-receptor signaling pathway that appears to have been well preserved throughout evolution due to its exceptional structural characteristics and limited similarity to other known cytokines and receptors. Comprehensive studies have revealed that IL-17 is a key immunological stimulator of an inflammatory network that powerfully synergizes with other cytokines. IL-17 has been linked to the development of inflammation, autoimmunity, and host defense against certain infections, according to studies in mice and human models (Lv et al., 2022). And hence this is the reason why many inflammatory diseases are profoundly linked to IL-17 expressions and or amount.

3. Inflammatory diseases and IL-17

As mentioned previously, IL-17 has been the target of numerous studies indicating its role in inflammatory diseases. IL-17 has since become a notable biomarker in these inflammatory diseases. This has facilitated many researchers better evaluate the pathogenic nature of particular diseases, in addition to all this, IL-17 has allowed for more accurate therapies. Many studies have revealed that only IL-23, not IL-12, caused activated memory T cells to secrete IL-17. In light of this, it was postulated that IL-23 stimulates the growth of a specific fraction of effector CD4+ T cells that are distinguished by the release of IL-17, which leads to the development of inflammatory diseases (Bianchi et al., 2019).

3.1 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is characterized by a symmetrical increasing inflammation of the afflicted joints that causes bone erosion, cartilage damage, and impairment, therefore RA is another chronic inflammatory disease that affects the joints (Ruiz de Morales et al., 2020). In addition to stimulating proinflammatory pathways such as inflammatory cytokine production, pannus development, and synovial neo angiogenesis, IL-17 also triggers negative feedback regulation through the activation of prostaglandin E2 (PGE2). This leads to structural deterioration of rheumatic joints. According to recent studies, individuals with early RA who have not yet started therapy had dramatically higher levels of IL-17 in their synovium, blood, and synovial fluid (Wu et al., 2019). Several studies have identified dysregulated amounts of IL-17A in the serum of patients. Mast cells make up the majority of IL-17A+ cells in the synovial tissue in the case of RA, while neutrophils and T cells are much less common (Ruiz de Morales et al., 2020). Another study showed how the IL-17Rs were able to bind to the same receptor; IL-17RA and IL-17RC subunits that caused inflammation and granulopoiesis. The same study showed in an in vitro experiment that the production of IL-6 and IL-8 is stimulated by IL-17A leading to migration and invasion that result in cartilage destruction. Moreover, the receptor activator of NF-κB ligand (RANKL) binds to RANK and stimulates osteoclastogenesis before causing osteoclasts to damage bone, which is expressed more often on osteoblasts as a result of IL-17 (Robert et al., 2019). The IL-17A and IL-17F and their receptor genes have functional polymorphisms that may influence their expressions' quality and or quantity, which may affect how susceptible they are to developing autoimmune disorders. In fact, several research has looked at the possible significance of IL-17 levels and IL-17 gene polymorphisms in RA susceptibility. Therefore, polymorphic association analysis has also been studied, such as a study done on the Tunisian population suffering from RA; linking any association between the disease and IL-17A. This

resulted in high levels of IL-17A plasma contents but no specific statistical association, but results predicted RA occurrence due to elevated amount of IL-17A (Dhaouadi et al., 2018). Similarly, another systematic review is based on the polymorphism of IL-17A and IL-17F to RA. Fifteen different studies were reviewed out of which five depicted a positive association between IL-17 and RA, however, one of them showed an inferior association with RA (Agonia et al., 2020). Likewise, another study is based on Fibroblast-like synoviocytes (FLS) which are cells that invade bone and cartilage and are relatively immune to apoptosis, a key feature of RA. These cells were treated with IL-17 to examine the behavior of the mitochondrial dysfunction and autophagy, which yielded that IL-17 caused mitochondrial malfunction and the development of autophagosomes in RA FLS, indicating that they were resistant to apoptosis. Autophagy-related antiapoptosis elicited by IL-17 was reversed by autophagy suppression, implying a link between mitochondrial malfunction and cell survival in RA FLS (Kim et al., 2018). Studies have concluded that patients with RA had a higher IL-17 circulating in them as compared to healthy individuals (El-Maghraby et al., 2019). All of these studies direct a strong relation to IL-17 and RA.

3.2 Inflammatory Bowel Disease and Crohn's Disease

Inflammatory Bowel Disease (IBD) is a collection of gastrointestinal tract inflammation conditions, which significantly reduce the quality of life. Abdominal discomfort, more frequent stools, and rectal bleeding are symptoms associated with these disorders (Gracie et al., 2019). Three primary manifestations of IBD are Crohn's disease (CD), ulcerative colitis (UC), and IBD unclassified (IBDU). The causes of these diseases are multifaceted. IBD is characterized by periods of remission interspersed with persistent recurrent disease activity. The long-term effects of chronic intestinal inflammation include tissue destruction, fistulizing and structuring inflammation in CD, and potentially fatal bouts of acute severe UC. Anti-inflammatory, immunomodulatory, and immunosuppressive medications, as well as biological therapy that targets inflammatory cytokines, are all used to treat individuals with IBD (Vries et al., 2019; Uhlig et al., 2018). As with any other inflammatory and cytokine-driven disease, IBD is linked to IL-17 expressions. A study concluded that stabilizing and or inhibiting IL-17 suggested finer effects to treat IBD (Li et al., 2019). Another study aimed a similar approach; to assess if inhibiting IL-17 would benefit IBD treatment. The study determined that anti-IL-17 drugs have been linked to IBD exacerbation. Thus, for individuals with IBD; these drugs should be prescribed with caution (Hohenberger et al., 2018). However, the exact role of IL-17 in IBD is currently being debated in the literature. A study showed that mice lacking in IL-17 or treated with anti-IL-17 had significant epithelial damage in the colon, implying that IL-17 has a protective effect (Bunte et al., 2019).

A part of IBD, the CD is a progressive and destructive inflammatory bowel disease defined by persistent inflammation of any portion of the gastrointestinal system. In most cases, the illness's development at a young age demands immediate but long-term therapy to prevent disease flares and disease progression with intestinal consequences. Inflammation can develop everywhere from the mouth to the anus. CD and UC are distinguished by certain clinical and diagnostic features. Diarrhea, stomach discomfort, rectal bleeding, fever, weight loss, and exhaustion are all common symptoms (Roda et al., 2020; Veauthier et al., 2018). Numerous studies have associated IL-17 with the pathogenesis of CD. As IL-17 signaling results in pro-inflammatory production resulting in the expression of inflammatory genes. IL23R signaling stimulates various pathways in CD, resulting in the production of many effector cytokine genes such as IL17A and IL17F. Studies have also shown that the submucosa and muscularis propria of CD patients had amassed cells able to produce IL-17 (Schmitt et al., 2021). Studies have also indicated amplified amounts of IL-17A and IL-17F expressions (Verstockt et al., 2018). Many studies have concluded

diverse outcomes on the use of anti-IL-17 drugs and or agents, relatedly Yamada et. al. (2019) conducted the effects on 16,690 patients with anti-IL-17 drugs, which showed no such difference in the onset of IBD (and CD), which made them conclude that onset of IBD is rare in the case of using anti-IL-17 agents.

3.3 Psoriasis

Psoriasis is a chronic inflammatory and autoimmune skin condition that is distinguished by erythematous plaques with a white scale. One of the most common chronic inflammatory skin illnesses is psoriasis. More than 80% of occurrences of psoriasis are caused by the most prevalent kind, plaque psoriasis (Armstrong et al., 2020; Kamiya et al., 2019). Psoriasis is associated with many factors and comorbidities. However, there are abundant reports and extensive studies that indicate IL-17 as a mediator for psoriasis. The Th17 pathway and its significance in psoriasis inflammation were discovered as a result of advancements in the understanding of the pathophysiology of psoriasis (Loft et al., 2020). Analogous to the aforementioned studies on inhibiting IL-17 to infer the function of IL-17 in various diseases – studies conducting the same tests were done on psoriasis. That gave comparable results. Psoriasis can be treated by targeting IL-17, as the IL-17 signature is more highly expressed in the skin of psoriatic patients (Furue et al., 2018; Furue et al., 2019). According to a study, dermal dendritic cells that produce IL23 stimulate the production of IL17, which then prompts keratinocytes to produce an inflammatory response by activating the CCAAT enhancer-binding protein. The progression of psoriasis is accelerated by this feed-forward reaction. TNF and IL17 work together to enhance the transcription of several proinflammatory genes. Which finally results in the promotion and the development of Th17 cells in draining lymph nodes and the skin (Tokuyama et al., 2020). IL-17 and IL-23 have been identified as important pathogenesis-promoting factors in psoriasis by immunological and genetic research (Griffiths et al., 2021). When exposed to IL-17A and tumor necrosis factor, keratinocytes can become activated and release secondary cytokines and chemokines in inflamed skin (Moos et al., 2019). Certain cells are activated by IL-17-producing lymphocytes, which increases their cytokine output and pathogenicity. The majority of the time, IL-17 works in tandem with other cytokines to massively activate immune pathways and other cytokines notably IL-22, IL-20, and IL-24, which promote epidermal alterations (Ghoreschi et al., 2021). In vitro studies in addition to clinical studies specify IL-17A to be the chief cytokine that alters the biological processes of dermal tissues which are affected. The same study also depicted that, IL-17A triggered more transcriptional activity than IL-22 in an in vitro investigation utilizing reconstituted human epidermal sheets, aligning with the psoriasis transcriptome (Blauvelt et al., 2018). In a recent study, researchers observed the mutations of a gene; CARD14, that encoded CARD-containing MAGUK protein 2 (CARMA2) under psoriatic conditions. A mice model was examined with the same conditions, provided with the constant activation by the IL-17A cytokine axis. The results determined that CARMA2 is a crucial modulator of IL-17A signaling, and the development of psoriasis is caused by its constitutive activation in keratinocytes (Wang et al., 2018). Another study carried out on experimental mice; compared the skin and arteries of mice with psoriasis with healthy mice since psoriasis increases the risk of cardiovascular disease in humans. According to the investigation, skin lesions caused by psoriasis induce IL-17-producing T cells in draining lymph nodes to migrate to proximal skin and then to arteries (Huang et al., 2019). Thus, keratinocyte proliferation, neo-angiogenesis, mast cell recruitment and activation, neutrophil and macrophage activation, and the expression of adhesion molecules can all be increased by IL-17 stimulation, which favors the breakdown

of the epidermal barrier (Boutet et al., 2018). In one report, the authors speculated that psoriasis, obesity, and depression may indeed be connected manifestations of a state of immune dysregulation, of IL-17 and its related cells (Zafiriou et al., 2021). Studies have also shown that Innate Lymphoid Cells (ILC) 3 and Mucosal-Associated Invariant T (MAIT) cells are additional significant producers of IL17 in the skin in response to inflammatory stimuli. Given that chronic mucocutaneous candidiasis in humans is characterized by inborn deficits in IL17 or IL17R, IL17 certainly appears to play a significant role in skin local immunity (Bugaut et al., 2021).

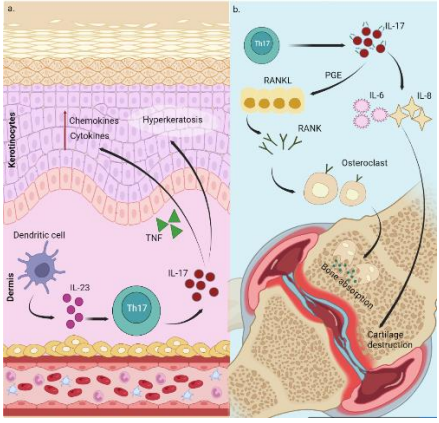

Figure 1 IL-17 release is related to many inflammatory diseases. **a.** In Psoriasis, dermal dendritic cells synthesize IL-23 which stimulates the Th17 which releases IL-17. IL-17 promotes inflammatory action and causes hyperkeratosis (thinking of the outer layer of skin) and TNF increases the chemokines and cytokines in inflammated skin. **b.** In Rheumatoid Arthritis, IL-6, IL-8, and Th-17 release the IL-17 which activates the PGE and RANKL. The RANKL release RANK which binds to the osteoclast and starts bone absorption. In addition, IL-17 causes cartilage destruction in rheumatoid arthritis.

4. IL-17 based therapeutics

There have been many therapies, clinical trials, and treatment methods to target IL-17 by inhibiting its expression or pathways. There is a multitude of experimental data that indicate the progressive conclusion, as previously mentioned. In most inflammatory diseases inhibiting the key cytokine has been documented to be successful, however certain drugs or agents may pose adverse aftereffects (Kany et al., 2019). Monoclonal antibodies against IL-23, IL-17, and TNF are now being used to treat patients with psoriasis, atopic dermatitis, and hidradenitis suppurativa, according to emerging data from clinical trials (Liu et al., 2020). Some of the more common IL-17 targeting agents, mostly for treating psoriasis are Secukinumab, Ixekizumab, Bimekizumab, and Brodalumab (Table 1).

Table 1 Different Monoclonal Antibodies against IL-17 for the treatment of different diseases

Name	Compound	Target	Dosage	Disease	References
Secukinumab	Immunoglobin G1 monoclonal antibody	IL-17A	300mg, 150mg	Moderate to severe Psoriasis	(Augustin et al., 2020)
Ixekizumab	Immunoglobin G4 monoclonal antibody	IL-17A	160mg, 80mg	Ankylosing spondylitis, Psoriasis arthritis, Plague Psoriasis	(Craig et al., 2020; J.-X. Huang et al., 2020)
Brodalumab	Immunoglobin G2 monoclonal antibody	IL-17RA	210mg, 140mg	Moderate to severe chronic Psoriasis	(Galluzzo et al., 2021)
Bimekizumab	Immunoglobin G1 monoclonal antibody	IL-17A, IL-17F	320mg	Plague Psoriasis	(Andrew Blauvelt et al., 2020; Oliver et al., 2021)
Netakimab	Immunoglobin G1 monoclonal antibody	Anti-IL-17A	120mg	Ankylosing spondylitis, Plague psoriasis, and Psoriasis arthritis	(Erdes et al., 2020; Puig et al., 2021)

Secukinumab is a human immunoglobulin G1 monoclonal antibody against IL-17A; a potent and secure biologic treatment for psoriasis. It can selectively bind to and neutralize IL-17A. The European Medicines Agency (EMA) has approved and recommended its use in treating psoriasis (Wcisło-Dziadecka et al., 2019). A humanized immunoglobulin G4 monoclonal antibody called Ixekizumab specifically inhibits IL-17A (Liu et al., 2020) and much like Secukinumab, functions to target IL-17A. Whereas Brodalumab is another complete human immunoglobulin G2 IL-17RA antagonist or inhibitor (Tomalin et al., 2020) and Bimekizumab is a humanized monoclonal immunoglobulin G1 antagonist that can inhibit IL-17A and IL-17F (Blauvelt et al., 2020).

Secukinumab at dosages of 300 to 150 mg is effective and safe for the treatment of moderate-to-severe psoriasis, according to evidence from randomized trailed studies (Reich et al., 2021). Inhibiting IL-17 comes

at a cost, as it halts its function. For instance, Individuals suffering from psoriasis may have an increased risk of contracting Candida spp. Infection; IL-17 prevents such infections. Consequently, anti-IL-17 antibodies, which are known to be effective in the treatment of psoriasis, may be linked to a rise in Candida spp. infections (Papini et al., 2018). However, according to that study, there were no such incidents of an amplified infection, this may be due to the limited number of patients that were examined. In another trailed study Secukinumab was administered to patients with Netherton syndrome, hence anti-IL-17 therapy may be a potential treatment choice. There have been various other studies demonstrating Secukinumab as a positive anti-IL-17 therapy (Bilal et al., 2018; Caldarola et al., 2020; Gasslitter et al., 2019; van der Heijde et al., 2020; Yin et al., 2020). According to one study, Ixekizumab is more persistent and had a lower rate of drug discontinuation as compared to others (Lockshin et al., 2021). However, paradoxical reactions due to the use of Ixekizumab have been indicated by a study, nevertheless, the treatment was effective (Marasca et al., 2021). One study determined the safety and efficiency of Bimekizumab which turned out to be greater in response to and efficiency than even Secukinumab (Ruggiero et al., 2022). Allying Bimekizumab is another novel inhibitor of IL-17A, Netakimab (Mosca et al., 2021), both are still under phase III clinical trials before approval. However both are quite resilient as anti-IL17 agents (Li et al., 2021).

Another rather unorthodox approach is the use of the novel Quercetin, a plant-based polyphenol inhibitor that lowered IL-17-induced RANKL protein levels. The study also observed that the extracellular signal-regulated kinase, mammalian target of rapamycin, and inhibitor of kappa B-alpha were all activated by IL-17 less when quercetin was present (Kim et al., 2018). Quercetin is even able to block the pathway of MAPK, this is possible since Quercetin can inhibit the nuclear protein known as high mobility group box 1 (HMGB1), a non-histone protein, that is connected to inflammation, thus reducing the production of Th17 cells, and decreasing procytokine and IL-17 production (Hashemi et al., 2018). Quercetin has also been examined in vitro and in vivo environments of RA manifestations, Quercetin generally reduced inflammatory cytokines and mediators, decreased oxidative stress, inhibited proliferation, migration, and invasion, and promoted apoptosis to prevent synovial membrane inflammation (Tang et al., 2022). In one study Quercetin's therapeutic outcomes were tested on mice immunized with type II collagen (CII); inflammatory mediator levels in the knee joint were found to have significantly decreased (Haleagrahara et al., 2018). Hence, quercetin may be utilized as an additional treatment for RA patients and may have the ability to prevent joint inflammation.

As described earlier IL-17 serves as an important component of the immune system, to defend the host against various foreign threats. Particularly in epithelial barrier locations, IL-17 is essential for the preservation of tissue integrity and the induction of immunological defenses against pathogenic microbes. The proinflammatory characteristics of IL-17 also make it an important immunopathology and mediator of inflammation. One of the most promising immunotherapeutic target options for the treatment of a wide range of disorders, including malignancies, autoimmune diseases, and infectious diseases, is IL-17 due to its remarkably diversified roles (Brembilla et al., 2018; Carney, 2018; Ma et al., 2019). Type 2 diabetes, insulin resistance, and inflammation have all been associated with IL-17 activity (Abdel-Moneim et al., 2018). However, genetic and environmental factors, mechanical stress, and dysbiosis can result in a pathological up-regulation of IL-17 and the emergence of inflammatory disorders. IL-17 is a protein that initially only functions during an immune response (Tsukazaki et al., 2020).

5. Conclusion

IL-17 has a significant role in immunity and several vital biochemical pathways, while also maintaining specific immune responses. IL-17 like all other cytokines IL-17 is highly responsible for the development of various inflammatory

diseases. Since the IL-17 responds by elevating certain other pro-inflammatory cytokines that it results often in autoimmune diseases as well. However, novel methods to treat such disorders or diseases are being tested and trailed, with many being approved and used. Most notably the use of IL-17 inhibitors, that either neutralizes IL-17 or its receptors. These treatment methods come with specific risks and may in turn have several unwanted effects. Nevertheless, these agents have yet caused any serious adverse effects; still, selective inhibition of IL-17 has yielded better outcomes. Soon, safer and more effective means to treat inflammatory diseases would be targeted. According to many findings these novel approaches, do require more tests and a greater understanding of IL-17.

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7. Declarations

7.1 Conflict of Interest

All authors declare that they have no conflict of interest.

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Acinetobacter as Model Organism: Environmental and Biotechnological Applications

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Abstract

Among different microbial groups present in nature, *Acinetobacter* holds an important place due to its profuse presence in different environments like freshwater, soil, solid wastes, and wastewater. Versatile metabolic characteristics of different species of genus *Acinetobacter* have been fascinating emergent interest in different fields of environmental, medical, and biotechnological perspectives. Various members of this genus are recognized to be involved in biodegradation, treatment, and removal of various inorganic and organic hazardous wastes. Some *Acinetobacter* strains are also well-characterized to produce industrially valuable bioproducts. Because of its ecological importance, the genus *Acinetobacter* is also considered a model organism for environmental and industrial microbiological studies. Various Bioproducts including Biopolymers, Bioemulsifiers, Bioreporters, and Biosurfactants are also produced by different species of *Acinetobacter*. This review recapitulates the practicality and various applications of *Acinetobacter* strains in the field of environmental biotechnology.

Keywords: *Acinetobacter*, biodegradation, bioemulsans, bioproducts, biosurfactant, lipases, polysaccharides.

1. Introduction

Acinetobacter is a Gram-negative bacterium that belongs to the family of γ-Proteobacteria and Pseudomonadales order. It is an oxidase-negative, and strictly aerobic bacterium. Both pathogenic and nonpathogenic species are included in this genus (deBerardinis et al, 2009). *Acinetobacter* spp. are prevalent in nature and can be isolated from human skin and other living organisms. They can also be obtained from water and soil. They are strictly aerobic, non-motile bacteria. While examine under the microscope, they appear as gram-negative coccobacilli which are usually arranged in pairs. They can utilize different carbon sources for their growth. For their culture, comparatively simple media, like trypticase soya agar and nutrient agar are usually used. (de Breij et al, 2010). The genus *Acinetobacter* includes various species which have been appealing much attention in both biotechnological and environmental applications. Different *Acinetobacter* strains are identified to be concerned with the biodegradation of a variety of various pollutants like amino acids (analine), benzoate, and biphenyl along with chlorinated biphenyl, phenol, acetonitrile, and crude oil. They are also involved in heavy metals and phosphate removal from wastewater. Particular strains of diverse *Acinetobacter* spp. are developed for the bioremediation purpose of recalcitrant and other detrimental organic chemicals in recent years. These strains are also used for the bioengineering of different enzymes and diagnostic constituents (Luckarift et al, 2011). Conventionally, the main focus of research regarding the genus *Acinetobacter* includes

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Table 1 Examples of usage of *Acinetobacter* spp. for the bioremediation of heavy metals contaminated soils and effluents

Contaminated environment	**Species/strains used**	**Reference**
Effluent from tannery or Textile industry containing heavy metals	*Acinetobacter* spp.	Ugoji and Aboaba, 2004 Srivastava and Thakur, 2007
Digested sewage sludge containing Lead	*Acinetobacter calcoaceticus var. anitraus*	Mak et al, 1990
Wastewater or Activated sludge contaminated with Chromium	*Acinetobacter* spp. *A. haemolyticus*	Francisco et al, 2002 Pie et al, 2010
Silver contaminated photographic wastewater	*Acinetobacter baumannii* BL54	Ohadi et al, 2017

naturally occurring transformation, hydrocarbon degradation, and organic compound utilization. The latest research areas for the genus *Acinetobacter* are various applications in the field of biotechnology, genomes analysis, and their evolution, the pattern of antibiotic resistance, and identification of pathogenic strains. (Jung & Park, 2015). Different *Acinetobacter* strains are also sound acknowledged fermentable bacteria because of their involvement in the manufacturing of various intra-and-extracellular economic products like bio emulsifiers, proteases, lipases, cyanophycine, and multiple types of biopolymers (Abdel-El-Haleem, 2003).

Therefore, the main emphasis of this review is on the use of different *Acinetobacter* strains in the bioremediation of several environmental pollutants and different applications in the field of biotechnology. This review also concerns various bioproducts which are produced by the genus *Acinetobacter*.

2. Bioremediation of Industrial Contaminants

Numerous toxic compounds can disperse and persist to a higher magnitude in the environment (Vasudevan & Mahadevan, 1992). Bioremediation is a comparatively economical method. In this process, hazardous substances can be converted into non-toxic or less-toxic forms by using microorganisms. Among different microorganisms, *Acinetobacter* is the one which can reduce and eliminate a widespread array of inorganic and organic compounds.

2.1 Heavy Metals

Heavy metals are the most important toxic component present in industrial wastewater. Before disposal they should be treated properly, otherwise, they become hazardous to the environment. Among several kinds of heavy metals, chromium (VI) has oxidizing, mutagenic, and carcinogenic properties and so it is considered an extremely hazardous metal. (Cheung and Gu, 2007). For developing an efficient bioremediation approach, a few things should be considered such as the choice of a suitable microbial strain, which can tolerate and minimize high levels of toxicants, and also to study the interaction between microbe and toxicant. (Das and Mishra, 2010). A study of moderately halophilic eubacteria regarding metal intolerance showed that strains of different species of *Acinetobacter* were found to be most tolerant against heavy metals. The majority of stains show tolerance to eight different kinds of metal ions (Kholodii et al, 2004). Because of the ability to transform or store heavy metals, *Acinetobacter* strains could hypothetically be misused for the bioremediation purpose of soil and water contaminated with

metals. (Table 1).

2.2 Pesticides

Some strains of *Acinetobacter* also have applications in the bioremediation of water and soil contaminated by a wide range of pesticides. The degradation of pesticides can be determined by a feature of the plasmid. Lamb et al in 2000 stated the genetic engineering strain BD413 of *Acinetobacter* spp. which represents the cytochrome P450 xenobiotic-metabolizing enzyme CYP105D1 derived from specie *Streptomyces griseus*. The genetically engineered strain can degrade various organic pollutants as well as chlortoluron, which is considered a greater achievement in the process of bioremediation

2.3 Hydrocarbons & Aromatic Compounds

Among different characteristics of *Acinetobacter*, one common characteristic is aromatic compound utilization, since the starting of taxonomic studies. In the natural environment, the aromatic compound's degradation ability is also active because of the interaction of microorganisms diverse in the environment (Simarro et al, 2013).

Pseudomonas, Ralstonia, Sphingomonas, and various other genera are among the well-reputed degraders of aromatic compounds. (Lee and Lee 2001; Coronado et al, 2012; Arora et al, 2014). The strains of the above-mentioned microorganisms are usually capable of degrading anthropogenic compounds which were recently synthesized; however, when talking about *Acinetobacter* strains, they usually can vitiate such aromatic compounds that contain solely natural products mainly from plants origin (Parke and Ornston, 2004; Young et al, 2005). During the catabolism of several aromatic compounds, many intermediate metabolites like catechol and protocatechuate and catechol, feeding the pathway of β-ketoadipate are produced. Several strains of environmental origin like *A. baylyi, A. calcoaceticus, and A. oleivorans* DR1) and pathogenic species like *A. baumannii* have pathways of catabolic metabolism for various aromatic compounds, sideways with the β-ketoadipate pathway, with almost similar syntenic planning. This property was reflected by sequencing data of genomes and comparative genomic studies. (Jung et al, 2011a). Aromatic compounds biodegradation can occur in contaminated soils, river or sea water (AlAwadhi et al, 2002; Hashizume et al, 2002; Ruzicka et al, 2002), or in the air (Juteau *et al*, 1999; Zilli et al, 2000).

Acinetobacter also has the property of degradation of hydrocarbons, particularly regarding alkanes of multiple chain lengths. Members of the genus *Acinetobacter* are often found in various sites of hydrocarbon contamination, including soils, Antarctic marine sediments, mangrove sediments, and pristine environments, which showed that *Acinetobacter* has the prospective for degradation of alkanes (Kuhn et al, 2009; Kang et al, 2011; Rocha et al, 2013). Different strains of *Acinetobacter* isolated have applications in the biodegradation of a variety of compounds, such as phenols, toluene, cresols, cyclohexane, furan, and lignin and lignocellulosic hydrolysate containing phenolic compounds (Vasudevan and Mahadevan, 1990, Jain et al, 1997; Lopez et al, 2004), acetonitrile, polychlorinated biphenyls (PCBs) (Rojas-Avelizapa et al, 1999), polymers and acrylic oligomers (Kawai, 1993), dichloroethenes (Olaniran et al, 2004), 4-chlorobenzoic acid (Kobayashi et al, 1998), and polycyclic aromatic hydrocarbons (Yu et al, 2005), because these strains have an extensive range of metabolic versatility (Towner, 1991b). For a better understanding of hydrocarbon degradation, some areas need to be studied further such as various environmental factors, sensing and signaling of substrate, and several other genetic factors in *Acinetobacter* spp.

2.4 Removal of Phosphates from Wastewater

Treatment of wastewater for the removal of Phosphate is a basic step of any sewage treatment facility because phosphate accumulation can result in eutrophication. Because of the frequent isolation of *Acinetobacter* spp. from activated sledges, it is considered that the concerned organism plays an important part in phosphate removal from wastewater by biological

treatment. This property of phosphate removal is dependent on the activated sludge enrichment with polyphosphate accumulating sternly aerobic *Acinetobacter* sp. Auling et al in 1991 and Wagner et al in 1994 also mentioned that *Acinetobacter* as the main microorganism accounted for phosphate removal biologically (Auling et al, 1991; Wagner et al, 1994).

3. Oil Degradation

Various ingredients of oily sludge are found to be potent immunotoxicants and carcinogenic. Alkanes, aromatic compounds, asphaltene fractions, and NSO (nitrogen-, sulfur-, and oxygen-containing compounds), together make a complex mixture of oily sludge. Unprocessed oil results in several threats during environmental release. Many toxic compounds which are present in unprocessed oil, include aromatic polycyclic hydrocarbons, benzene, and its substituted cycloalkane rings. When these compounds are present in comparatively high amounts, they can cause physical, chemical, and biological damage to the marine environment. Among various microorganisms, *Acinetobacter* strains are thought to be the most competent oil degraders.

4. Stabilization of Bioemulsans and Oil-Water Emulsions

All bacteria which are hydrophobic in nature can stabilize oil-water emulsions. Two phenomenons were perceived for *A. venetianus* RAG-1 which were possibly liable for the emulsion gel structure including strong interactions of the cell to cell and the strong binding between the oil droplets and cells (Dorobantu et al, 2004). Also, the production of bioemulsans by *Acinetobacter* spp. is accountable for the emulsifying and biosorption properties of *Acinetobacter* spp. Several species of *Acinetobacter* also inhibit the property to produce polymeric Bio emulsions or Biosurfactants. Considering bio emulsions production by multiple Acinetobacter strains, the best-known examples are emulsan, alasans, and biodispersan, which have a key role in many industrial applications.

The main composition of bio emulsions produced by various *Acinetobacter* strains includes heteropolysaccharide, protein-polysaccharide complex, lipoglycan, proteoglycan, lipoprotein, and lipo-hetero-polysaccharide in environments. *Acinetobacter* spp. utilizes ethanol, crude oil, methylnaphthalene, n-hexadecane, glucose, peptone, lactic acid, n-heptadecane, glycerol, edible oil, C-heavy oil, and olive oil, as a source of carbon. (Hayder, 2015, Zhao, 2016). Along with carbon source, another important parameter to enhance emulsifier production is nitrogen source (Zhao, 2016), and the most common nitrogen sources used for the production of bio emulsions are $(NH_4)_2SO_4$ and Na_2HPO_4. Other sources of nitrogen used for the production of bio emulsions are urea and ammonium hydrogen carbonate (Navon-Venezia, 1995, Phetrong, 2008). It is noteworthy that nitrogen and carbon sources not merely increase the production yield but they also affect the emulsification activity of Bioemulsans (Zhao et al, 2016, Amaral et al, 2010). The temperature range for the stability of Bio emulsions produced by multiple strains of *Acinetobacter* was found to be 20°C to 40°C (Patil & Copade, 2001). While the pH ranges from 3 to 12 results in the stability of bio emulsions produced by *Acinetobacter* spp.

In 2001, Ron and Rosenberg reviewed the biological functions of emulsions. They reported about the bioremediation and mentioned that the use of *Acinetobacter* spp. enhances the bioavailability of water-insoluble hydrophobic substrates like polyaromatic hydrocarbons. It is also reported that these species can also bind heavy metals. Clinically, bioemulsions produced by various *Acinetobacter* species play a role in surface attachment and detachment and also promote biofilm formation (reviewed in Ron and Rosenberg, 2001).

Because of their biological properties, Bioemulsifiers could substitute some of the chemically synthesized emulsifiers in bioremediation regarding enhanced oil recovery and clean-up of vessels and pipes contaminated with oil. These bioemulsifiers can also use as additives in cleaning products and formulations of laundry, in the food

industry as emulsion stabilizing agents, and in pharmacy and cosmetics products (Martínez-Checa et al, 2007, Dastgheib et al, 2008, Franzetti et al, 2012, Zheng et al, 2012, Monteiro et al, 2013, Amaral et al, 2006, Luna-Velasco et al, 2007)

5. Alasan

Alasan is a bioemulsifier mainly produced by the KA53 strain of *A. radioresistens* which is isolated from soil contaminated with oil. It has a high molecular weight and is made up of three proteins (AlnA, AlnB, and AlnC) and polysaccharides. First protein AlnA showed OmpA-like protein properties and is accountable for the alasan,s activity of emulsification. (Toren et al, 2002a). AlnB is the second protein that belongs to the family of thiol-specific antioxidant enzymes which are also called peroxiredoxins. There is no emulsifying activity that is inhibited by this Recombinant AlnB protein but it can stabilize AlnA generated emulsions of oil-in-water. (Bekerman et al, 2005). In the growth cycle, alasan is mainly in bound form to the cells during the log phase while it is released into extracellular space during the stationary phase. This growth pattern is also inhibited by the emulsan.

In purified form, alasan increased the aqueous solubility and polyaromatic hydrocarbons degradation rates, this may be due to hydrophobic reaction between alasan and these substances. Until now, alasan is mainly used for research purposes such as the recombinant surface-active protein production from a definite gene. This recombinant protein helps to study the structure and function of bioemulsions for the very first time (Toren et al, 2002b).

6. Emulsion

The emulsion is a bioemulsion mainly produced by an oil-degrading microorganism known as the RAG-1 strain of *A. venetianus*. The main function of emulsion is to form and stabilize the interaction between oil-water emulsions and a range of hydrophobic substrates. It is a complex of proteins and polysaccharides. Structurally it has a backbone of polysaccharide which is usually unbranched along with side chains of fatty acid which are complexed to proteins. Among these proteins, functionally significant is an esterase (Bach et al, 2003). In the biodegradation of oil, better inhibitory as well as stimulatory effects have been stated after the pretreatment of substrates with purified emulsan. In a biodegradation comparison of untreated unprocessed oil by *Acinetobacter* with emulsan-treated oil, it was observed that emulsion treatment enhanced aromatic mineralization. It was also observed that the mineralization of linear alkanes as well as other hydrocarbons which are mostly saturated was reduced both by mixed bacterial populace and by pure cultures. The lack of physical interaction between cells and the hydrophobic substrate may be the reason for this inhibitory effect. Among different emulsions produced by *Acinetobacter*, the most efficient emulsan in removing hydrophobic compounds from soil slurries is produced by strain RAG- 1 of *Acinetobacter*.

The availability of bioemulsifier and their manufacturing cost chiefly defines effective applications and technologies for the utilization of emulsan. While discussing applications of Emulsion, it has been reported that in the petroleum industry, the emulsion has the property to reduce petroleum viscosity and viscosity of other products of petroleum during their transport through the pipeline by establishing heavy oil–water emulsions. It also plays its role in direct combustion with dewatering by synthesizing fuel oil–water emulsions (Zhao, 2014). In the food industry, Emulsion exhibits potential application as an emulsifier. It has been proposed that emulsion incorporation in toothpaste and mouthwash could considerably minimize the formation of dental plaque. Immunologically, Emulsion can activate macrophages in a dose-dependent manner, and for this reason, it could be used to boost the immune response to a vaccine as an adjuvant (Panilaitis et al, 2002).

7. Biodispersion

The role of biodispersion is to adhere to the surfaces and disperses inorganic minerals. Structurally, biodispersion has a comparatively low molecular weight average of 51,400 Da when compared with emulsion produced by different strains of *Acinetobacter* spp. In purified form,

anionic polysaccharide was found to be the active component of this biodispersion. Limestone is extensively used in the production of various products like paint, paper, and ceramics. In the context of this, purified biodispersion has impending application in the above-mentioned industries too. In grinding limestone into fine particles, biodispersion addition results in two possible benefits. The first one is to enhance efficiency by minimizing the required grinding time by more than 50%. And second, is the production of more uniformly ground products. Biodispersan produced by strain *A. calcoaceticus* A2 has the unusual ability to disperse TiO_z and $CaCO_3$ in water and so this biodispersion is widely used in different industries such as paint, paper, ceramics, and textile industries (Busi et al, 2017).

8. Biodegradation of Halogens and Xenobiotics

Many of the Xenobiotics pollutants like phenol, benzene, styrene, and toluene along with halogenated organic compounds like polychlorinated biphenylls and pentachlorophenol are usually present in wastewater in impartially low concentrations. These pollutants may also exist in higher concentrations in the form of spills. They may also be present in larger amounts in the soil as well as in water at unrestrained industrial sites. Xenobiotics and halogens are among the highly toxic compounds and their disposal is extraordinarily difficult.

Various studies have reported the role of multiple microorganisms in phenol biodegradation. Different strains of the genus *Acinetobacter* are also among the phenol degraders. Such strains use phenol as a sole source of carbon and energy. In 2002, a study was conducted by Abd-El-Haleem et al, on different Egyptian ecosystems and reported that out of twelve phenol-degrader microorganisms, four species are closely linked to *Acinetobacter* (Abd-El-Haleem et al, 2002a). Among these four species, one specie has been used in different applications of environmental studies (Abd-El-Haleem et al, 2002c; Beshy et al, 2002).

Various *Acinetobacter* strains can metabolized different xenobiotic compounds like toluene (Zilli et al., 2001), 2-chloro-N-isopropylacetanilide (Martin et al, 1999), 4-hydroxybenzoate (Allende et al, 2000), benzoic and p-hydroxybenzoic (Delneri et al, 1995), 4-hydroxymandelic and 4-hydroxy-3-methoxymandelic acids, 4- chlorobenzoate and 3- chlorobenzoic acid into their respective benzoates. Certain strains of *Acinetobacter* also can consume biphenyls together with chlorinated biphenyls.

Moreover, certain *Acinetobacter* spp. found to be efficient in the thorough mineralization of mono-halogenated biphenyls. But such species are usually isolated from mixed cultures. Degradation of lignin and amino acids has also been reported by different strains of *Acinetobacter* (Buchan et al, 2001, Kahng et al, 2002; Kim et al, 2001).

9. Phenol Biodegradation

Phenol is organic in nature and a vital raw chemical used in the production of many products such as preservatives, fungicides, resins, and pharmaceuticals. Phenol is also important in the production of dyes, synthetic rubbers, synthetic fibers, and other important materials for industrial uses (Gheni et al, 2018; Sepehr et al, 2019). In the end, phenol, however, is released into the environment from choking plants and refineries with the sewage discharge, becoming an important environmental pollutant (Cetinkaya and Ozdemir., 2018). Phenol is a highly toxic and carcinogenic chemical that can burn the skin and damage tissues following exposure or ingestion. It has also been reported that phenol can cause diarrhea, blurred vision, and liver damage (El Gaidoumi et al, 2019).

As phenol can cause serious damage to the environment and humans, three methods physical, chemical, and biological have been suggested for the treatment of phenol removal. Among these methods, the biological method is considered the best one for reducing phenol pollution. The reason is that biological treatment tends to be more feasible and environment friendly (Singh

et al 2018; Zhou and Nemati, 2018). Strains of *Pseudomonas* and *Acinetobacter* were found to be more effective microorganisms for this purpose. These degrading strains are well-known for the bioremediation of various water bodies contaminated with phenol successfully (Iqbal et al. 2018; Ke et al, 2018;)

10. Role in Experimental Research

Another important application of the genus *Acinetobacter* is possibly in the experimental research field. An example of this is ADP1 strain of *Acinetobacter* spp. has been used in genetics as well as in genomics studies and microbiology laboratories and the field of molecular biology as a model organism because it has metabolic versatility as well as an extraordinary tendency to endure natural transformation. Many field scientists generally considered the strain ADP1 a non-toxic and non-pathogenic strain, so it can be used in the laboratory training of undergraduates (Metzgar et al, 2004; Young et al, 2005). The capacity of *Acinetobacter* to undergo a natural transformation and its unique behavior in the environment make it be used as an ideal sensor/model system. This system can be used to detect horizontal gene transfer from animals, plants, or other microorganisms.

11. Byproducts from *Acinetobacter*

11.1 Biopolymer Production

The use of fertilizers and effluent discharge from industries has increased in recent years, which results in the accumulation of phosphate to higher levels in the water bodies. It is also noteworthy that phosphate is the bioavailable form of phosphorus. Accumulation of phosphate in water bodies leads to eutrophication and algal bloom. (Xu K, 2012, Mishra 2010) Biological treatment for the removal of phosphate tends to be much better than physical and chemical methods because it is more efficient and disposal is easy. (Cloete, 2001, Albertsen et al, 2012, Onnis-Hayden et al, 2011)

Acinetobacter spp. is identified to be a principal organism in high phosphorus-containing sludge. This specific organism also can produce biopolymers and biopolymers in turn have the property to bind phosphorus. For the bioremediation purpose of phosphate removal, the use of biopolymers has several advantages over the usage of live microorganisms. These include the stability of biopolymers over a vast temperature range and for this reason, biopolymers do not require any specific arrangements for handling, storage, and transportation, another advantage is that biopolymers can be reused after bound phosphate removal. Also, the biopolymers are non-toxic and biodegradable as well as easy to dispose off (Boswell et al, 2001, Sathasivan et al., 2009, Liu et al, 2006).

Multiple strains of *Acinetobacter* species also have the property to accumulate esters of wax, cyanophycin, and polyhydroxyalkalonic acids (Vinogradov et al, 2002, Krehenbrink et al, 2002; Pirog et al, 2002;). Such kinds of biopolymers produced from these *Acinetobacter* spp. are extensively used in the production of fine chemicals like candles cosmetics, coating, printing inks, and lubricants.

11.2 Bio-emulsifiers

Bioemulsifier structurally contains both hydrophilic and hydrophobic groups. These are extensively used in different industries such as cosmetic, food industry, agrochemical, and pharmaceutical industries. Several microorganisms including the different strains of *Acinetobacter* can synthesize a variety of bioemulsifier. The well-known and most studied strains of *Acinetobacter* for the production of "bioemulsans," are *A. calcoaceticus* BD4 *A. calcoaceticus* RAG-1 and *A. radioresistens* KA53. Different glycolipids like sophorolipids, rhamnolipids, and trehalose lipids, as well as several lipopeptides like polymyxin, surfactin, and gramicidin, are usually considered bioemulsifiers of low molecular mass. While examples of bioemulsifiers having high molecular mass comprise proteins, amphipathic polysaccharides, lipoproteins, lipopolysaccharides, as well as complex mixtures of such polymers. (Toren et al, 2001).

RAG-1 *A. calcoaceticus* strain is an essential strain for two different industrial applications which includes its characterization to grow on hydrocarbons and it also plays a significant role in the production of emulsion, which is a bioemulsifier of high molecular mass. The emulsion that is produced by the RAG-1 strain is chemically composed of proteins and a lipoheteropolysaccharide non-covalently linked complex. The polysaccharide, that is present in lipoheteropolysaccharide is called apoemulsion, which is about 990kD in molecular weight. Taylor and Juni in 1961 initially isolated *A. calcoaceticus* BD4 strain which can produce a huge polysaccharide capsule. When this capsular polysaccharide is released into the medium, a complex is formed between proteins and capsular polysaccharide which eventually becomes an active emulsifier. The amphipathic properties of emulsan BD4 derive from the complex of proteins with a hydrophilic anionic polysaccharide.

Another strain of *Acinetobacter* known as the KA53 strain of *A. radioresistens* produces alasan which is also a bioemulsifier. This bioemulsifier is 100 to 200 kD in molecular weight. Preheating at 60 to 90°C generally increases its emulsifying activity (Toren et al, 2002). Kim et al in 1996 reported a bioemulsifier known as mannoprotein produced by *Acinetobacter* spp. BE-254. Mannoprotein can produce stable emulsions with several hydrocarbons, waste oils, and organic solvents. For this reason, the respective emulsifier can also be used as an effective cleaning agent.

11.3 *Acinetobacter* as Bioreporter

Among different kinds of nano-technologies, bioluminescent bioreporter is the most promising because it is economically feasible and a real-time technique for the detection as well as monitoring of environmental contaminants. While talking about the composition of Bioreporters, it refers to live, intact microbial cells which have been genetically engineered. Due to this, the bioreporter in response to certain physical or chemical agents has to ability to produce measurable signals. The composition of bioreporter revealed that it is composed of a reporter gene like green fluorescent protein or luciferase and an inducible promoter gene. (Hay et al, 2000). For bioreporter construction, different types of catabolic genes along with their regulatory systems can also be used. Nevertheless, bioreporter made up of *Acinetobacter* spp. comprises the use of whole-microbial-cell, provides most promising applications than the conventional bioreporter host like *E.coli*. The reason is that the species of *Acinetobacter* have different physiological characteristics regarding survival and growth. These features permit the use of ADP1 bioreporters for exploring and detecting oil spills in soil and water environments (Zhang et al, 2012).

11.4 Polysaccharides, Lipases, and Polyesters

Different *Acinetobacter* strains can produce various extracellular polysaccharides of variable sizes which can be up to several million Daltons. These polysaccharides can comprise of D-galactose, 3-(L-2-hydroxypropionamido)-3,6-dideoxy-D-galactose, rhamnose, D-2-acetamido-2-deoxy-D-glucose, 3-deoxy-3-(D-3-hydroxybutyramido)-D-quinovose, S-(+)-2-(4'-Isobutylphenyl) propionic acid or lipopolysaccharide (Kunii et al., 2001). Additionally, certain strains of *Acinetobacter* also show the property to cultivate on ethanol and then synthesize exopolysaccharides known as ethapolan from ethanol (Johri et al, 2002; Pirog et al, 2002; Pyroh et al, 2002).

Different species of *Acinetobacter* are worth mentioning lipase sources. Several strains of *Acinetobacter* were found to be lipolytic and they are isolated from variable sources (Snellman and Colwell, 2004). The activity of lipase in *Acinetobacter* species can be stabilized or maximized by the presence of Ca2+ ions. This led to the correct configuration of the active site because of the presence of a Ca2+-binding pocket. Moreover, an enormous number of lipases produced by *Acinetobacter* have potential applications

in different procedures such as esterification, hydrolysis, and triglycerides transesterification, and in the selective chiral synthesis of esters (Chen et al, 1999; Li et al, 2001).

11.5 Production of Carnitine and Adjuvants

Different species of *Acinetobacter* have been suggested for the production of several other chemicals such as immune adjuvants, single-cell proteins, carnitine, and glutaminase-asparaginase which is used in the treatment of cancer. It has also been used for manganese leaching from ores. Other important uses of various *Acinetobacter* spp. or their products include promoters for the growth of plants and bio-control agents for several fungal and bacterial plant pathogens. Another specie of *Acinetobacter* known as *A. iwoffi* has been suggested to be used as a sensitizer for allergy protection.

11.6 Biosensors

Different *Acinetobacter* species have been broadly used as a biosensor. An example of this is, ADP1 which was used as a microbial sensor to detect sumithion, pesticide metaphos, and PNP in aqueous media (Guliy et al, 2003). It is also used as an indicator of *planta* bioluminescent which is non-destructive in nature and used for the production of methylsalicylate and salicylate. These two compounds are a part of the response system of plants against pathogens and are fundamental in acquiring systemic resistance in plants (Huang et al, 2006). A DF4 strain of *Acinetobacter* was nominated as bioluminescent biosensor in the form of whole cells and its role is to check heavy metals toxicity in water and wastewater (Abd-El-Haleem et al, 2006).

11.7 Production of Biosurfactant

Another important feature of different species of *Acinetobacter* is the production of Biosurfactant, along with lipase production and usage as a bioreporter. These *Acinetobacter* derived Biosurfactants have several applications in different industrial products like biodiesel (Noureddini et al, 2005), therapeutical accessories (Ono et al, 2001), production of biopolymers (Gross et al, 2001), and of cosmetics (Kiyota et al, 2001; Satpute et al, 2010). Different *Acinetobacter* species are known to produce biosurfactants including *Acinetbacter* spp. D3-2 (Bao et al, 2014). Among different species, *Acinetobacter venetianus* is best known and characterized for this purpose.

Biosurfactant are reported to be more powerful chemical surfactants than bioemulsans because of various properties. These include higher biodegradability, maximum efficacy at very low CMC, capability to reduce surface tension, specific and selective activity at extreme temperatures, salinity and pH, and higher foaming ability, (Roy, 2017). Many scientists have reported the effective applications of BS in multiple industries, for example. Cosmetics, paint, textile, detergent, medical and pharmaceutical, petroleum and petrochemical, food, and beverages, (Bannat et al, 2000).

12. Conclusion

There are several applications of different strains of *Acinetobacter* in the removal of environmental pollutants as well as the treatment of hazardous waste. They are also known to produce many important bioproducts which are economically feasible too. Potential improvements are expected from the genetic engineering of *Acinetobacter* strains from natural environments with widespread applications in environmental and industrial use.

13. Declarations

13.1 Conflict of Interest

All authors declare that they have no conflict of interest.

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Computational Design and Analysis of a Novel Inhibitor for *FERMT1* Gene: A Novel Treatment Strategy for the Kindler Syndrome

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Abstract

Epidermolysis Bullosa is a rare genetic condition that can lead to the blistering of the skin which appears due to a minor injury just by scratching and rubbing the skin Epidermolysis bullosa is a polygenic disorder since different types are caused by different combinations of genes. The Kindler syndrome, which is caused by disease-causing variants in the *FERMT1* (FERM Domain Containing Kindlin 1) gene, is being considered in this research. Due to the lack of a suitable drug, topical treatments have been the only option so far. Therefore, this study aims to develop a drug by the utilization of in-silico approaches to so the designed drug can act as an inhibitor and can be used to treat Kindler Syndrome. The protein of human FERMT1 gene was utilized in this study that is available under the specifically allocated UniProt ID Q9BQL6. The secondary and tertiary structure of the protein was predicted by MESSA and AlphaFold online web servers. Additionally, the ligands were designed by ELEA3D online web server and the interaction analysis was carried out utilizing the SwissDock docking server. The molecular dynamic simulation was performed by IMODs online web server to validate the results of docking. In the end, an ADMET analysis was carried out to determine the physiochemical characteristics, water solubility, toxicity, and drug-likeness of the proposed medication. It follows that a medicine developed utilizing the CAAD technique can function as an inhibitor of the Kindler Syndrome-causing gene and that if designed in vivo and in vitro, it will produce remarkable outcomes. Kindler Syndrome may one day be treatable with the help of the computer-aided medication design that has been developed.

Keywords: Kindler Syndrome, Bioinformatics, Drug Design, Inhibitor Design, Molecular Docking

1. Introduction

Epidermolysis bullosa is a genetically transmitted skin condition that has been either inherited from one of the parents who has the disease, which is autosomal dominant inheritance, or one can get the disease from both parents and that would be autosomal recessive inheritance (Mellerio, 2019). It is a rare condition that can lead to the blistering of the skin which appears due to a minor injury just by scratching and rubbing the skin (Alharthi et al., 2022). Epidermolysis has been classified into four types namely epidermolysis bullosa simplex, junctional epidermolysis bullosa, dystrophic epidermolysis bullosa, and Kindler syndrome (Boeira et al., 2013). They are distinguished by the extent of blister fragmentation and classified into several subgroups based on the genetic inheritance pattern, morphology, and topography of the diseases (Mariath, 2019). This study focuses on the *in-silico* analysis of Kindler Syndrome and the gene mutation that will

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aid in the development of a drug to treat it. A rare form of EB known as Kindler syndrome is inherited in an autosomal recessive pattern and is brought on by disease-causing variants in the *FERMT1* gene also known as the *KIND1* gene (Alharthi et al., 2022b). The symptoms of Kindler syndrome may begin to develop in young infants.

Up to now, molecular genetic engineering techniques like comprehensive genome testing or multigene paneling have been used to assess many clinical implications based on both alleles of pathogenic disease-causing variants and disease-causing variants of *FERMT1* (Youssefian et al., 2022). Photosensitivity, hyperkeratosis, Pseudo syndactyly, malignancy, mucosal involvement, poikiloderma, mucosal, variable hypermobility, and skin hyperextensibility are only some of the symptoms that have been identified in the approximately 400 patients who have been diagnosed. The gene *KIND1* that encodes kindling-1, an attachment protein for actin cytoskeleton to the matrix of basal keratinocytes, is mutated in this condition. The *KIND1* gene is likewise located on the short arm of chromosome 20. The loss of the Kindlin-1 function in this example was due to a combination of two disease-causing variants: *R271X* and *KIND1* (1755delT) (Bruckner-Tuderman, 2019). Allowing pro-inflammatory cytokines to proliferate in KS skin as a result of UV-B irradiation is proposed as a novel therapeutic method (Edrees et al., 2023). Additional studies link the actin-extracellular-matrix linker protein UNC of kindlin-1 in Caenorhabditis elegans to illness. Genetic linkage analysis and genomic screening, cell culture with RNA extraction and cDNA synthesis, multiple tissue northern blotting and cDNA panels, mutation detection, anti-kindlin antibody formation and immunohistochemistry, kindling-1 transfection, and results can be seen respectively. Clinical diagnostic criteria for Kindler syndrome have not yet been defined. Bioinformatics methods such as ELISA, immunofluorescence, flow cytometry, and PolyPhen-2 and SIFT all agreed that it is probably harmful. Therefore, this study is being conducted in preparation for the formulation of a specialized medicine based on mutational analysis, for which no recognized treatment existed previously.

Kindler syndrome is caused due to the mutation in the *FERMT1*gene at C20orf42 that encodes for the kindlin-1, a protein that is primarily expressed in keratinocytes and is an actin cytoskeleton-focal contact-associated protein (Arita et al., 2007). In individuals with Kindler syndrome, the *FERMT1* gene has been found to contain more than 70 (*FERMT1 gene: MedlinePlus Genetics*). Different bioinformatics tool have been used in this study to design the drug to treat the kindler syndrome. The first step in the drug synthesis for kindler syndrome involves the retrieval of the mutated protein encoded by the *FERMT1 (*Has et al., 2015*)*. The various physicochemical properties of the protein are then analyzed. The secondary and tertiary (3D) structures of the protein are predicted using advanced bioinformatics methods and its structural stability is scaled (Zhang et al., 2008). The tertiary structure is also refined and improved with the help of galaxy refine. As kindlin-1 is present at centrosome and it induces mitotic spindle formation its role depends on integrin binding and activation and can be identified by si-RNA. Specifically, ligand was generated from kindlin-1 by using elea3D as the targeted region will be epithelial cells for which ligand can be Ep-CAM for leukocyte associated immunoglobin like receptor and have used AutoDock vina, discovery studio and python for molecular docking and a drug can be designed according to the nature of the protein used in the specific process and PrEST – antigen as affinity ligand is used until now for affinity purification.

The Swiss Target Prediction, which forecasts the characterization of the ligand and its compatibility with its targets inside the body, was used to anticipate a drug's

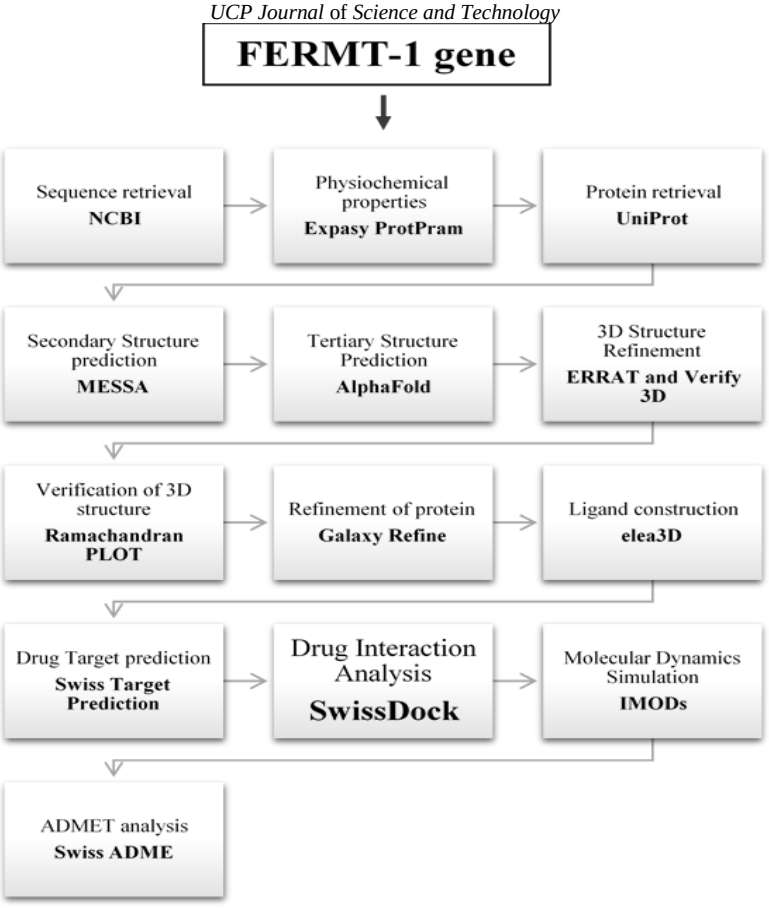

Figure 1 The methodology flow chart followed in the designing of *FERMT1* gene.

targeted locations in the following stage. The optimal docking complex was then found through docking using the SwissDock, which was afterwards recreated using IMODs. In the final step, the substance is put through additional testing to determine its many features, particularly the ADMET (chemical absorption, delivery, metabolism, excretion, and toxicity) properties. Based on how much the substance resembles a drug, an ADMET score is given to the substance. This stage brings the drug *in-silico* investigation to a close. If positive outcomes are obtained, the drug can then be *tested in vitro* and *in vivo*.

In this study, the drug has been developed using computational *In-silico* approach to treat the disease caused by the mutation in *FERMT1* gene. The kindler syndrome has never been successfully treated with a medication prior to that time. Therefore, the *in-silico* techniques used in the study has assisted in recognizing the polymorphisms of drug targets, designing the targeted drugs, binding affinity of the drug with the target and also to find out the other pathways that can get effected with the designed drug. *In-silico* drug design is cost effective in research and development of drugs (Jabalia et al., 2021).

This contemporary approach to drug development is expected to build an effective drug candidate, as it helps to analyze the molecular recognition process of targets and ligands. Nevertheless, for the effectuality and precision of the designed drug is yet to be evaluated in vivo and in vitro.

2. Methods and Methodology

This study was conducted using *in-silico* methods and protocols, the figure 1

below indicates the methodology flow chart of this study.

2.1 Sequence Retrieval

NCBI which stands for National Centre for Biotechnology Information (https://www.ncbi.nlm.nih.gov/) has been was utilized to retrieve the sequence of *FERMT1* whose NCBI reference sequence is NC_000020.11**.** The NCBI database comprises data from over 2400 species and includes over one million proteins exhibiting great biological variety encompassing prokaryotes, eukaryotes and viruses (Pruitt et al., 2005)

2.2 Physiochemical Properties

In order to get the physiochemical properties of the selected protein, the ExPaSy bioinformatics resource portal has been used which is an online tool. Expasy stands for "expert protein analysis". This portal was developed by the Swiss Institute of Bioinformatics. It provides an uninterrupted access to a large range of data in a variety of fields, including proteomics, genomics, phylogeny/evolution, systems biology, population genetics, transcriptomics, and so on (Artimo et al., 2012). ExPaSy-ProtPram is the tool of Swiss Institute of Bioinformatics(https://web.expasy.org/protparam) that was utilized to calculate the physiochemical parameters of the FERMT1 protein.

2.3 Uniprot

UniProt (https://www.uniprot.org) is the most comprehensive library of protein sequence and functional annotation, as well as the major resource for storing and combining information from huge and varied sources (Consortium, 2018). It stands for The Universal Protein Resource. Uniprot id of the protein used in this study is **Q9BQL6**. UniProt is a collaboration between the European Bioinformatics Institute , the SIB Swiss Institute of Bioinformatics and the Protein Information Resource.

2.4 MESSA

MESSA stands for MEta Server for Sequence Analysis. (http://prodata.swmed.edu/MESSA/MESSA.cgi) It is used to predict the structural and functional features of the desired protein. The server predicts the different properties of the protein such as its secondary structure, coiled coils, structurally disordered regions, signal peptides and transmembrane helices (Cong & Grishin, 2012). In this study, the input sequence of the protein is inserted in the MESSA which predicted the secondary structure of our protein.

2.5 Tertiary Structure Prediction

The tertiary structure of the protein is predicted by using the AlphaFold 2 (https://alphafold.ebi.ac.uk/) online tool. It is developed by the DeepMind which computationally predict the three structure of protein with unmatched accuracy and speed (Jumper et al., 2021). AlphaFold greatly improves the accuracy of structure prediction by incorporating novel neural network architectures and training procedures based on the evolutionary, physical and geometric constraints of protein structures.

2.6 Verification of 3D Structure

Ramachandran plot (https://swift.cmbi.umcn.nl/servers/html/ramaplot.html) gives the two dimensional plot which is used to verify the protein structure whether it is correct or not. The plot was developed in 1963 by G. N. Ramachandran. It shows the result in the form of chart with torsion angles calculated of the protein (*Ramachandran Plot - Proteopedia, life in 3D*, 2010). Two values are used one is taken on x-axis and the other on at y-axis.

2.7 Refinement of The Protein Model

Galaxy refine webserver(https://galaxy.seoklab.org/cgibin/submit.cgi?type=REFINE) is freely available online that aids in refining the structure of protein. In order to predict the structure, the need of developing template-based model structures beyond the accuracy provided by template information has been emphasized (Heo et al., 2013). Hence, the protein quality *FERMT1* was enhanced by using the galaxy refine tool.

Table 1 The composition of amino acid present in the selected protein.

Amino Acid	Number of residues	% Composition
Ala (A)	10	5.2%
Arg (R)	11	5.7%
Asn (N)	2	1.0%
Asp (D)	3	1.6%
Cys (C)	3	1.6%
Gln (Q)	10	5.2%
Glu (E)	7	3.6%
Gly (G)	23	12.0%
His (H)	12	6.2%
Ile (I)	4	2.1%
Leu (L)	24	12.5%
Lys (K)	5	2.6%
Met (M)	6	3.1%
Phe (F)	19	9.9%
Pro (P)	7	3.6%
Ser (S)	21	10.9%
Thr (T)	11	5.7%
Trp (W)	2	1.0%
Tyr (Y)	3	1.6%
Val (V)	9	4.7%
Pyl (O)	0	0.0%

2.8 Ligand Construction

Cheminformatic Tools and Databases for Pharmacology (https://chemoinfo.ipmc.cnrs.fr/) was used to construct the suitable ligand for our protein. ELEA-3D was used where protein was given as an input and got 20 different ligands out of which the model which had the highest population score was chosen as our ligand for the further analysis.

2.9 Drug Target Prediction

Swiss target prediction (http://www.swisstargetprediction.ch/) online tool had been utilized to predict the drug target. The Swiss Target Prediction engine assesses the similarity between the user's query drugs and those compiled in regulated, cleansed collections of known actives in well-defined experimental binding assays (Daina et al., 2019).

2.10 Drug Interaction Analysis

SwissDock (http://www.swissdock.ch/) is an online web server available to analyze the interaction of the designed drug with the protein. It is developed by the Molecular Modelling group and supported by the SIB Swiss Institute of Bioinformatics. The main aim of this server is to provide the protein-ligand docking that can be used to design an efficient drug. The complex with the minimum energy was selected.

2.11 IMODs

iMODS (https://imods.iqfr.csic.es/),which is an internal coordinates normal mode analysis server,contains new visualization features for depicting collective motions, such as an improved affine-model-based arrow representation of domain dynamics (**López-Blanco et al., 2014**). The IMODs server provides an easy-to-use interface for the improved NMA approach in internal coordinates.

2.12 ADMET analysis

Swiss-ADME (http://www.swissadme.ch/) is an online tool that is used to check find out the properties of the designed drug like its toxicity, excretion, absorption,

metabolism and distribution activities are checked to make sure the designed drug is safe to use. The designed drug must be capable of reaching the targeted molecule in sufficient concentration in order to carry out its biological function to either degrade or inhibit the targeted molecule (Daina et al., 2017). In this study, the ADMET score was analyzed to find out the drug like properties of the designed inhibitor.

3. Results

3.1 Sequence Retrieval

The whole sequence of the *FERMT1* gene was retrieved from the National Centre for Biotechnology (NCBI) with the accession no of NC_000020.11. The gene of the homo sapiens had been taken which was further analyzed to find the mutation which caused the Kindler Syndrome**.** The length of the selected gene has the 48186 bp.

3.2 Analysis of Physiochemical Properties

The longest open reading frame of the *FERMT1* gene was put as the input on the Expasy ProtPram which analysis the physiochemical properties of the protein. **Table 1** shows the composition of the amino acid present in the selected protein. The theoretical Pi was given to be 9.66, the point at which the pH is neutral. The aliphatic index was calculated as the 75.68 and the instability index was computed to be 35.54. GRAVY (Grand Average of Hydrophobicity) was found to be 0.011. Other physiochemical properties that were calculated by the ProtPram were the molecular weight, atomic composition, extinction coefficient, estimated the half-life of the protein in different organisms that is 30 hours in mammalian reticulocytes (in vitro), >20 hours in yeast (in vivo) and >10 hours in Escherichia coli (in vivo).

3.3 Retrieval of 3D Structure of Protein

Universal Protein Data Bank (UNIPROT) had been used to retrieve the 3D structure of the protein. Particularly Uniprot (Universal Protein Data Bank) gives in-depth useful information about the structure and function of the protein. Uniprot id: **Q9BQL6** · FERM1_HUMAN of *FERMT1* gene with *kind1* protein formation, responsible for Kindler Syndrome is analyzed. FERMT 1 has 677 amino acids. It plays significant role in adhesion of keratinocytes to fibronectin and laminin, activation of ITGA2B with talin, requires normal basal keratinocyte polarization on skin along with normal cell shape and integrin activation. It also induces keratinocyte migration to tumor site and mediates in tumor prolifer interaction with TGF –beta 1 signaling. The figure 2 below represents the 3D structure of *FERMT1* protein.

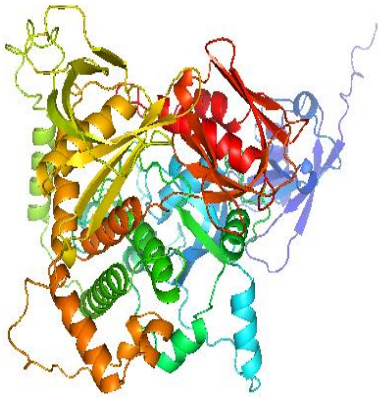

Figure 2 The 3D structure of *FERMT1* protein taken from UniProt representing the coils, helices and beta-strands in the protein structures

3.4 Secondary Structure Prediction

Another tool for structure prediction is MESSA, which have local sequence feature prediction, along with domain architecture, gene ontology terms prediction and function prediction. It also predicts Enzyme commission number and spatial structure. In this case, no EC number was not assigned to protein. It helped in predicting the secondary structure of protein and gave the score of

each residue of amino acid involved in the formation of either strand, alpha or coil.

3.5 Tertiary Structure Prediction

AlphaFold 2 was used to predict score residue along with alignments and expected position error in angstrom which provides useful information about protein folding, stability and design. Figure 3 shows the tertiary structure obtained of the protein of interest from the AlphaFold tool.

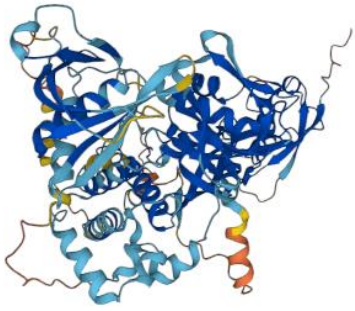

Figure 3 The tertiary structure of *FERMT1* protein obtained from the AlphaFold 2 tool

It gives the per-residue confidence score (pLDDT) which ranges from 0 to 100. Any score which is less than 50, that residue may be unstructured in insolation. The score greater than 90 is in dark blue color, the score between 90 and 70 is shown as light blue color, the score between 70 and 50 is in yellow color and less than 50 score is indicated with orange color. Given this confidence score, our most of the protein's residue had the score greater than 90 as shown in Diagram 2.

3.6 3D Structure Refinement

3-D structure can easily be refined by verify 3-D which determines compatibility of atomic 3-D model with its own amino acid sequence depending on external factors and its location (alpha, beta,loop,polar) and can compare them with good structures. ERRAT had been used in this study to check the quality of the 3D structure of the selected protein. The pdb format of the protein was uploaded and the ERRAT gave the quality factor of 92.3208. The ERRAT score greater than 90 is considered a good score.

3.7 Ramachandran Plot

Ramachandran plot explains the angles of the proteins. It gives the two-dimensional plot of all the torsion angles phi and psi that are present inside the structure of all peptides of the given protein. The plot has been typically divided into four quadrants, with three quadrants as the allowed region while the one quadrant at the bottom right is the not allowed region. The score greater than 85% is considered an outstanding one. In the study, 89.2% score was obtained of the residues that were present in the most favored region. The figure 4 below represents the Ramachandran plot for the protein of study.

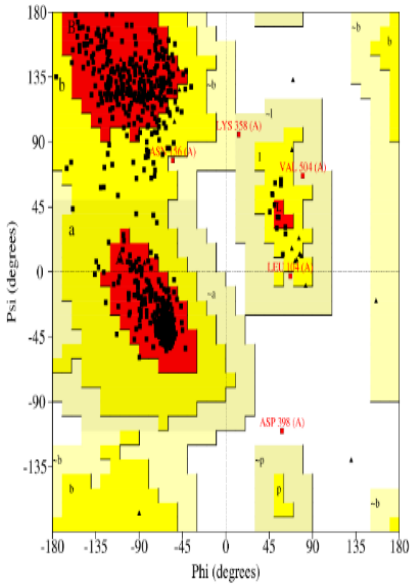

Figure 4 The results of Ramachandran plot with 89.2% of residues present in the most favored region

3.8 Refinement of the Protein Model

The degree of similarity between the target and available template structures substantially determines the quality of model structures created by modern protein structure prediction algorithms. Hence, galaxy refine tool was used to improve the model of the protein. It remodels side chains, conducts side-chain

Table 2 The ADMET properties predicted by SwissADME online tool

ADMET parameters	Parametric values
Formula	C23H25ClO4
Molecular weight	400.90g/mol
Num. heavy atoms	28
Num. arom. heavy atoms	13
Fraction Csp3	0.35
Num. H-bond acceptors	
Num. H-bond donors	1
Molar Refractivity	116.27
TPSA	63.58 Å²
Liphophilicity Log Po/w (SILICOS-IT)	7.20
Water Solubility Log *S* (ESOL)	-6.00
Class	Moderately soluble
(Pharmacokinetics) GI absorption	High
(Pharmacokinetics) BBB permeant	No
(Drug likeness) Lipinski	Yes; 0 validation
(Drug likeness) Ghose	No; 1 violation
(Drug likeness) Egan	No; 1 violation
Bioavailability score	0.55
Synthetic accessibility	4.61

repacking, and then relaxes the overall structure using molecular dynamics modeling. Out of 5 models obtained, model 2 was chosen as it had the highest score of the Rama favored with poor rotamers of about 0.2. The refined model of protein is given below in figure 5.

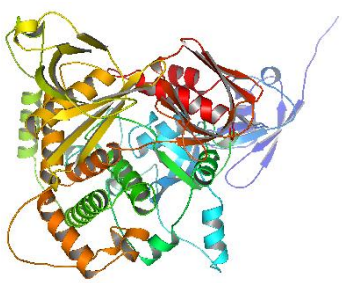

Figure 5 The Model 2 that refined by the galaxy refine online server indicating a refined protein structural model with a better quality and high Ramachandran Score

3.9 Ligand Construction

Ligand had been constructed for our selected protein *FERMT1* with the help of elea3D tool. The protein downloaded in the pdb format had been purified by utilizing Discovery Studio tool by removing water and ligand molecules. The protein had been submitted as an input in the pdb format on the elea3D and coordinates were added which were obtained from the AutoDock Vina. Twenty models were obtained, out of which 18 was the best model as it had the highest population (mean) score and it was then used as a ligand for further study. The figure 6 below represents the 3D model of the designed ligand molecule.

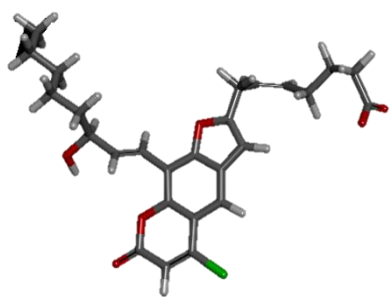

Figure 6 The selected model of designed ligand (g18 molecule) obtained from the Elea3D

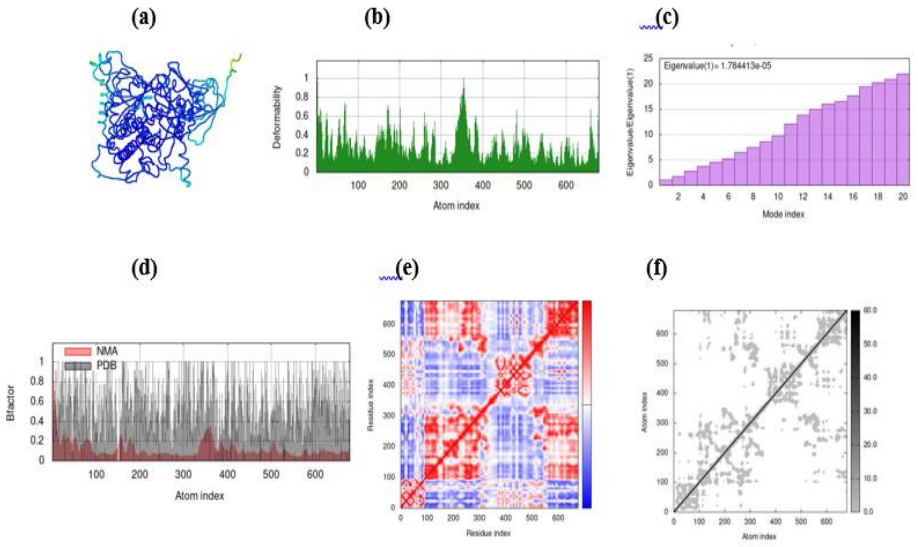

Figure 7 The results of molecular dynamics obtained via IMODs. **(a)** Stimulated 3D structure **(b)** B-factor or mobility **(c)** Eigenvalues **(d)** Deformability **(e)** Covariance map; red indicates correlated motion and blue indicates anti-correlated motion **(f)** Elastic network; the darker the color more stiffness

3.10 Swiss Target Prediction

Swiss target prediction is the tool that was used to find the compatibility and binding of the designed drug with its target. The ligand file was uploaded as an input on this tool which gave the result in the form of the pie chart. Protease are the main target of our ligand as it had the 33.3% binding capability with it followed by the kinase which had the 26.7% binding capacity with the ligand.

3.11 SwissDock

SwissDock had been used to analyze the drug interaction with the ligand. The *FERMT1* protein was uploaded in the pdb format along with the ligand in mol2 format. The docking had been run and the complex which had the minimum predicted energy **ΔG.** In our study, the complex with -10.69 kcal/mol had been downloaded and its structure had been visualized with the help of the UCSF Chimera.

3.12 IMODs

IMODs online tool had been used to run the molecular dynamics simulation of our complex to find out the changings in the protein receptor upon the binding of ligand. The eigenvalue calculated of the complex structure was 1.784413×10^{-5}. The lower the eigenvalue the more easily the protein can get deformed. The more the high peak of the B factor graph the higher are the chances of the residues to get deform. Figure 7 below represents the graphical representations of the molecular dynamic simulations.

3.13 ADMET analysis

SwissADME online tool had been used to carry out the ADMET analysis of our designed drug. The complex obtained from the elea3D had been downloaded and given as the input on the SwissADME tool. Properties like physiochemical, lipophilicity, water solubility, pharmacokinetics, medicinal chemistry and drug likeness were predicted by this tool. The table 2 represents the predicted ADMET properties of the designed ligand molecule.

Boiled egg model provides a straightforward and quick means to determine the gastrointestinal absorption and the uptake of the small molecules by the brain barrier that is associated with the drug discovery and its development. The white part of the egg represents the gastrointestinal absorption and yellow part

depicts the brain barrier having access to the molecule. In this study, the molecule 2 (*shown in red dot)* is present inside the white part. The boiled egg is shown below in the figure 8 representing the nature and absorption features of designed molecule.

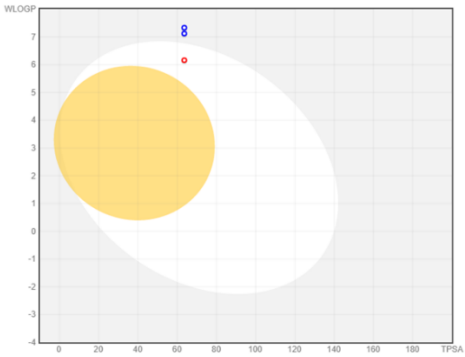

Figure 8 The image of the boiled egg retrieved from the SwissADME online *tool*

4. Discussion

The present study delves into the discipline of computational drug designing, specifically targeting Kindlin-1 associated with the *FERMT1* gene Uniprot id: Q9BQL6 (Yates et al., 2012).The method of research applied in this study involves pharmacophore modeling (Yang et al., 2010). The efficacy of any particular drug in treating Kindler Syndrome, a condition associated with disease-causing variants in *FERMT1* that result in the production of insufficient kindlin-1 protein, remains uncertain (Youssefian et al., 2022). However, ongoing research is shedding light on the root cause of the disease and potential solutions to address it. Clinical trials are presently underway to evaluate the expected outcomes for patients afflicted with Kindler Syndrome (Tabor et al., 2017).The art of computational biology was employed to develop a medicinal product through the use of pharmacophore modeling. The development of drug ligands for KIND-1 involves the utilization of both structure-based and ligand-based techniques (Has et al., 2011). Through the utilization of a structure-based approach, one can identify potential ligands by docking compounds and subsequently determining the interactions between the receptor and ligand (Yates et al., 2012). The present study encompasses advanced bioinformatics and technological methodologies to offer mankind a potential cure for Kindler Syndrome. The FASTA format of the Kindler Syndrome *KIND 1* mRNA cds with accession NC_000020.11 has been retrieved from NCBI, containing the protein sequence. The physicochemical properties of the protein were cautiously analyzed using ExPASy ProtPram. This involved a thorough examination of the amino acid composition, molecular weight, extinction coefficient, estimated half-life, and theoretical Pi, which was determined to be 9.66. With a calculated aliphatic index of 75.68 and a computed instability water index of 35.54. The grand average of hydrophobicity was determined to be 0.011.The Universal Protein Data Bank (Uniprot) was utilized to forecast the three-dimensional configuration of the protein, which provided comprehensive insights into its structure and functionality. The *FERMT1* gene, specifically *FERM1*_human, which is responsible for Kindler Syndrome, has been subjected to analysis. This particular gene is comprised of 677 amino acids. The adhesion of keratinocytes to fibronectin and laminin, as well as the activation of ITGA2B with talin, are of great importance (Ussar et al., 2008).For the skin to maintain its optimal state, the basal keratinocytes must exhibit proper polarization, with cells maintaining their natural shape and integrin activation. The prediction of a protein's secondary structure is facilitated by MESSA, which provides a score for each amino acid residue. This score offers valuable insight into the protein's spatial arrangement and enzyme commission number. In addition, it furnishes prognostications for sequence and function characteristics, as well as domain architecture and gene ontology. The protein under study has not been assigned an Enzyme Commission number.

The Alpha Fold 2 has successfully predicted the tertiary structure of a protein, providing valuable insights into the scoring residue, alignments, and expected position error. This information is crucial in determining the protein's folding, stability, and design. The algorithm provides a per-residue confidence score, revealing that a significant majority of the protein's residues received a score exceeding 90. The process of refining the 3-D structure involves the verification of its compatibility with the atomic model and its amino acid sequence, considering external factors and its specific location. The protein's PDF format was successfully uploaded, and its quality factor was determined to be 92.3208 by ERRAT. In our study, we employed the Ramachandran plot to analyze the distribution of residues. The results were quite promising, with a remarkable 89.2% of the residues found to be situated in the most favored region. It elucidates the various angles of proteins, accompanied by a two-dimensional plot of the torsion angles phi and psi that comprise the structure of the protein in question. The refinement of the protein model was carried out using the Galaxy refinement tool, which is a cutting-edge protein structure prediction algorithm. Among the five models that were procured, the second model was deemed most suitable, given its superior score of the Ramafavored with poor rotamers, which amounted to approximately 0.2. The construction of the ligand for *FERMT1* was executed utilizing the ELEA 3D tool. The input for ELA 3D includes the protein in pdb format, along with the coordinates from auto dock vina. Out of a pool of twenty models, the most exceptional was discovered. With the highest population or mean score, this particular model was selected as the ligand for further investigation. The Swiss Target Tool was employed to ascertain the compatibility of the proposed medication with its intended target. Upon uploading the ligand file, the outcome was presented in the form of a pie chart. Our ligand primarily targets proteases, given their binding capacity of 33.3%, while kinases exhibit a binding capacity of 26.7% with the ligand. The Swiss dock is employed for the analysis of drug interaction with ligands. The PDB format of the *FERMT1* protein, along with its corresponding ligand, was uploaded. The complex boasting a remarkable -10.69 Kcal/mol was procured for our study and subsequently rendered in visual form with the aid of UCSF Chimera. The IMODs online tool was employed to conduct a molecular dynamics simulation of our complex, to elucidate the alterations in the protein receptor that occur upon ligand binding. The computed eigenvalue of the intricate structure amounted to 1.784413 * 10^-5. The Swiss ADMET online tool was employed to conduct an in-depth analysis of the ADMET properties of our newly designed drug. This included the prediction of various crucial parameters such as physiochemical attributes, lipophilicity, water solubility, pharmacokinetics, medical chemistry, and drug-likeness. The intricate structure derived from ELEA3D is submitted as input within the SwissADME tool. Within the confines of our investigation, it has been determined that the molecule denoted as 2 is situated within the albumen of a boiled egg model. Within the confines of this study, a diminutive molecule was employed to fashion a medicinal substance. The ligand adjoined itself to the protein of concern, and the molecular recognition of targets was scrutinized in depth (Prieto-Martínez et al., 2019).The utilization of *in-silico* approaches in the study has proven to be advantageous in the identification of drug target polymorphisms, development of targeted medications, analysis of ligand interactions with proteins, and exploration of potential pathways affected by the created drug. The initial phase in the development of medication for Kindler syndrome necessitates the retrieval of the mutant protein generated by the *FERMT1* gene. The various physicochemical

characteristics of the protein are then scrutinized. The alignment of the principal sequences of kindlin was accomplished through a thorough blast search, as detailed by Kloeker et al. in 2004. Various instruments are employed in the process of drug design. Over seventy disease causing variant have been unearthed in the *FERMT1* gene among individuals afflicted with Kindler syndrome. Recent advancements in molecular modelling techniques have enabled the application of computer-assisted drug design in the quest for novel mechanism- or structure-based medications.ssss The present study employs a non-testing methodology, which has yielded results of remarkable precision. As such, it holds great promise in the realm of disease treatment. The novelty of the subject matter renders it advantageous, for it remains untouched by previous inquiry. Although scant research has been conducted, regrettably, no remedy exists to alleviate its symptoms. The drug in question exhibits promising efficacy in the treatment of Kindler Syndrome, as evidenced by in vitro studies conducted (Fatima et al, 2021). Prior to utilization, pre-clinical testing is conducted to ascertain that the medication possesses all the requisite characteristics or attributes. Its role in the advancement of technology cannot be overstated, and it is also a highly effective method for treating complex diseases. Its contribution to the progression of technology is noteworthy, and it also serves as a promising approach to addressing intricate medical conditions.

5. Conclusion

Epidermolysis bullosa is a hereditary condition that manifests in four distinct forms. The subtype of this disorder that was examined in this study was Kindler Syndrome. This condition results from a -disease-causing variant in the FERMT 1 gene and is extremely rare. Unfortunately, the only effective long-term solution now available is the topical cream. As a result of this scientific understanding of the Kindler Syndrome-causing gene mutation, a targeted, particular medication was designed *in-ssilico* using several bioinformatics tools. To build a medication that can inhibit the *FERMT1*, the most effective ligand was selected and interacted with the protein. The potential to deliver enrichment in identifying molecules and genes for the target of interest is increasingly being demonstrated by these methods, and while they have not yet been confirmed to develop drugs on their own, they do represent progress. However, additional analysis of the development.

6. Declarations

6.1 Conflict of Interest

All authors declare that they have no conflict of interest.

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- **Journal article**
  Derwing, T. M., Rossiter, M. J., & Munro, M. J. (2002). Teaching native speakers to listen to foreign-accented speech. *Journal of Multilingual and Multicultural Development,* 23(4), 245-259.
- **Article by DOI**
  David, H., & Juyuan, J. (2013). A study of smog issues and PM 2.5 pollutant control strategies in China. *Journal of Environmental Protection*, 21(3), 16-21 DOI:10.4236/jep.2013.47086

- **Book**
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  Gazda, G. M., Balzer, F. J., Childers, W. C., Nealy, A. U., Phelps, R. E., & Walters, R. P. (2005). *Human relations development: A manual for educators* (7th ed.). Boston, MA: Pearson Educational

- **Book chapter**
  Easton, B. (2008). Does poverty affect health? In K. Dew & A. Matheson (Eds.), *Understanding health inequalities in Aotearoa New Zealand* (pp. 97–106). Dunedin, New Zealand: Otago University Press.

- **Dissertation**
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