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From The Editor;

Dear Readers and Authors,

As "International Journal of Science Letters (IJSL)", we are pleased and honored to present the second issue of 2021. IJSL, is an international double peer-reviewed open access academic journal published on the basis of research- development and code of practice.

The aims of this journal are to contribute in theoretical and practical applications in relevant researchers of Life Sciences, Biology, Biotechnology, Bioengineering, Agricultural Sciences, Food Biotechnology and Genetics institutions and organizations in Turkey, and to publish solution based papers depending on the principle of impartiality and scientific ethics principles, focusing on innovative and added value work, discussing the current and future.

With these thoughts, we are especially thankful to academicians honoring with the articles, valuable scientists involved in editorial boards and reviewers for their contributions to the evaluation processes with through their opinions/ideas/contributions/criticisms in this issue of International Journal of Science Letters.

24.08.2021

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Review

Assessment of tools for sustainability appraisal of buildings/building groups

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Abstract

The construction sector is one of the significant users of energy and natural resources. It was estimated that the sector uses nearly 40% of the total raw-material inflow to the global economy each year. Based on this fact, the construction sector is an essential contributor to environmental pollution and poses challenges in meeting sustainable development goals. This paper discusses the building assessment tools or models used to assess whether a building meets environmental standards with the view to explore the applications of these tools and their benefits. The paper notes that the environmental assessment models and the assessment itself are worthwhile as it offers several benefits to the society and environment, especially the first among them, the Building Research Establishment Environmental Assessment Method (BREEAM) in the UK. The BREEAM model has created awareness among the stakeholders and has achieved high levels of success. Over 1000 buildings have been assessed in the UK and over 1800 individuals involved as assessors. Thus, it creates jobs in addition to protecting the environment. Mitigation measures are integrated into the certification. It is cost-effective, especially in the long run and more environmentally friendly, unlike the conventional ones. Moreover, efforts should be geared towards harmonising the rating scales and standards across continents or climate regions.

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1. Introduction

The actions of humans on the environment have impacted it negatively such that environmental quality is compromised and its resources endangered. Anthropogenic activities have contributed immensely to the disruption of climate, freshwater system, ecosystem and forests and have had devastating impacts on local communities, especially in developing regions of the world (Madu and Nwankwo, 2020). The construction sector is one of the significant users of energy and natural resources. It was estimated that the sector uses nearly

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40% of the total raw-material inflow to the global economy each year (Uttam, 2014). Based on this fact, the construction sector is an essential contributor to environmental pollution and poses challenges in meeting sustainable development goals. Carbon dioxide emission is evident in all the phases of a building's life cycle, from material production through construction to demolition (Uttam, 2014). However, the earth's natural resource base is finite (Rockström et al., 2013), hence the need for the sector to improve its environmental performance (Tam et al., 2006; Uttam, 2014). This implies minimising the negative environmental impacts of its activities and products while still maintaining quality service delivery. Also needed is the preservation of local heritage and access to green space (Uttam, 2014). Such actions are critical as protecting the environment is a global agenda that has remained topical in the twenty-first century (Nwankwo, 2018).

Several critical policies formulated to curb the degradation include but are not limited to green public procurement (GPP) (Haapio and Viitaniemi, 2008), Sustainable public procurement (SPP) and Environmental Impact Assessment (EIA). GPP is "the approach by which public authorities integrate environmental criteria into all stages of their procurement process, thus encouraging the spread of environmental technologies and the development of environmentally sound products by seeking and choosing outcomes and solutions that have the least possible impact on the environment throughout their whole life cycle" (Bouwer et al., 2005). A growing concern on social aspects yielded SPP (McCrudden, 2004) since the social dimension is an aspect of sustainable goals. One of the main objectives of SPP is to achieve blended value via the integration of social, economic and environmental objectives (Williams et al., 2007). EIA is simply a systematic process to identify, predict and evaluate the effects on the environment of proposed actions and projects (Sadler, 2004). An extended sort of EIA is the SEA which aims to integrate the environmental and sustainability consideration in strategic decision-making (Therivel, 2012). A more potent synergy between EIA and GPP has been posited to promote coordination between planning and construction phases (Uttam, 2014). BREEAM is a good example where such linkage has yielded results.

Propelled by meeting sustainable development goals, the building sectors started putting up measures to mitigate environmental impacts (Haapio and Viitaniemi, 2008; Ahmad and Thaheem, 2018). Thus, sustainability has increasingly become central to building development (Happio, 2012; Kawakubo et al., 2018). Another important driver was the growing demand for environmentally sound products and services which was not unconnected

to the public policy to mitigating environmental impacts and achieving sustainability. Hence, the yardstick became the quality of building performance. This was difficult to define, as investors are interested in economic performance while tenants are interested in health and comfort aspects (Haapio and Viitaniemi, 2008). These were instrumental in the emergence of building assessment tools. This paper discusses the building assessment tools with the view to keep abreast scholars and planners with the applications of these tools and their benefits. The following section discusses the building assessment tools and applications before the conclusion is presented.

2. Building Assessment Tools and Applications

Various indicators and associated tools were developed to meet different interest groups. The first of these was the Building Research Establishment Environmental Assessment Method (BREEAM) in the UK in the 1990s (Grace, 2000; Lee, 2013; Ilhan and Yaman, 2016). Many of the tools have gained global recognition and have formed discourse in specific conferences like the Green Building Challenge (GBC) (Haapio and Viitaniemi, 2008). Hitherto, according to Haapio and Viitaniemi (2008), the International Organisation for Standardisation (ISO) was at the forefront of defining standard requirements for environmental assessment of buildings. Efforts to improve the building quality have been on the increase ever since. The European Committee for Standardisation provided a voluntary guide for assessing sustainability aspects of new and existing construction works and products (CEN, 2005 cited by Haapio and Viitaniemi, 2008). Several environmental assessment tools for buildings abound and range in applicability, covering different phases of a building's life cycle and different environmental issues. The tools (Table 1) are developed for different purposes such as research, consulting, decision-making and maintenance (Haapio and Viitaniemi, 2008). The tools used depend on the building type (residential, commercial or office) and its stage of development- whether new or existing. The list is inexhaustible as there are still others like SBAT (South Africa), BEAM (Ireland and Hong Kong) (Calquin 2017; Hui et al., 2017), SBTool (EU) (Larsson, 2015; Bernardi et al., 2017; Atanda and Öztürk, 2020), Rapid Sustainability Assessment (RSAM, Kazakhstan) (Karaca et al., 2020), Building sustainability assessment method (BSAM, Sub-Saharan Africa) (Olawumi et al., 2020), Ecology, Energy saving, Waste reduction and Health (EEWH, Taiwan) (Liu et al., 2019), Gesellschaft fur Nachhaltiges Bauen (DGNB, Germany, Denmark) (Stender and Walter, 2019; Al-Qawasmi et al., 2019).

Table 1. List of some of the building assessment tools

Tool	Developer
ATHENA^{TM} (EIE)	ATHENA sustainable material Institute Canada
BEAT 2002	Danish Building Research Institute (SBI) Denmark
BeCost	VTT Finland
BEES 4.0	US National Institute of Standards and Technology (NIST)
BREEAM	Building Research Establishment (BRE) the UK
EcoEffect	Royal Institute of Technology (KTH) Sweden
EcoPrifile	Norwegian Building Research Institute (NBI) Norway
EcoQuantum	IVAM, the Netherlands
Envest 2	Building Research Establishment (BRE) the UK
Environmental Status Model (Miljostatus)	Association of the Environmental Status of Buildings Sweden
EQUER	Ecoles des Mines de Paris,Centre d' Energetique et procedes France
ESCALE	CTSB and the University of Savoie France
LEED	US Green Building Council, USA
LEGEP	University of Karlsruhe, Germany
PAPOOSE	TRIBU France
TEAM^{TMa}	Ecobilan, France
CASBEE	Japan
BEAM Plus	HongKong modelled from BREEAM
ESGB	China modelled from LEED

TEAM^{TMa} is a professional LCA tool for evaluating life cycle, environmental and cost profiles of products and technologies, including buildings. It is the only tool here that is not specifically for the environmental assessment of buildings (Haapio and Viitaniemi, 2008; Lee, 2013).

In recent times, it has progressed from sustainability to Green Building assessment which assesses buildings to learn if they meet the needs of reducing adverse impacts on the environment and the occupants throughout its life cycle (Li et al., 2017; Liu et al., 2019). Thus, a building is only certified green if it meets the following attributes; energy saving, efficiency in water and other resources use; pollution and waste reduction; carbon emission reduction; materials re-use and recycling; renewable energy usage; healthy indoor environment and air quality; use of green and sustainable materials and consideration of biodiversity in building designs (Liu et al., 2019). It has been argued that most of the tools fail to incorporate economic and social aspects (Lopez et al., 2019). Social and economic sustainability assessments have been advocated for residential buildings (Ahmad and Thaheem, 2018; Stender and Walter, 2019) integrated with Building information modelling (Solla et al., 2016). For instance, a collaboration by Danish Building Research Institute integrated social sustainability into the Gesellschaft fur Nachhaltiges Bauen (DGNB) assessment tool (Stender and Walter, 2019). Also, there has been advocacy for a tool to assess heritage and recreational buildings due to their specificity (Raslanas et al., 2016; Al-Sakkaf et al., 2020). Due to the multiplicity and diversity of rating systems in sustainability building

assessment, Mahmoud et al. (2019) devised a global assessment tool for assessing buildings. However, the limitation here is that there are significant environmental differences among countries, variations in building qualities between developed and developing nations, among other factors. Nevertheless, a harmonised assessment tool at larger scales probably based on similarity of environmental factors or climate is needed. Such a tool will aid comparability of performances (Mahmoud et al., 2019), might minimise costs and enhance the achievement of sustainability in buildings across regions.

3. Method Applications

The tools are grouped below based on the kind of buildings they can assess (Table 2) and phases of the life cycle (Table 3). A dot indicates a building type the given tool can assess.

Table 2. Tools and the building types they assess (Haapio and Viitaniemi, 2008)

Tools	Existing Building	New building	Refurbishment of Building	Building product component	Building	Residential building (Multi-unit)	Residential building (Single unit)	Office	Others
BEES 4.0				•					
TEAM				•	•				
ATHENA	•	•	•			•	•	•	•
BEAT 2002	•	•	•	•	•				
BeCost				•	•				
EcoQuantum					•		•		
Envest 2								•	
EQUER		•				•	•	•	•
LEGEP	•	•		•					
PAPOOSE				•	•	•	•	•	•
BREEAM	•	•	•			•	•	•	•
EcoEffect	•	•	•	•		•		•	•
EcoProfile	•					•	•	•	•
ESM	•					•	•	•	
ESCALE		•				•	•	•	•
LEED	•	•	•			•	•	•	•

Some of the tools can be utilised for product comparisons and an environmental assessment of a whole building. Envest 2 is only applied to assess office buildings (Table 2).

Table 3. The tools and the life cycle phases of buildings they assess (Haapio and Viitaniemi, 2008)

Tools	Production	Construction	Operation	Maintenance	Demolition	Disposal
BEES 4.0	●		●	●		●
TEAM	●	●	●	●		●
ATHENA	●	●		●	●	●
BEAT 2002	●	●	●	●	●	●
BeCost						
EcoQuantum	●	●	●	●	●	●
Envest 2	●	●	●	●	●	●
EQUER	●	●	●	●	●	●
LEGEP		●	●	●	●	
PAPOOSE	●		●	●	●	●
BREEAM	●	●	●	●	●	●
EcoEffect	●	●	●	●	●	●
EcoProfile			●	●		
ESM						
ESCALE	●	●	●	●		
LEED	●	●	●	●	●	●

From Table 3 above, most of the tools cover nearly all the phases of the buildings' life cycle. BREEAM is used for all (Table 3), while the Environmental Status Model (ESM) cover none of the phases. From Tables 2 and 3, it is apparent that only a few of the tools cover over 70% of the building types that can be assessed. These are ATHENA, EcoEffect, BREEAM and LEED. However, in this study, the evaluation will focus on BREEAM since it has higher global usage (BREEAM, 2014); a pioneer tool and the most used in the UK. Therefore, this study will critically review it as the study is literature-based. It is basically to highlight its applications and finally outline its strengths and limitations.

4. The Building Research Establishment Environment Assessment Method (BREEAM)

The BREEAM model is built in pursuance of the following aims and objectives (Table 4). It is developed to assess new and existing buildings. The latest version, new construction, can assess diverse kinds of buildings (Tables 1 and 2). Variants of the model like EcoHomes is for refurbished homes, BREEAM schools' assessment Tool replaced in 2008 by BREEAM Education is for assessing educational institutions. Also, the NHS Environmental Tool was introduced in 2008 for assessing healthcare (Islington, 2012). There is a new variant for

assessing infrastructure. The minimum required standard for BREEAM infrastructure is very good. It employs a scoring system that relates to core areas (Figure 1).

Table 4. Aims and Objectives of BREEAM (BREEAM, 2014)

Aims	Objectives
• Mitigate life cycle impacts of buildings on the environment • That building be recognised based on their environmental benefits • Provide a credible environmental label for buildings • Stimulate demand for sustainable buildings and their products	• Provide market recognition of buildings with low environmental impacts • Ensure best environmental practice is part of planning, design construction and operations • Define a robust, cost-effective performance standard surpassing that required by regulations • Raise awareness amongst diverse interest groups in the building sector • Challenge market to provide innovative, cost-effective solutions that minimise the environmental impact of buildings

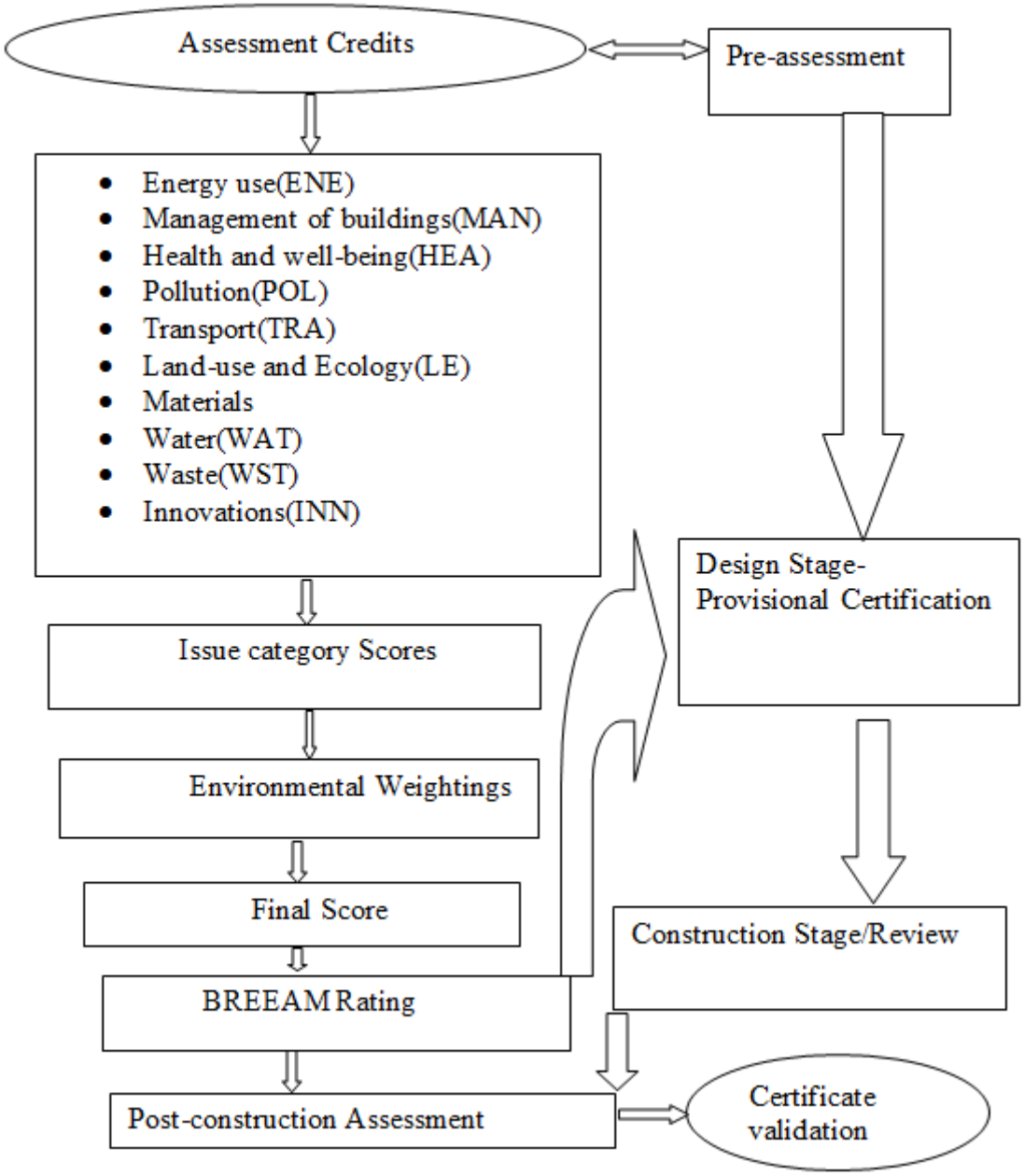

Figure 1. Process of BREEAM rating calculations (Lowe and Watts, 2011; Islington, 2012; BREEAM, 2014; 2016).

The assessment involves awarding credits to each of the areas according to the building's performance against specific criteria (Islington, 2012). The credits are then summed up to produce an overall rating based on a weighting system. Based on this, a certificate is awarded depending on the performance on a scale of PASS (>=30%), GOOD (>=45%), VERY GOOD (>=55%), EXCELLENT (>=70% overall score) and OUTSTANDING (>=85% overall score)- a later inclusion. It has a mandatory minimum requirement for water and energy (ISLINGTON, 2012). A certified BREEAM assessor does BREEAM assessment. It involves

a two-stage process to get a certification. The first is the design stage, followed by the post-construction stage. This ensures that the plans are executed from design to construction devoid of compromise to the set standards before the provisional certificate issued at the design phase is validated.

However, in 2007, in pursuant of sustainability, EcoHomes was replaced by the Code for Sustainability for assessing new housing in England (ISLINGTON, 2012), but EcoHomes is still used for refurbishment of buildings. This emphasises carbon emissions and energy use from/in homes providing greater regulatory certainty for the builders (ISLINGTON, 2012). It is now mandatory for new homes to undergo a rating based on the code even if the outcome is nil-rating, denoting not assessed (ISLINGTON, 2012). The government aimed to have all homes built to zero carbon standard by 2016 (Table 6) (ISLINGTON, 2012). The scoring for sustainable homes is for nine design categories and their scores (Table 5). Greater emphasis is put on energy, material and health.

Table 5. The BREEAM categories and scores (Parker, 2012; BREEAM, 2014)

Category	**Score(%) Old**	**Score(%) New**
Energy	19	15
Health and Well-being	15	15
Materials	12.5	13.5
Management	12	12
Land-use and Ecology	10	10
Pollution	10	10
Transport	8	9
Waste	7.3	8.5
Water	6	7
Total	100	100
Innovation (additional)	-	10

The ratings have code levels 1 to 6 depending on the building's performance (Table 5). Code level 6 implies zero carbon emissions (Table 6). Level 6 is the goal of the latest BREEAM (Table 6), and greater weight cum importance is assigned to energy and health (Table 5).

Table 6. Code levels for Sustainable Home rating (Islington, 2012)

Code-level	Minimum reduction in dwelling CO_2 emission rate over target emission rate (%)	Maximum Water consumption(1/person/day)	Total point scored on code (%)
1	10	120	36
2	18	120	48
3	25	105	57
4	44	105	68
5	100	80	84
6	Zero carbon	80	90

The use of the BREEAM model has many advantages and shortcomings (Table 7).

Table 7. Advantages and disadvantages of BREEAM Model (Lowe and Watts, 2011)

Pros	Cons
Robust	Complicated
Detailed	Rigid
Famous	Poorly understood
Easy to specify	Often poorly specified
Independent	Extra cost
Tailored to a particular building	A lot of differentiation

5. Examples of Applications - Case studies

BREEAM was applied to reconstruct the demolished Primary Care Centre on East Gate Road in Driffield that would accommodate GP surgery facilities to serve the community (Lowe and Watts, 2011). The ratings were done and yielded approximately 74%, which implies excellent. However, the acquisition of green building materials raised the building cost. It was equally applied in constructing a two-storey shopping centre in Italy in 2015 (Jacobs, 2017). It has been used to certify over 260,000 buildings across the building lifecycle and is being applied in over 50 countries (BREEAM, 2014).

6. Strengths and Limitations

A lot of benefits accrue from the use of BREEAM for the assessment of buildings. One of the strengths is the actualisation of the aims for its establishment, one of which is to mitigate the life cycle impacts of buildings' environment. It has created awareness and raised consciousness among citizenry stimulating demand for sustainable buildings (Parker, 2012). It

has ensured that buildings have ratings depending on their performance towards carbon emission reduction (Tables 4 and 5). It has promoted teamwork and dialogue among diverse players in the building sector (Lowe and Watts, 2011). It has led to building efficiency by using energy-efficient fittings and appliances and pollutant-free appliances like chlorofluorocarbon-free air conditioners(Roodman et al., 1995). Some studies have evidence that BREEAM assessment is cost-effective (Lowe and Watts, 2011). For instance, Roodman et al. (1995) indicate that the use of unbaked brick has lowered pollution to 0.2 per cent. They noted a housing development in Dallas, the USA, that slashed utility bills by 450 dollars upon incorporating solar heating.

However, some argue that the cost premium arising from designing a building to meet the BREEAM rating standards can be excessive (Lowe and Watts, 2011). This rising cost is due to the higher cost of acquiring the green building materials introduced to replace the non-compliant materials (Lowe and Watts, 2011). Nevertheless, it is still more cost-effective when considered in the long run. Furthermore, significant weakness is the non-existence of standard measurement scales. That is, there is no logical basis for assigning the maximum number of points for each case. There is an element of subjectivity in the weighting system. Thus, there is the need for a uniform or consistent scalar system which will enhance comprehension and enable data handling (Cole, 1998). This is complicated by the broad scope of data for assessment and which weights are assigned (Kajikawa et al., 2011). These criteria include a mixture of quantitative and qualitative data, and as such, ambiguity may arise in handling them.

7. Discussion and Suggestions

There are substantial variations among the various building assessment tools, which vary according to scope, rating scales and performance (Kajikawa et al., 2011; Mahmoud et al., 2019). There are also variations based on the building types assessed, age or life cycle. Additionally, some of the tools focus on the energy demands and consumption or total environmental quality that focus on the ecological and socio-economic aspects (Berardi, 2012; Mahmoud et al., 2019). Green building assessment tools address several issues such as recycling materials, conservation of water and energy, healthy air and temperature, illumination of the indoor environment, rainwater harvesting and recycling, reduction of carbon emission and below-ground reservoir (Liu et al., 2019). However, the use of the tools

and the factors considered is dependent on the type, size and use to which the building is to be put.

Furthermore, Al-Qawasmi (2019) and Braulio-Gonzalo and Bovea (2020) reveal marked variations and inhomogeneity in the breadth and length of coverage of attributes. As such, out of the dimensions of sustainability, the social aspect gets the least representative coverage (Al-Qawasmi 2019). The most widely used tools are the LEED, BREEAM, CASBEE and SBTool, according to Bernardi et al. (2017). Therefore, to minimise the discrepancies and enhance applicability and more reliable results, some building tools can be integrated. Such integration will promote broader spatial usability at the regional or continental level. It will also enhance the achievement of sustainability in building as more attributes will be considered. Additionally, the use of BIM with sustainable building assessment tools has been argued to give better results (Carvalho et al., 2019). Active stakeholders' participation is key to achieving the desired integration in the sustainability assessment of buildings (Roostaie et al., 2019).

8. Conclusion

The construction sector, one of the significant users of energy and natural resources, is estimated to use nearly 40% of the total raw-material inflow to the global economy each year. As a result of this, the construction sector is a crucial contributor to environmental pollution and poses challenges in meeting sustainable development goals. Hence, the environmental assessment tools of the building were reviewed, which shows that the exercise is worthwhile as it offers several benefits to the society and environment. Its introduction has resulted in increased pre-contract design work which may be due to the requirements of meeting the energy dimension in the BREEAM model (Parker, 2012). The model has created awareness among the stakeholders and has achieved high levels of success. Over 1000 buildings have been assessed in the UK, and over 1800 individuals as assessors (Parker, 2012). Thus, it creates jobs in addition to protecting the environment. Mitigation measures are integrated into the certification. It is cost-effective, especially in the long run and more environmentally friendly, unlike the conventional ones (Roodman et al., 1995).

Moreover, assessment cost can be reduced by subsidising the cost of acquiring green building materials, and the exercise made a continuous one like EIA and backed by

legislation. That is, monitoring should be a part of the BREEAM assessment to routinely check if developers keep the buildings to set standards, especially in the post-construction period. There is a close affinity between BREEAM and EIA. Both pursue the same goal of promoting and enhancing environmental quality. However, in EIA, the emphasis is on mitigating impacts, whereas, in BREEAM, the emphasis is on maximising benefits. Furthermore, EIA has statutory backing. That is, legislation drives EIA, while in BREEAM, certification is voluntary. Nevertheless, both have measures that can be fed in to achieve similar goals (Jacobs, 2017).

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Research Article

An investigation of the toxic effects of water samples collected from 3 different regions of Antarctica on *Drosophila melanogaster*

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Abstract

In this research, it was aimed to investigate the ecotoxicological effects of seawater from Galindez Island, lake sediment samples collected from Ardley Island, and green algae ice samples collected from Horseshoe Island on *Drosophila melanogaster*, which is an important model organism. Newly hatched *Drosophila melanogaster* larvae of the same age and adult individuals were used. While the individuals in the control group were tested in standard media, the individuals in the experimental group were tested under 3 different conditions at the rates of 25%, 50%, and 100% of each water sample. The effects of polar water added to the media on mortality rates on *Drosophila melanogaster* eggs, larvae, and adults were investigated. The effect of water samples collected from Ardley and Horseshoe Islands on the survival percentage in *Drosophila melanogaster* larvae was found to be similar to the control group. Furthermore, while the viability rate in Ardley and Horseshoe Island was 92% and 96%, respectively, in the control group individuals, similar results were obtained in all rates in the experimental group. The water samples obtained from 3 different points from the Antarctic region have not reached a level that will adversely affect the lives of the larvae and adults of the creature as of the present day. Nevertheless, although pollution was detected in some areas in the Antarctic region in the literature, we consider that this pollution can be prevented before it reaches dangerous levels with some measures to be taken.

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Antarctic marine pollution, *Drosophila melanogaster*, Ecotoxicity

1. Introduction

In earlier times, the oceans were considered a reservoir where pollutants can be easily discharged. Organochlorine compounds, domestic and industrial wastes, and petroleum products cause adverse effects in the ocean environment even when they are released at low

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levels (Pinto et al., 2003). As mining and industrial activities increased in the 19th century, environmental pollution caused by organic compounds increased and has also continued to increase since then (Colepicolo et al., 2008).

Different methods have been developed to determine the level of this increasing pollution. The use of marine sediments as markers to detect the presence of environmental pollutants is one of these methods (Ergin et al., 1991). Many toxic and bioaccumulative pollutants accumulate in sediment at higher amounts than in water (Binelli and Provini, 2003). Important organic compounds that accumulate in sediments include PAH (Polycyclic Aromatic Hydrocarbons) and dl-PCB (Dioxin-Like Polychlorinated Biphenyl) compounds (Lacorte et al., 2006). Toxicity caused by organic pollutants leads to an increasing concern due to its bioaccumulation and persistence in the environment (Zhou et al., 2014).

Polar regions are considered clean environments without significant sources of pollution. Especially since Antarctica is far from any urbanization, the pollutant rate of this region is considered to be very low. Antarctica has been considered by humans as an undisturbed region for years (Kim et al., 2006). Nevertheless, global warming, population growth, and industrial development in the Southern Hemisphere countries increase the effects of pollutants on the Antarctic environment (Bargagli, 2008). The main human activities in Antarctica are scientific research and the bases established for this purpose, shipping traffic, waste disposal, fishing, and tourism (Aislabie et al., 1999).

Antarctica is a continent located in the southernmost part of the Southern Hemisphere and includes the South Pole. The Antarctic Peninsula, the only extension of the continent, is also the closest part of Antarctica to South America. It is the only continent without a country. It is the world's driest place in that no rain fell in some parts of the continent for 2 million years. Antarctica is covered by an ice sheet with an average thickness of 2000 m. The ice thickness at the South Pole reaches 4335 m. This ice mass constitutes approximately 90% of all ice on the earth (Kırkıncı et al., 2021).

The Antarctic Peninsula has many research bases since it has the continent's mildest climate. The Antarctic Peninsula consists of a series of rocky islands under the ice sheet that covers it. These rocky islands are connected by an ice sheet that acts as soil (Stewart, 2011). Of Antarctica, 98% is covered with the ice sheet. It has been calculated that if all of this ice

sheet, which constitutes approximately 90% of the world's ice and 70% of the freshwater, melts, the sea level will rise by approximately 60 m all over the world (Howstuffworks, 2017).

In polar regions, no pollution is expected since there is no source of pollutants. However, persistent organic pollutants have been encountered in Antarctica since the beginning of the 1960s (Bidlemen et al., 1993). Human activities are among the main causes of pollution in the environment. Human activities in Antarctica began with the establishment of research bases. Subsequently, situations such as increasing research activities, tourism, fishing, shipping and vehicle traffic, and waste disposal have caused pollution in Antarctica (Kim et al., 2006). Air currents are considered one of the most important ways for pollutants to reach Antarctica (Wania and Mackay, 1993). These transported pollutants are trapped on ice or land by precipitation or atmospheric precipitation and begin to accumulate. During the summer months, the pollutants released by the melting of ice and snow mix directly with seawater (Geisz et al., 2008). Different studies are conducted on the determination of pollutants. One of these studies was conducted by Kim et al. (2006). In this study, 621 ng/g – 5024 ng/g T-PAH value was determined in dry weight in the sediment around the McMurdo Research Station. Borghesi et al. (2008) found 15.1 ng/g dl-PCB in dry weight in the liver tissue of *Trematomus bernacchii* organism.

Our study aimed to determine the possible effects of water samples obtained from three different stations in the Antarctic Peninsula on the larvae and adult individuals of *Drosophila melanogaster* individuals.

Drosophila melanogaster, also known as the vinegar fly, is one of the most commonly used model organisms in life sciences. Thomas Hunt Morgan won the Nobel Prize in Physiology and Medicine in 1933 with his study on the chromosomal theory of inheritance using *Drosophila melanogaster* for the first time, showing that the white gene is inherited on the X chromosome (Morgan and Bridges, 1916). Hermann Müller received another Nobel Prize in 1946 with his study investigating the effect of X-rays on mutation rates (Muller, 1928). Based on these discoveries, balancer chromosomes that inhibit recombination through DNA inversion emerged, and these developments showed that mutations were conserved over generations on a single chromosome and made *Drosophila melanogaster* the first genetic system used in genetic research (Lindsley and Zimm, 2012). Successive genetic discoveries

allowed for conducting more complex genetic studies. These genetic discoveries in the vinegar fly have made significant contributions to many medical and scientific studies, including gene biology, cell biology, developmental biology, population genetics, molecular genetics, toxicology, and resistance development in insects (Wilson, 1988).

Drosophila melanogaster is used in genetic studies due to its simple genetic structure with 4 pairs of chromosomes. The sequencing of the *Drosophila melanogaster* genome was completed in 2000. Thus, 13600 genes were identified, 95% of which were encoded in 3 of 4 pairs of chromosomes. As a result of the analyses, it was found that different genes associated with human diseases had 77% similarity with *Drosophila melanogaster*. Nevertheless, it was determined that the proteins involved in the regulation of gene expression and metabolism were similar to human genes (Rand, 2010).

2. Materials and Methods

2.1. Materials

2.1.1. Sampling studies

The samples used in the study were provided in 2019 within the scope of the project carried out by Assist. Prof. Yılmaz KAYA and supported in the TAE-III expedition carried out under the auspices of the Presidency, under the responsibility of the Ministry of Industry and Technology, and by the coordination of ITU Polar Research Center. The samples obtained were kept in the cold in the laboratory of the Faculty of Agriculture of 19 Mayıs University until the period of use in our study. The obtained samples were collected from the regions with the coordinate system in Table 1.

Table 1. Effects of seawater sample collected from Galindez Island on *D. melanogaster* eggs, larvae, and adult individuals

No	Region	Coordinate		Sample type
YO	Ardley Island	62º 12' 48,81" S	58º 56' 21,09" S	Lake Sediment
Y13	Galindez Island	65° 14' 41,5" S	64º 15' 22,5" S	Seawater
Y15	Horseshoe Island	67º 49' 42,39" S	67º 13' 29,88" S	Green Algae Ice

In the study, lake sediment, seawater, and green algae ice samples were collected from 3 different stations (Figure 1). Galindez Island, Horshoe Island, and Ardley Island were some of the regions visited by our Turkish scientists to conduct scientific studies within the scope of the National Polar Science Program (2018-2022) carried out under the auspices of the Presidency, under the responsibility of the Ministry of Industry and Technology and the coordination of TÜBİTAK Marmara Research Center (MAM) Polar Research Institute (KARE) (Figure 2).

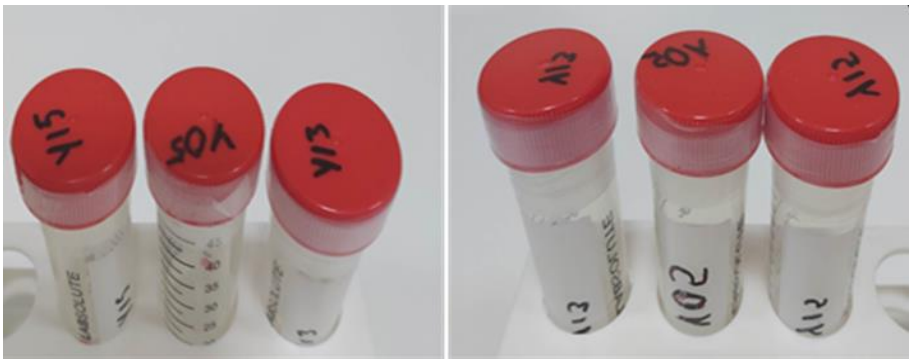

Figure 1. Samples collected from 3 different regions from the Antarctic Peninsula (original)

Figure 2. Islands visited by Turkish scientists to conduct studies (Wikipedia, 2021)

2.1.2. Drosophila melanogaster

The fruit flies to be used in the study were obtained from Amasya University Faculty of Arts and Sciences Biological Research Laboratory. The Oregon (wild type) strain of *D. melanogaster* was used in the experiments. Before the studies as an experimental organism, female and male individuals of the Oregon stock were crossed, and the eggs obtained from

this crossing and the larvae and adult individuals obtained from the eggs were used.

The instant *Drosophila* medium was used in the control and experimental groups. The instant medium was prepared using 1.5 grams of the medium and 5 ml of distilled water. The water obtained from the poles, the effect of which was investigated, was added to the medium content by 0%, 25%, 50%, and 100%, and the effect of the 3rd-stage larvae on the mortality rates of the adult individuals obtained from the larvae was investigated.

2.2. Methods

In the study, 24 *D. melanogaster* eggs were used in the control and experimental groups. In the experimental groups, the samples at the rates of 0%, 25%, 50%, and 100% at 4 different concentrations were added to the habitat of the eggs (Figure 3). The percentage of transformation from eggs to larvae and adult individuals and the data obtained by keeping adult individuals alive in media prepared chronically for 15 days were determined. During the experiment, the ambient conditions were maintained at 25 °C and 60% humidity, which are the standard survival conditions of *D. melanogaster*.

Figure 3. Addition of eggs to the instant *D.melanogaster* medium and standard survival conditions

3. Results

The possible effects of the samples collected from 3 different stations in the Antarctic Peninsula were tested on *D.melanogaster* individuals. Twenty-four eggs were used for each test group. The percentage of transformation of water samples collected from Galindez Island

of *D. melanogaster* eggs to larvae and adult individuals and the data obtained by keeping adult individuals alive in the same medium for 15 days are presented in Table 2.

Table 2. Effects of seawater sample collected from Galindez Island on *D. melanogaster* eggs, larvae, and adult individuals

Application Groups	Number of eggs	Number of larvae	Number of adult individuals	Viability Rate
Control G.	24	24	22	92%
25% P.W*	24	21	20	83.3%
50% P.W	24	12	12	50%
100% P.W	24	9	7	29.1%

*PW, polar water

The percentage of transformation of lake sediment water collected from Ardley Island of *D. melanogaster* eggs to larvae, and adult individuals and the data obtained by keeping adult individuals alive in the same medium for 15 days are presented in Table 3.

Table 3. Effects of lake sediment sample collected from Ardley Island on D. melanogaster eggs, larvae, and adult individuals

Application Groups	Number of eggs	Number of larvae	Number of adult individuals	Viability Rate
Control G.	24	24	24	96%
25% P.W*	24	23	23	100%
50% P.W	24	23	22	92%
100% P.W	24	24	23	96%

*PW, polar water

The percentage of transformation of *D. melanogaster* eggs to larvae and adult individuals and the data obtained by keeping adult individuals alive in the same medium for 15 days are presented in Table 4. In the study, egg-larva-prepupa-pupa and adult stages of all individuals in the control and experimental groups were examined one by one under a stereomicroscope and evaluated (Figure 4).

Table 4. Effects of green algae ice sample collected from Horseshoe Island on *D. melanogaster* eggs, larvae, and adult individuals

Application Groups	Number of eggs	Number of larvae	Number of adult individuals	Viability Rate
Control G.	24	24	22	92%
25% P.W	24	23	23	96%
50% P.W	24	24	22	92%
100% P.W	24	24	23	96%

Figure 4. Stages of transformation from eggs to pupae and adult individuals in the control and experimental groups (original)

4. Discussion

In our study, the possible effects of water samples obtained from 3 different regions of the Antarctic Peninsula were evaluated on *Drosophila melanogaster*, an organism used in ecotoxicology studies.

When Table 2 was examined, the effects of the seawater sample collected from Galindez Island were taken into account only at low rates because the water prepared for fruit flies was freshwater. While the survival percentage in eggs and larvae kept alive in the medium containing 25% seawater sample was 83.3%, this percentage decreased due to the increase in seawater. When Table 3 was examined, while the effect of the lake sediment sample collected from Ardley Island on the survival percentage of flies was 96% in the control group, the survival percentages in adult individuals in the experimental groups (25%, 50%, 100%) were found to be 100%, 92%, and 96%, respectively. The fact that the viability rate was determined as 96% in adult individuals kept alive for 15 days in the medium prepared with the water sample obtained from Ardley Island indicated that the water sample from this region did not

have any pollution that could have an adverse effect on fruit flies. When Table 4 was examined, the viability rate in the media prepared with water obtained from green algae ice obtained from Horseshoe Island was determined as 96%, 92%, and 96%, respectively (25%, 50%, 100%). The viability rate was determined as 92% in the control group individuals. The same or higher data were obtained with the control group at all rates in the experimental groups. These results showed that the water did not have any toxic effect on the living thing, as in Ardley Island.

When the effect of water samples collected from seawater, green algae ice, and lake sediment samples on the eggs, larvae, and adult individuals of fruit flies was evaluated in general, it was observed that freshwater samples (green algae ice-lake sediment sample) did not cause any negative effects and that the seawater sample caused an increase in mortality rates due to its salinity content.

Although the results we obtained were considered positive for the Antarctic Peninsula, some effects on the increase in the pollution rate in the Antarctic region and the source of pollution in the region were found in the literature review (Light, 2017).

Although Antarctica has been considered an undisturbed region by humans for years, it is also known that the effects of pollutants on Antarctica are increasing every day due to global warming, population growth, and industrial development in countries in the Southern Hemisphere (Kim et al., 2006; Bargagli, 2008). The main human activities in Antarctica are scientific research and the bases established for this purpose, shipping traffic, waste disposal, fishing, and tourism (Aislabie et al., 1999).

Antarctic tourism has expanded with the modern cruise industry that started in 1969 (IAATO, 2020). Large-scale Antarctic tourism increased its popularity at the beginning of the 1990s and also continues to grow nowadays. While the number of tourists was 1000 per year with 12 ships in the 1990-1991 summer season, this ratio increased to 50,000 with a total of 50 ships in the 2017-2018 season (Mc Carthy et al., 2019). According to the latest statistics, the total number of tourists in the 2018-2019 season was 56,168, and the total number of tourists in the 2019-2020 season was expected to reach 78,520 (IAATO, 2020). It is also considered that plastics, which are given to the environment as garbage in proportion to the increasing tourism, pose a threat to the Antarctic environment (Bessa et al., 2019; Lacerda et

al., 2019). Therefore, studies are conducted to ensure that all human activities, especially tourism activities, are carried out within the framework of strict rules as far as possible.

Unless the plastic wastes on land are disposed of carefully, they mix with rivers and other water bodies and become a major source of marine pollution (Jambeck et al., 2015). According to global estimates, 80% of plastic waste in the ocean comes from land, while 20% comes directly from the use of plastic in the ocean (Li et al., 2016). Although the first report on marine plastic pollution appeared in the early 1970s, little attention was paid to this problem in the scientific community until the mid-2000s. However, the awareness of marine plastic pollution has increased with the discovery of plastics on the planet's most remote islands (including the Southern Ocean) (Waller et al., 2017).

Plastics in Antarctica can come from a variety of sources. Direct sources such as the disposal of waste from research stations and ships (Waller et al., 2017) and indirect sources such as transport by ocean currents that can transport microplastics from low latitudes to high latitudes of Antarctica can be considered in this context (Fraser et al., 2018). In the study conducted by Eriksson et al. (2013), approximately 6500 samples were collected from six islands in Antarctica (Macquarie Island and Heard Island). It was determined that the lost or discarded fishing gear constituted 22% of the plastics collected on both islands. They were reported to consist mostly of ropes, bait box straps, monofilament ropes, and buoys. In the study conducted by Convey et al. (2002), it was observed that the ocean waste in South Georgia was closely associated with local fishing activities and that the source of plastic waste in the South Sandwich Islands was mainly polystyrene from fishing buoys and remote sources.

Our results showed that no adverse effect occurred in the model organism used, which indicated that the level of pollution in these regions did not reach critical values for the living things used. However, as shown in the examples given above, pollution is occurring in the region and continues to increase due to main human activities, scientific research and bases established for this purpose, ship traffic, waste disposal, fishing and tourism activities.

Although Antarctica is the continent that is the farthest from human impacts, it is adversely affected due to the growing world population and consequently the increasing needs, touristic trips made with the increase of transportation opportunities, and the increasing use of

chemicals in industrial applications. Careful implementation of the decisions limiting/prohibiting the use of such chemicals, which have adverse effects by being transported over very long distances, is important in minimizing the ratio of this negative effect.

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Review

Food additives and genotoxicity

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Abstract

In today's changing conditions, there has been an increase in the consumption of ready-made food with the change in eating habits. Moreover, parallel to the increase in ready-made food production, there has been an increase in the food additives used. The dose amounts of food additives are determined as a result of experimental analyses. However, some additives show long-term toxic effects on the human body in genotoxicity tests. In this review, definition of substances, purposes of usage, classification, genotoxicity, definitions of tests and publications of genotoxicity studies in food additives were discussed. The search was conducted in peer-reviewed journals using Science-Direct, Web of Science and Google Scholar. In this study, genotoxicity studies conducted with food additives between 2015-2021 were complied. For this purpose, the keywords ‘‘food additive’’, ‘‘genotoxicity’’ were used together and research articles were included in this study.

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1. Introduction

It has become inevitable that food items have been changed with the development of technology. Businesses have increased the production of ready-made foods and also food additives according to people's demand for snacks and more practical foods. Shelf life and preserving properties are important parameters for using food additives. However, excessive consumption of ready-made foods and also additives cause carcinogenic and mutagenic effects on human body (Ayaz and Yurttagül, 2012).

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1.1. Food Additives

The preference for additives in the food market has emerged from the diversity of the consumers' taste and therefore different production methods brought by the development of technology. Current production techniques such as increase in yield, minimising losses, increasing product quality, standardisation and the shelf life of products and producing new foods with different formulas have been commonly used in food industry. The definiton of food additive is "not consumed as a food on its own but a characteristic component of the food with or without nutritive value" according to Turkish Food Codex and Regulation on Food Additives. These are substances that are expected to be a preferred food componentin itself or its by-products as a result of its packaging, addition to food at transport or storage levels (Ayper and Binokay, 2010).

The classification of food additives according to their main functions can be summarized as follows colourants, preservatives, sweeteners, antioxidants, emulsifiers and acid-base providers.

Colourants: It is a class of food additives that can correct the colour without affecting the taste of the food (EFSA, 2018; Brancato et al., 2018).

Preservatives: It is the preferred class with the function of preventing the spoilage of food, ensuring the safety of food by keeping the long shelf life and preventing microorganisms production.

Sweeteners: It is used to improve the taste of foods and remove the irritating odours at the level of food production.

Antioxidants: They are preferred to delay or prevent colour loss related to undesirable odours, aromas, flavour changes, enzymatic darkening, to prevent or delay the bitter taste that occurs in fatty foods.

Emulsifiers: They provide uniform mixing of the fat and water-soluble substances in the food.

Acid-base providers: They are preferred to control and regulate the pH level of the food at different stages during production and also the final product (EFSA 2018; Brancato et al., 2018).

1.2. EFSA (The European Food Safety Authority)

EFSA is the international organisation that guarantees the food safety of the European Union since 2002. This organisation evaluates the risks in the food chain scientifically and therefore help guarantee safe food in the European continent. The Food Safety Authority's work covers all areas involving the food chain: nutrition, food and feed safety, animal health and welfare, phytosanitary and plant protection. EFSA provides independent high-quality scientific advice and evaluation based on up-to-date scientific data to the European Commission, the European Parliament and European Union (EU) decision-makers such as Member States. Thus, it helps risk managers in Europe, making informed decisions to improve EU food safety. By working closely with partners and stakeholders, EFSA contributes to a high level of consumer protection while ensuring the reliability of EU food sources (Erkmen, 2010).

1.3. Effects of Food Additives on Health

It has been observed that non-food chemicals have negative effects on human health. Numerous studies have found that consuming excessive amounts of synthetic food additives can cause gastrointestinal, respiratory, dermatological and neurological reactions (Chassaing et al., 2015). In some studies, it has also been observed that emulsifiers and sweeteners change the composition of the intestinal microbiota and facilitate the translocation of bacteria in intestinal epithelium. It is thought that the consumption of food additives such as carboxymethylcellulose or polysorbate 80 significantly reduces the thickness of the mucus layer and has a role in inflammatory bowel diseases such as ulcerative colitis and Chron's disease as well as colon cancer, obesity and diabetes (Cowan et al., 2013; Tayfur, 2014). It prevents the growth of *Clostridium botulinum* which is a harmful bacteria that develops in meat and meat products and prevents high toxicity to the consumer. *Clostridium botulinum* is the most important bacterium with a highly toxic effect on humans and animals (Skypala et al., 2015). Nitrite passes into the blood and combines with haemoglobin to form methemoglobin. Methemoglobin inhibits the oxygen-carrying function of haemoglobin.

Nitrites and nitrates can turn into cancer-causing components such as nitrosamines and become active in many organs such as liver, respiratory system, kidney, urinary bladder, pancreas, stomach and cause cancer (Chassaing et al., 2015). Natural food ingredients and added food additives may cause allergic reactions depending on the dose taken and the individual's particular sensitivity. Clinical symptoms after ingestion of food additives are angioedema or chronic urticaria. However, symptoms may also include severe anaphylactoid or anaphylactic reactions such as atopic dermatitis, flushing, abdominal pain, diarrhoea, hypotension, and asthmatic reactions (Stevens, 2013). Food colourants are thought to release prostaglandins and histamine in urticaria with a direct pharmacological effect in sensitive individuals rather than an allergic reaction. It has been reported that colourants also cause behavioural disorders such as hyperactivity, especially in children aged 3-9 years (JECFA, 2013).

1.4. Genotoxicity Tests

Standard *in vitro* and *in vivo* mutagenicity tests can provide a link between the carcinogenic and mutagenic effects of the genetic system and the substances. There are different genetic toxicity tests and some of them are discussed below.

The Ames (Salmonella/Microsome Mutagenicity) assay is a short-term and frequently applied test for detecting mutations at the gene level and preferred as a genotoxicity assay (Zeiger, 2019). The Ames assay is based on the effect of a mutation that confers the ability to synthesise histidine to strains with mutations that have lost their ability to synthesise histidine. Because the chemical component is estimated to have a mutagenic effect, it provides clear information about the genotoxicity level (Omurtag et al., 2013).

Comet assay is an easy, fast and reliable sensitive genotoxicity test applied to calculate the damage and level of DNA (Azqueta and Collins, 2013). The method of the comet assay is that the negatively charged DNA fragments in the nucleus generally isolated from living tissues are fixed into the thin agarose gel and the protocol is applied in the electrophoretic environment. If there is a break in the chain of single or double-stranded DNA, the broken one has a different molecular weight and electrical charge. It is based on the principle that fragile DNA molecules migrate at a variable rate in the electrophoretic field. It can be applied

in different types of cells and DNA breaks formed in the cell can be determined visually and create a comet appearance (Güner and Muranlı, 2013).

Sister Chromatid Exchange assay is expressed as the symmetrical exchange of DNA replica products between homologous locus of sister chromatids. Double-stranded DNA breaks are repaired by the general recombination method. Bromodeoxyuridine acts like a thymine analogue in DNA is added to the cell culture to make DNA breaks visible. During the cell cycle, bromodeoxyuridine passes between sister chromatids and mutual exchange of DNA fragments in homologous chromosomes is observed. Staining difference causes sister chromatids to stain differently from each other and thedifferences between sister chromatids in DNA are detected (Sezginer and Feruzan, 2016).

Micronucleus is generally formed from deficiencies in gene that controls the cell cycle and errors in the mitotic spindle. It arises from its different parts in the kinetochore or mitotic apparatus and chromosomal damage. It occurs during the mitosis division of the cell and not included in the main nucleus. Whole chromosomes are formations consisting of acentric chromosome fragments (Hayashi, 2016).

Chromosomal aberrations assay occurs as a result of damage to the level of DNA. Chromosome breaks are caused by unrepaired double-strand breaks in DNA while chromosomes with new structures are caused by incorrect repair of chain breaks in DNA. High chromosome abnormalities frequencyoccurs when such damages in the genetic material can not be repaired, indicating an increased cancer risk (Şekeroğlu and Atlı, 2011). The chromosome aberrations assay is a standard method frequently used for the detection of various structural and numerical chromosomal abnormalities induced by mutagens. Chromosomal abnormality frequency can be evaluated in mammalian cell cultures by *in vitro* chromosome aberrations assay and bone marrow cells. In addition, *in vivo* chromosome aberrations assay also allows the evaluation of factors such as metabolism, pharmacokinetics and DNA repair mechanisms which may vary depending on the species and tissue, especially in the determination of mutagenic damage (Şekeroğlu and Atlı, 2011).

1.5. Some of the Genotoxicity Studies with Food Additives

Food additives are substances that directly or indirectly contribute to human nutrition. For this reason, studies on genotoxic effects along with its effects on health are quite abundant. Among these studies, those carried out between 2015-2021 were summarised (Table 1). One of these studies is related to the genotoxic effects of monopotassium glutamate, calcium diglutamate, monoammonium glutamate and magnesium glutamate in root tip cells of *Allium cepa* (Türkoğlu, 2015). Different concentrations of the mentioned food additives were applied at different times. All concentrations of these chemicals have been reported to exert an inhibitory effect on cell division in root tips of *Allium cepa* and cause a decrease in the mitotic index. Micronucleus assay and comet assay techniques were used. In addition, these compounds have been reported to increase the frequency of chromosomal aberrations in the assay material (Türkoğlu, 2015). In another study conducted in 2016, *in vitro* genotoxic effects of monosodium glutamate on human peripheral lymphocytes were determined by a chromosomal aberrations, sister chromatid exchange, micronucleus, comet assay. It was observed that monosodium glutamate significantly increased chromosomal aberrations, sister chromatid exchange and micronucleus frequency compared to control. According to the comet assay results, tail density, tail length and tail moment also increased significantly when compared to the control. It has been reported that obtaining results indicated that monosodium glutamate has a genotoxic effect on human lymphocytes (Ataseven et al., 2016). Allura Red is used as a food color additive. *In vivo* micronucleus assay (bone marrow) and comet assay (liver, stomach, and colon) were performed using male young adult mices. As a result, it was determined that Allura Red did not have genotoxic activity in both assays (Bastaki et al., 2017). In a study conducted in 2017, the genotoxic effects of potassium propionate (E283), a food preservative, were investigated. *In vitro* micronucleus assay technique was used in human peripheral blood lymphocytes. According to the test results, micronucleus frequency was increased as a result of potassium propionate treatment (Ataseven et al., 2017). Sodium benzoate and potassium sorbate are widely used today. Genotoxic potential of their mixture was investigated by micronucleus assay in human peripheral blood lymphocytes *in vitro*. As a result, it was observed that the mixture could have a genotoxic effect (Mamur et al., 2018). In a different study, the genotoxic effects of sunset yellow were investigated. Human peripheral lymphocyte cultures were studied with chromosomal aberrations assay and micronucleus assay. As a result of this study, it was determined that sunset yellow were showed dose-dependent genotoxic potential in both assays (Haverić et al., 2018). Due to the widespread use

of stevia extracts, it was investigated in human peripheral blood lymphocytes. Chromosomal aberrations assay and micronucleus assay were used. No genotoxic effects were observed in either assay (Uçar et al., 2018). With the development of the food industry, the use of potassium nitrate has become widespread. Somatic mutation and recombination test (SMART) and comet assay techniques were used. *In vivo* experiments were performed in the animal model of *Drosophila melanogaster*. Both assays showed a tendency to high levels of genotoxic potential of potassium nitrate (Aledwany et al., 2018). In an another investigation conducted in 2018, genotoxicity and cytotoxicity of sodium acetate on Human Umbilical Vein Endothelial Cells (HUVEC) were determnined. Cytotoxicity was investigated by MTT assay and genotoxicity was investigated by DNA fragmentation. In conclusion, sodium acetate did not show cytotoxic and genotoxic effects in this study (Mohammadzadeh-Aghdash et al., 2018). Aspartame is one of the most preferred sweeteners today. It was evaluated by Ames assay and *in vivo* micronucleus assay. As a result, no genotoxic or mutagenic potential was observed in either test (Otabe et al., 2019). The genotoxic effects of ascorbic acid, benzoic acid, citric acid and sorbic acid in human peripheral blood lymphocytes were investigated using *in vitro* micronucleus assay. It was concluded that high concentration of benzoic acid, citric acid and sorbic acid were shown cytotoxic and genotoxic effect (Bogar and Tuylu, 2019). Methanyl yellow and carmoicine are widely used two azo dyes. There is a lot of controversy about these two dyes. To evaluate the genotoxicity of these food dyes, *Allium cepa* test was performed and mitotic index and chromosomal aberrations were examined. It was determined that methanol yellow and carmoicine had a significant decrease in the mitotic index. In addition, it was determined that different kinds of chromosomal aberrations were induced, especially at high concentrations. For this reason, it was emphasized that these two food additives should be used in limited doses (Khan et al., 2020). In different investigation, *in vitro* genotoxic effects of monopotassium glutamate (MPG) and magnesium diglutamate (MDG) were studied in human peripheral blood lymphocytes. Chromosomal aberrations assay were studied with sister chromatid exchange, micronucleus assay and comet assay. In these four tests, clastogenic, mutagenic and cytotoxic effects were detected in human peripheral lymphocytes *in vitro* (Avuloğlu-Yılmaz et al., 2020). In another study in 2020, the genotoxicity and cytotoxicity effects of glycerol triacetate (E1518) were investigated. Mitotic index and chromosomal aberrations assay were used in *Allium cepa* root tip cells. As a result, cytotoxic and genotoxic effects of E1518 were observed (Kaya, 2020).

Titanium dioxide (E171) is considered an inert and indigestible substance. It is also used in food packaging, pharmaceutical and cosmetic fields. Biological effects of E171 on germination percentage, root elongation, mitotic index, comet assay and micronucleus were observed in *Lens culinaris* and *Allium cepa*. As a result, it detected dose-related genotoxicity (Bellani et al., 2020). Silver food additive (E174) has recently increased its use in many consumer products, including cosmetics and food packaging. The genotoxic effects of E 174 were analysed using comet (mouse liver, blood, spleen, duodenum and kidney tissues) and micronucleus (mouse spleen lymphocytes) assays. In all tissues tested, no genotoxic or tissue damage was detected in either assay (Narciso et al., 2020). The genotoxic effects of sodium sulfite, boric acid and benzoic acid, which are frequently used in daily meals, were investigated. The genotoxic effect of these three food preservatives was investigated in *Drosophila melanogaster* by SMART and comet assay. All three food additives caused increased tumor induction and frequency in the SMART, and also induced DNA damage in the comet assay (El-Hefny et al., 2021). Potassium sorbate is used as a food preservative. The genotoxic (with chromosomal aberrations and micronucleus assays) and cytotoxic (with MTT test) effects of potassium sorbate and its ability to induce oxidative stress (with superoxide dismutase activity) in human lymphocytes were investigated. As a result, it was determined that potassium sorbate induced cytotoxic and genotoxic effects in human cells and caused oxidative stress (Pongsavee and Mishra, 2021). Flavoring food additives have an important area in the food industry. Merismatic stem cells of *Allium cepa* L. were preferred in order to evaluate the toxicity of the aroma synthetic chocolate additive. As a result, the aroma of chocolate caused cytotoxic, genotoxic and mutagenic effects on root meristems (Frâncica et al., 2021).

Table 1. List of some of the building assessment tools

Food Additivies	Research / Result	References
-Monopotassium Glutamate -Calsium Diglutamate -Monoammonium Glutamate -Magnesium Glutamate	CA / Mitotic Index / MN (*Allium cepa*) + / + / +	Türkoğlu, 2015
-Monosodium Glutamate	CA / MN / SCE In human peripheral lymphocytes + / +/ +	Ataseven et al., 2016
-Allura Red	Comet assay / MN *In vivo* - / -	Bastaki et al., 2017
-Potassium Propionate	MN In human peripheral lymphocytes +	Ataseven et al., 2017
-Sodium Benzoate -Potassium Sorbate	MN In human peripheral lymphocytes +	Mamur et al., 2018
-Sunset Yellow	CA / MN In human peripheral lymphocytes + / +	Haverić et al., 2018
-Stevia Extracts	CA / MN In human peripheral lymphocytes - / -	Uçar et al., 2018
-Potassium Nitrate	SMART / Comet assay Drosophila Melanogaster system + / +	Aledwany et al., 2018
-Sodium Acetate	DNA Fragmentation / MTT Human Umbilical Vein Endothelial Cells(HUVEC) - / -	Mohammadzadeh-Aghdash et al., 2018
-Aspartame	Ames assay / MN *In vivo* - / -	Otabe et al., 2019
-Ascorbic Acid -Benzoic Acid -Citric Acid -Sorbic Acid	MN In human peripheral lymphocytes +	Bogar and Tuylu, 2019
-Methanyl Yellow -Carmoisine	Mitotic Index / CA *Allium cepa* + / +	Khan et al., 2020
-Monopotassium Glutamate -Magnesium Diglutamate	CA / Comet assay / MN / SCE In human peripheral lymphocytes + / + / + / +	Avuloğlu-Yılmaz et al., 2020
-Glycerol Triacetate	CA / Mitotic Index *Allium cepa* root tip cells + / +	Kaya, 2020
-Titanium Dioxide	CA / Mitotic Index / MN *Allium cepa* and *Lens culinaris* + / + / +	Bellani et al., 2020
-Silver	Comet assay / MN *In vivo* - / -	Narciso et al., 2020
-Sodium Sulfite -Boric Acid -Benzoic Acid	Comet assay / SMART Drosophila Melanogaster System + / +	El-Hefny et al., 2021
-Potassium Sorbate	CA / MN İn rat and hamster cells, in human peripheral lymphocytes + / +	Pongsavee and Mishra, 2021
-Flavor Synthetic Chocolate Additive	Mitotic Index Meristematic root cells of *Allium cepa L.* +	Frâncica et al., 2021

*CA, Choromosomal Aberrations Assay; MN, Micronucleus Assay; SMART, Somatic Mutation and Recombination Test; +, Positive genotoxic results; -, Negative genotoxic results.

2. Conclusion

The rapid increase in the world population, environmental pollution, economic imbalance and lack of education negatively affect food problems. This makes it difficult to obtain safe food. Considering the effects of food additives as a whole, most of the commonly used food additives were found to be genotoxic. The purpose of testing the genetic toxicity of ingredients in food additives and other foods are to minimise the health risk to consumers. Genetic damage to somatic or germ cells is associated with harmful health effects such as cancer, hereditary diseases, and degenerative disease states. Even if the food additives that are frequently used in foods are used in amounts that do not harm health, it should be taken into account that food additives may accumulate in the body over time and may be harmful, thus threatening human health directly or indirectly. Therefore, care must be taken in their use and existing rules should be followed.

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Research Article

Anti-diabetic effects of *Berberis cretica* extract in INS-1E cells

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Abstract

Berberine has been used for the adjuvant treatment of type 2 *diabetes mellitus*, hyperlipidemia (high levels of fats), and hypertension (high blood pressure). Also, it has different effects on diarrhea, inflammation, and cancer. Berberine, is a profoundly common compound in Berberis species. Although *Berberis cretica* is one of the Berberis species, it is unknown whether it has anti-diabetic effects yet. Also, synergistic effects of various compounds together with berberin or similar chemical forms of berberine within Berberis species can lead to find new anti-diabetic agents. The aim of this study is to investigate possible drug potential of *Berberis cretica* extract containing berberine and, its potential signaling pathways on Rat Insulinoma (INS-1E) cells. According to our results, *Berberis cretica* extract has anti-apoptotic effects in INS-1E cells decreasing expression p53, p38 and Bax genes. Suppressive effects of *Berberis cretica* plant extracts on apoptotic signalling pathways in β cells show that the extract contents can have a drug potential for treatment of diabetes.

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1. Introduction

Apoptosis is a form of programmed cell death regulated by genes. The Bcl-2 gene is an apoptosis inhibitor, and the Bax gene, an endogenous antagonist of Bcl-2, is an apoptosis

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promoter. The ratio of Bax to Bcl-2 (Bax / Bcl-2) is accepted as an indicator of cell survival or death following an apoptotic stimulus (Oltvai et al., 1993).

Oxidative stress is the formation of cellular damage in the organism as a result of the deterioration of the balance between oxidant and antioxidants in favor of the oxidant system, lipid peroxidation and the release of free reactive oxygen products. When the antioxidant mechanisms of the organism against oxidative stress are insufficient, oxidative damage develops in the cells and functions are disrupted significantly. This mechanism is responsible for the aging process and progression of many diseases such as cardiovascular diseases, cancer, sepsis, degenerative neurological diseases, kidney failure, infertility, muscle and liver diseases (Tabakoğlu and Durgut, 2013).

Lipid metabolism, insulin secretion, inflammatory response, response to oxidative stress, and apoptosis are interrelated cellular processes. Fatty acid (FFA) oxidation is a major metabolic pathway in which fatty acids are catabolized by breaking down into 2-carbon units in the mitochondria, which is very important in meeting energy needs. Increased FFA oxidation has been shown to increase hyperglycemia and the dysfunction associated with diabetes in the sarcoplasmic reticulum. Fatty acids or toxic intermediates released during fatty acid metabolism; It leads to deterioration in mechanical functions, cellular damage, dysfunction of the sarcoplasmic reticulum Ca^{2+} pump, and a decrease in the activities of ATPase and myosin isoenzymes (Onay-Beşikçi and Güner, 2006). Na^{+}-K^{+} ATP'ase activity decreases in the cell and sodium accumulates in the cell. As a result, edema and dysfunction occur in the cell. These changes cause inflammation and necrosis in tissues and initiate apoptosis in cells (Duran-Salgado and Rubio-Guerra, 2014).

Diabetes mellitus (DM) is a chronic disorder depended on the absence or insensitivity of insulin secretion (Altinoz et al., 2015). Type 2 *diabetes mellitus* is a disorder characterized by progressive loss of pancreatic beta cell function and resistance to the effects of insulin in organs such as muscle, fat and liver (Ferranini, 1998). Chronic systemic inflammation is the main reason of vascular complications in DM (Salcini et al., 2016). Berberine has been used for the adjuvant treatment of type 2 *diabetes mellitus*, hyperlipidemia, and hypertension (Lan et al., 2015). Also, it has different effects on diarrhea, inflammation, and cancer (Abrams et al., 2019). It was demonstrated to repressed Nuclear Factor kappa B (NF-κB) and Signal Transducer and Activator of Transcription 3 (STAT3) pathways in cholangiocarcinoma (Puthdee et al., 2017). On the other hand, it was shown that it can influence mitogen-activated

protein kinase (MAPK) signaling pathways (Li, et al., 2016). These studies support to its anti-diabetic potential. Berberine, is a profoundly common compound in Berberis species. Although *Berberis cretic*a is one of the Berberis species, it is unknown whether it has anti-diabetic effects. Also, synergistic effects of various compounds together with berberin or similar chemical forms of berberine within Berberis species can lead to find new anti-diabetic agents. In view of the above lines of evidence, the aim of the study was to investigate effects of *Berberis cretica* extract on insulin secretion and apoptotic signaling pathways in INS-1E cells.

2. Materials and Methods

2.1. Preparation of B. cretica Extracts

Plants have been collected from the West Taurus Mountains at a height of 1500-1700 meters (Between Gevenalanı Plateau and Karumca Saddle) located on the north of Seydikemer district of Muğla Province, Turkey. Identification of species was done by Professor Emine Akalın Uruşak, from Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Botany. Roots of *B. cretica* were used for the extract preparation. Roots were air-dried at room temperature. Dried roots were extracted using methanol in a Soxhlet extractor.

2.2. Cell lines and Culture Conditions

INS-1E cells (a rat insulinoma cell line) were a kind gift from Professor Claes B. Wollheim from University of Geneva Medical Center, Switzerland. Cells were cultured in RPMI 1640 medium supplemented with 10 mM HEPES, $5x10^{-5}$ M ß-mercaptoethanol, 1 mM sodium pyruvate, 100 IU/mL penicillin and 100 µg/mL streptomycin. Cells were passed weekly by gentle trypsinization, 0.25% tripsin-EDTA. Experiments were carried out using cells between passages 2 and 9.

2.3. Real-time PCR

Cells were incubated with an extract concentration of 10 µg/mL for 24 hours and RNA was isolated subsequently. Controls were only cells without extract. After RNA isolation, cDNA

synthesis was carried by using SensiFAST cDNA Synthesis Kit according to their manufacturer's instructions. Gene expression levels of p53, p38, Bax, and Bcl-2 were measured by using FastStart DNA Master SYBR Green I kit as stated in the manufacturer's instructions. The reverse primer 5' CCAGCCCATGATGGTTCTGAT 3' and the forward primer 5' CCCGAGAGGTCTTTTTCCGAG 3' were used to determine changes of Bax gene expression levels. For Bcl-2 gene, the reverse primer was 5' CGGTTCAGGTACTCAGTCATCC 3', and forward primer was 5'GGTGGGGTCATGTGTGTGG3'. For p38 gene, reverse primer was 5' CTGTAAGCTTCTGACATTTC 3', and forward primer was 5' GTGCCCGAGCGTTACCAGACC 3'. Also, the reverse primer 5' AGCTTCAAGAGCGACAAGTTTT 3', and forward primer 5' AACTGCGGGACGAGACAGA 3' were used to measure Bax gene expression levels (Kigili et al., 2019).

2.4. Real-time PCR

Insulin ELISA kit (ThermoFisher Scientific, Cat. No: ERINS) was used to analyze secretion of insulin from INS-1E cells incubated with *B. cretica* root extracts according to the manufacturer's instructions.

3. Results and Discussion

It is known that the aqueous extract of the roots of Berberis species is used as an anti-diabetic, and the cure obtained from the roots is used as a wound healer among the people (Durmuş et al., 2016). To further investigate the effects of *B. cretica* root extracts on insulin secretion of INS-1E cells, insulin protein levels were measured. A significant difference in insulin secretion was not observed after the extract treatment on INS-1E cells (Figure 1).

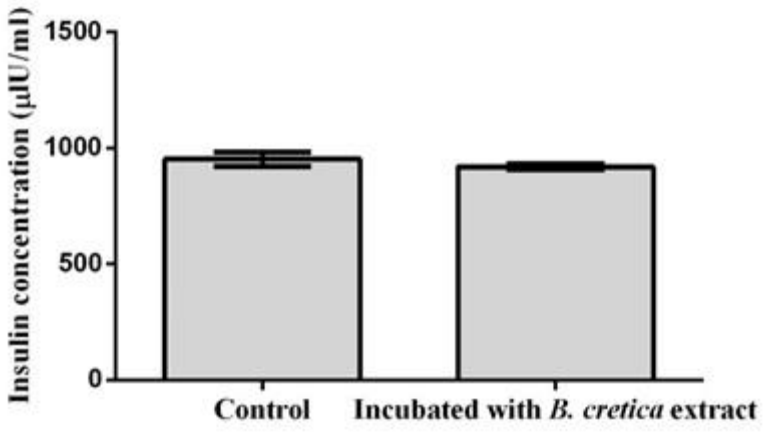

Figure 1. Insulin concentrations of control and *B. cretica* treated INS1-E cells are shown as bar graph.

In vivo and *in vitro* studies with *B. cretica*, which has been used in traditional medicine for many years, have shown that the root of *B. cretica* contains high levels of berberine and has anti-bacterial and anti-tumor activities (Alemardan et al., 2013). In a study with MCF-7 breast cancer and M4A4 metastatic breast cancer cell lines, berberine-chloride was reported to have an anti-cancer effect. In the same study, anti-bacterial properties of berberine were also demonstrated against various bacterial strains (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Enterococcus faecalis*) that are the source of common bacterial infections (Altundağ et al., 2020). Chu et al. (2016) showed that berberine administration in the presence of a caspase-1 inhibitor can reduce the viability and invasiveness of cancer cells by inducing apoptosis in liver cancer cells.

In addition to its anti-cancer properties, berberine is known to have high antioxidant properties (Kukula-Koch et al., 2013). Berberine has been shown to alleviate oxidative stress by increasing enzymatic and non-enzymatic anti-oxidant levels (İleritürk et al., 2021).

To understand anti-apoptotic effects of the extract, gene expression levels of common apoptotic and anti-apoptotic proteins for type 1 and type 2 diabetes after *B. cretica* root extract treatment were analyzed. According to the results, while *B. cretica* root extract significantly decreased expression levels of p53, Bax, and p38 genes, it did not change anti-apoptotic Bcl-2 gene expression (Figure 2). Following *B. cretica* incubation, p53 (4-fold), Bax (2-fold), and p38 (2-fold) gene expressions were reduced in INS-1E cells. This result implies that *B. cretica* root extracts can have diminishing effects on p53-dependent apoptotic

activity. Fatty acid (FFA) oxidation activates p53/Bax-mediated mitochondrial apoptosis (Li et al., 2015) Therefore, it's the 4-fold decreasing effect on p53 and Bax genes expression can suppress FFA-dependent p53/Bax-mediated mitochondrial apoptosis in INS-1E cells.

Reactive oxygen species (ROS) may activate Jnk/P38 in β-cells increasing insulin receptor substrate 2 (IRS-2) serine/threonine phosphorylation, and its degradation. Suppression of IRS-2 signaling, can cause β- cell apoptosis as well as insulin resistance (Rhodes, 2005). In our study, *B. cretica* root extract is found to reduce p38 gene activity by 2-fold in INS-1E cells (Figure 2). Therefore, contents of the extract have a potential for preventing p38 mediated IRS2 degradation.

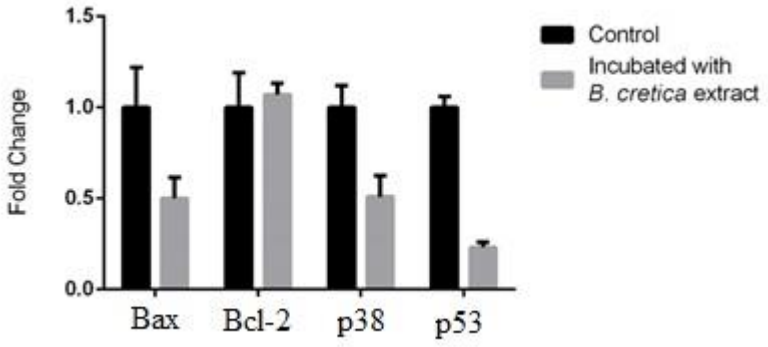

Figure 2. p38, Bax, p53, and Bcl-2 gene expression levels in control and *B. cretica* treated INS1-E cells.

Although the extract did not change Bcl-2 gene expression, its suppressive effects on expressions of apoptotic genes can lead to indirectly Bcl-2 activation on INS-1E cells. The apoptotic gene expressions results indicate that the extract can contain the molecules interacting with these pathways. It is known that berberine is a major compound of the extract. However, the extract also may have some new agents except from berberine and synergistic and antagonistic effects of their combinations can induce the differences in the apoptotic gene expressions.

4. Conclusion

Suppressive effects of *B. cretica* plant extracts on apoptotic signaling pathways in β cells show that the extract contents can have a drug potential for treatment of diabetes. Further

isolation of the molecules from the extract and investigation of their specific activity can give rise to identification of new drug molecules towards treatment of this disease.

Acknowledgements

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Research Article

Theoretical calculation of some chemical properties of the cannabidiol (CBD) molecule

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Abstract

Lately, many products with the active ingredient cannabidiol (CBD) have been sold around the world. Cannabidiol is an annual herb of the cannabis plant that is known to have no euphoric effects. Another type of terpenophenolic compound known as cannabinoids is also preserved in the structure of the cannabis plant. Products containing CBD are offered on the market as drugs, food supplements or dietary supplements. The US Food and Drug Administration FDA (Food and Drug Administration) has approved the oral solution of Epidiolex (cannabidiol) [CBD] for the treatment of epilepsy in patients aged two years and over. The legal assessment of commercial products containing cannabidiol (CBD) depends on the composition of the drugs available in pharmacies. Depending on whether the drugs also contain tetrahydrocannabinol (THC) in their structure in addition to CBD, criminal narcotics convictions are also decisive. In our study, some parameters related to the chemical structure of the cannabidiol (CBD) molecule were calculated using the Gaussian 09W program and the GaussView 5.0 interface program.

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Keywords

Cannabidiol,
Chemical reactivity,
FMOs

1. Introduction

It is believed that cannabinoids have an effect on epilepsy because they act on more than one nerve-to-brain transmission channel and are substances that can influence the receptor mechanism. In addition, studies are being conducted on the versatile biological effects of cannabidiol in various clinical applications, including its antioxidant and anti-inflammatory effects.

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Research on cannabidiol has increased lately. Under the keyword cannabidiol from 1963 to 2021, the PubMed search found 4107 publications and between 2000 and 2021,3666 publications. First isolated from cannabis in 1940, the structure shown in Figure 1 was not described until 1963 (Mechoulam and Shvo, 1963).

Studies on Cannibas have brought THC (tetrahydrocannabinol), the compound of Cannibas, to the fore. Later scientific studies show that another Cannibas compound, CBD, was ignored. This was undeniably due to the belief that this was due to the psychoactive property revealed by THC rather than CBD. For cannabidiol (CBD), this is unfortunate, as a number of studies on the potential therapeutic benefits of CBD have long been ignored (Burstein, 2015).

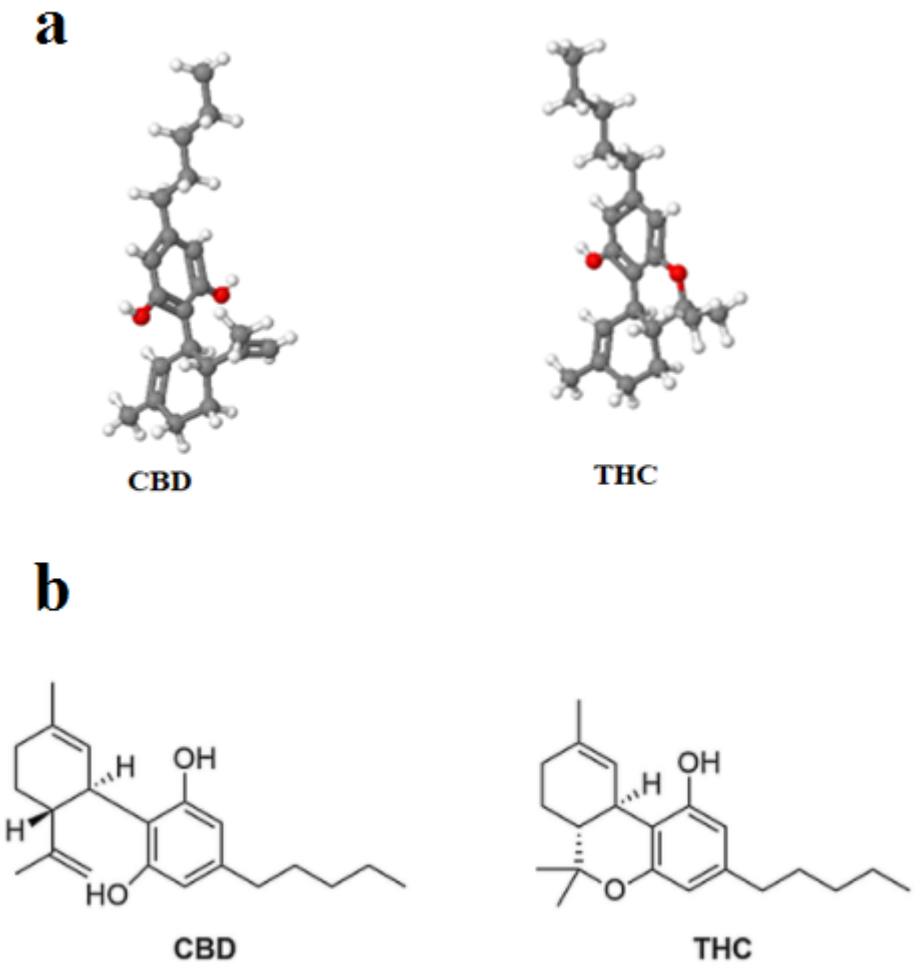

Figure 1. a. The molecular structure and b. chemical diagrams of CBD and D9-tetrahydrocannabinol (THC) (Kosović et al., 2021)

The D9 tetrahydrocannabinol molecule has a highly planar shape in space, while the cannabidiol molecule has a convoluted shape. The difference between these two molecules

when viewed in two dimensions in 1-B results in different pharmacological structures, although there is considerable structural overlap in both molecules.

2. Cannabinoid Receptors and Their Medical Legal Uses

Natural cannabis is found in the structure of the cannabis plant (*Cannabis sativa*) and its effect on cannabinoid receptors known as CB1, CB2 is known in the scientific community (Artuç et al., 2014). In 1964 Gaoni and Mechoulam discovered the chemical components of tetrahydrocannabinol (tetrahydrocannabinol-THC), the main component of the euphoric activity of cannabis (Burstein, 2015). Among the cannabinoid receptors, the CB1 receptor was discovered in 1988 and the CB2 receptor in 1993, and then endocannabinoids (anandamide, 2-arachidonylglycerol) and enzymes that synthesize and break down endocannabinoids (FAAH and MGL) were found (Devane et al., 1988; Munro et al., 1993). After all these studies, interest in the therapeutic use of cannabis has increased and there is an increasing demand for its use in clinical treatments. In 1996 a law was passed in the United States of America that allows the use of medicinal cannabis.

Today, the cannabis license has been legalized in California, Nevada, Massachusetts, and Colorado, as well as in 29 other states in the United States. Recently, many European countries have passed laws on the legal use of cannabis in the medical field. Doctors and scientists also see the legalization of cannabis in the medical field positively. Canada is one of the first countries to conduct legal studies on cannabis liberalization in the medical field and introduced the medical cannabis use license in 2001 (Ulugöl, 2018). In a study carried out in the Canadian state in 2019, it was found that doctors and pharmacists rate the medical use of cannabis positively and, on the basis of personal experience, also cause a lower rate of side effects compared to many treatments (Elias et al., 2019).

3. Components of Cannabis

In addition to the 420 cannabinoids in cannabis, the main active ingredients are the psychoactive compound Δ^9-THC (Figure 1), cannabinol (CBN) (Figure 2), which is less effective (10% percent) than Δ^9-THC, and, the itself Cannabidiol (Figure 1) (CBD) is not a psychoactive substance, which can modify the effects of Δ^9-THC and cannabinol.

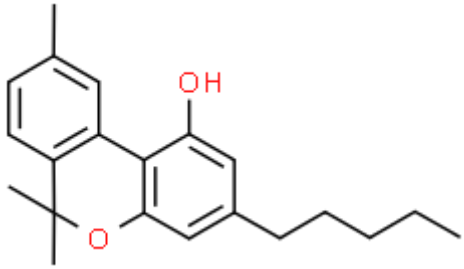

Figure 2. Chemical structure of cannabinol (CNB) (ChemSpider, 2021)

4. Chemical Structure of Cannabinol

The chemical structure of cannabis is extremely complicated, but its structure is well elucidated by today's technology (Lehmann, 1995). The cannabis plant produces different amounts of resin with different concentrations of active ingredients in female inflorescences. Chemical studies on the cannabis plant began in the 19th century. But it was a little late that its structure was finally clarified until 1964 the correct chemical structure of THC was investigated by Gaoni and Mechoulam (Table 1) (Gaoni and Mechoulam, 1964).

Table 1. Chemical and physical data of cannabinol (CBD) molecule (World Health Organization, 2018)

Cannabidiol (CBD)	
CAS:	13956-29-1
Empirical formula:	$C_{21}H_{30}O_2$
Molecular weight:	314.46 g/mol
Melting point	66–67 °C
log P	5.79 (octanol/ water)
Solubilities:	
Water	insoluble
Ethanol	soluble
Chloroform	soluble
Hexane	soluble

Recent research shows that cannabinoids can be counted among the beneficial sources of antioxidants. The CBD structure contains free oxygen atoms. This oxygen atom can bind free radicals and neutralize these radicals. Most likely, this oxygen atom gives CBD its antioxidant activity and can help fight free radicals (Borges et al., 2013).

5. The Medicinal Uses of CBD

Cannabidiol has found the following uses in medicine:

- Epilepsy: especially in certain genetic forms of epilepsy.
- Anxiety Disorders.
- Schizophrenic psychoses.
- Inflammation and pain due to inflammation.
- Movement disorders: dystonia, dyskinesia.
- Substance abuse.
- Nausea and vomiting.

In addition, there is evidence that it can be used in different areas of application, for example in the repair of bones, skin diseases, allergic diseases and the minimization of the inverse effects of the cytostatic agent doxorubicin. Many of these listed properties have hardly been explored for a long time. The effect of cannabidiol (CBD) is based on an antagonism on the CB1 receptor, which binds to the receptor on the vanilloid receptor type 1 (TrpV1).It depends on various effect systems, including the effects it creates.CBD has also been shown to facilitate neurotransmission mediated by the serotonin receptor 5-HT1A (De Gregorio et al., 2019).

6. Materials and Methods

The geometry parameters of the title compound were optimized using the polarized triple zeta split valence 6-311++G (d, p) basis set and the Becke3LYP (Figure 3) functional of density functional theory (DFT). All calculations made in the examination of this molecule were carried out in the Gaussian 09W package program (Frisch et al., 2009). Mulliken Charges and boundary orbitals and boundary orbitals (HOMO-LUMO) distribution and their energy values of CNB levels calculations were performed with the help of GaussView 5.0 imaging program (Roy et al., 2009).

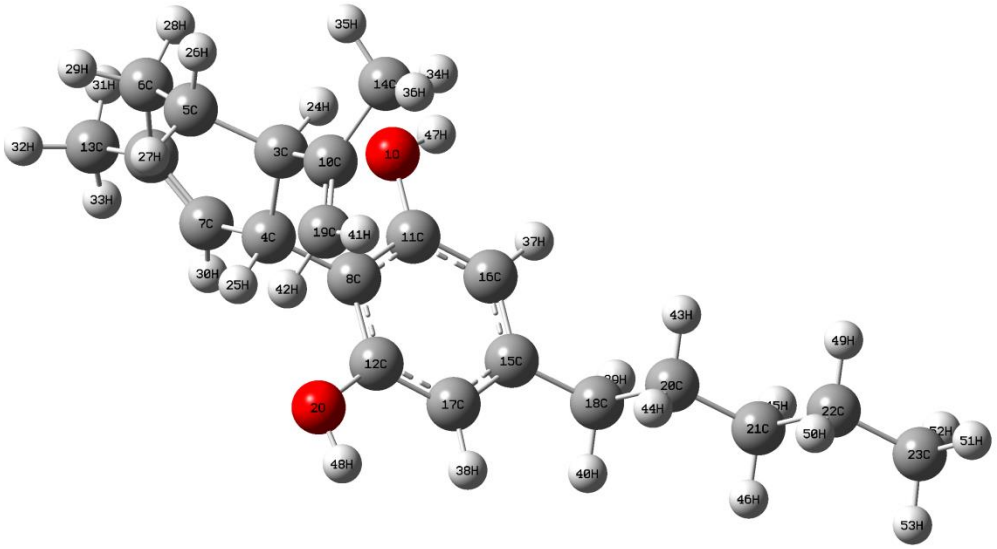

Figure 3. Optimized molecular geometry of cannabinol (CNB) optimized with B3LYP function and the 6–311 + + G (d, p) basis set.

7. Results

The optimized structure, theoretical parameters, boundary molecular orbitals, molecular electrostatic potential value of the title molecule were chosen as the basic set with the help of the DFT method / B3LYP function in the package program Gaussian 09 W and 6-311 + + G (d, p) (Table 2).

Table 2. Calculated bond lengths and angles of cannabinol (CNB) molecule

Parameters	Calculated	Parameters	Calculated
Bond lengths (Å)			
O1-H47	0.962	**C3-H24**	1.093
O2-H48	0.962	**C5-H27**	1.512
C3-C4	1.550	**C5-H26**	1.541
C3-C5	1.545	**C6-H28**	1.532
C5-C6	1.531	**C6-H29**	1.533
C6-C9	1.510	**C13-H31**	1.097
C9-C7	1.335	**C13-H32**	1.096
C7-C4	1.520	**C13-H33**	1.092
C9-C13	1.505	**C14-H34**	1.096
C3-C10	1.522	**C14-H35**	1.096
C10-C14	1.509	**C14-H36**	1.091
C10-C19	1.334	**C19-H41**	1.085
O1-C11	1.372	**C19-H42**	1.083
O2-C12	1.373	**C17-H38**	1.087
C8-C11	1.403	**C18-H39**	1.095
C8-H12	1.403	**C18-H40**	1.095
C12-C17	1.396	**C20-H43**	1.096
C17-C15	1.393	**C20-H44**	1.096
C15-C16	1.394	**C21-H45**	1.098
C16-C11	1.396	**C21-H46**	1.098
C4-C8	1.525	**C23-H53**	1.094
C15-C18	1.512	**C23-H51**	1.093
C18-C20	1.541	**C23-H52**	1.095
C20-C21	1.532	**C22-H49**	1.096
C21-C22	1.533	**C22-H50**	1.096
C22-C23	1.531	**C4-C25**	1.093
C7-H30	1.087		
Bond angles (^{0})			
C5-C6-C9	112.7	**C11-C16-C15**	120.7
C6-C9-C7	121.3	**C16-C15-C17**	118.1
C9-C7-C4	125.4	**C15-C17-C12**	120.5
C7-C4-C3	111.3	**C17-C12-C8**	122.4
C7-C4-C8	111.1	**C21-C22-C23**	113.2
C4-C8-C11	123.1	**H38-C17-C15**	119.9
C4-C3-C10	115.3	**H38-C17-C12**	119.4
O1-C11-C8	117.4	**O1-C11-C16**	120.2
H47-O1-C11	109.1	**O2-C12-C8**	117.3
H48-O2-C12	109.2	**O2-C12-C17**	120.2
C8-C11-C16	122.2	**H43-C20-H44**	106.0

7.1. HOMO-LUMO Analysis

The energy levels of a neutral cannabinol (CNB) molecule, the highest occupied molecular orbital (HOMO), and the lowest unoccupied molecular orbital (LUMO), play an important role in maintaining the properties of molecular structures.

From the HOMO-LUMO energy data and diagrams, we can obtain information about the chemical activity and the kinetic stability of the CNB molecule under study. HOMO is in electron donating orientation, LUMO is in electron accepting orientation and these orbitals can be used to calculate charge transfer. Here, the HOMOs and LUMOs diagrams and energy values of the CNB molecule are given in Figure 4 and calculated parameters in Table 3.

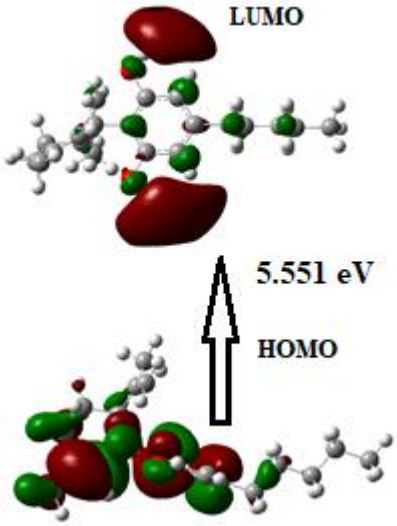

Figure 4. For cannabinol (CNB) according to the B3LYP / 6-311 ++ G (d, p) method, difference between calculated ΔE (E_{HOMO}-E_{LUMO})

Table 3. Calculated parameters of cannabinol (CBD)

Parameters	**Method** [B3LYP / 6-311 ++ G (d, p)]
E_{HOMO} (eV)	-6.027
E_{LUMO} (eV)	-0.476
ΔE (eV)	5.551
Dipol Moment (Debye)	2.5792
Energy of CBD (kcal/mol)	$-6.080 \cdot 10^5$

The DFT calculation approach shows that HOMO and LUMO for the compound examined correspond to the π and π * orbitals of the benzene ring (Figure 1). Due to their delocalization, these orbitals are mostly distributed over the adjacent double bond areas of the compound. Every HOMO energy calculation of the compound is consistent with previous publications (Kumer et al., 2019).

7.2. Molecular Electrostatic Potential (MEP)

The molecular electrostatic potential (MEP) can be defined as the interaction energy of the positive test charge and the charge distribution in the molecular system. The color coding system is used to interpret the molecular electrostatic potential. On the MEP map, the most negative potential (the area in which the entire surface of the molecule is electron-dense compared to the nucleus) is colored red, while the positive potential (the area in which partial positive charges are concentrated) is shown in blue (Cramer, 2004). The intermolecular interaction plays a key role in MEP, where the positions of the molecules are close together. Where a molecule is most negative on the MEP map indicates areas most susceptible to electrophilic attack (Levine, 2000). The MEP map of cannabidiol (CBD molecule is shown in Figure 5). The negative region of the molecule resides on the carbon atoms on the phenolic ring and on the oxygen atoms on the phenolic ring, while these areas are considered the most suitable areas for electrophilic attack. The arrangement of the positive areas can be seen around the methyl groups and hydrogen atoms (Figure 5).

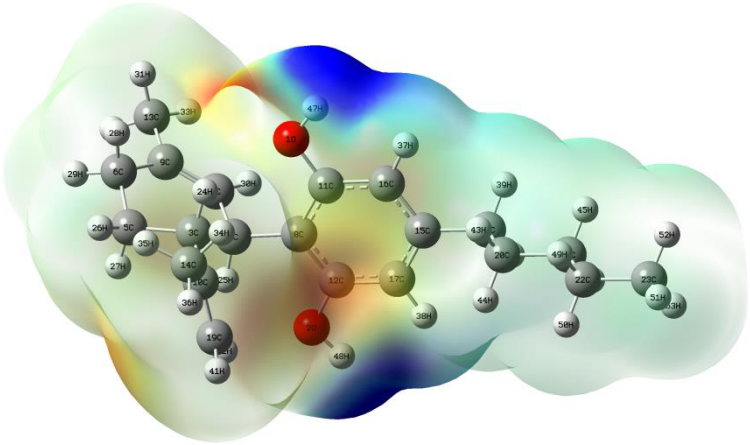

Figure 5. Molecular electrostatic potential (MEP) surface of the cannabidiol (CBD) molecule

7.3. Mulliken Atomic Charges of Cannabinol (CBD)

Molecular structures are an important parameter in the application area of calculations based on quantum chemical methods. While the charge transfer occurs via the distribution of the atomic charges, we can obtain information about the dipole moment and the electronic configuration of the molecular structure in the donor and acceptor areas of the molecule.

The Mulliken charge distributions of the molecule were calculated in the gas phase using the DFT / B3LYP / 6-31 ++ G (d, p) method (Table 4).

Table 4. Mulliken Atomic Charges of Cannabinol (CBD)

Atoms	Mulliken Charge	Atoms	Mulliken Charge
O1	-0.16	C13	-0.47
O2	-0.19	C14	-0.61
C3	-0.01	C15	0.96
C4	0.82	C16	-0.72
C5	-0.27	C17	-0.61
C6	-0.84	C18	-0.28
C7	-0.34	C19	-0.84
C8	0.61	C20	-0.32
C9	0.03	C21	-0.24
C10	0.39	C22	-0.16
C11	-0.32	C23	-0.64
C12	-0.48	H24	0.22

If you look at Table 4, when you look at the given charge distributions, you can see that the electronegativity of the negative charges from the gas phase is mainly concentrated on the carbon atoms on the benzene ring and on the O1 and O2 oxygen atoms, which are also bound to the benzene ring. The different values of the charge distribution between the atoms are an indicator of the polarization of the molecule.

7.4. Theoretical Raman Spectrum of Cannabinol (CBD)

The data of the vibrational movements corresponding to the peak points obtained from the simulations of the Raman spectra for the cannabidiol (CBD) molecule were carried out with the analysis program GaussView, which can calculate the Raman spectra. A simulated spectrum was obtained from the Raman frequencies calculated by this program, and it was possible to predict the vibrational motions representing each Raman peak. In addition, randomly selected peaks and various publications that may give characteristic frequencies of the molecule under study were examined to confirm the accuracy of the GaussView program. Table 5 shows the characteristic frequencies selected for the cannabidiol (CBD) molecule.

Table 5. Characteristic Raman frequencies of the calculated Cannabinol (CBD) molecule

Molecular Vibration	Raman Shift(cm-1)
Benzene Breathing/ C-C Stretch	1624.5
C=C Stretch (ring)	1655.45
C=C Stretch (alkene)	1704.0
O-H Stretch	3836.04, 3841.93
C-H Deformation	3065.3, 3037.09, 3129.79

4. Conclusion

With the B3LYP function and the 6–311 + + G (d, p) basis set, quantum chemical calculations of the calculation results of the optimized structure, such as boundary molecular orbitals, molecular electrostatic potential and nonlinear properties, were carried out (Figure 6).

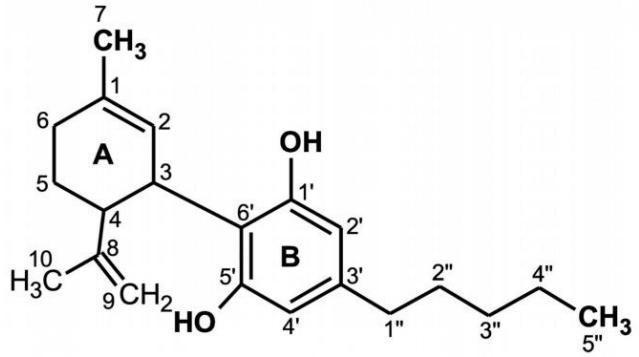

Figure 6. Numbered atoms of the cannabidiol (CBD) molecule (Atalay et.al, 2020).

Cannabinol (CNB) molecule has two rings as phenol and cyclohexene (Figure 5). The chemical activity of cannabinol (CNB) can generally be due to the attachment of hydroxyl groups to the C-1 'and C-5' atoms of the phenolic B ring and similarly the methyl group to the C-1 atom of cyclohexene-A-Ring. The attachment of the pentyl chain to the C-3 'atom of the B ring can also contribute to the reaction activity of the molecule under consideration. However, the cyclohexene ring in the Cannabinol (CNB) molecule is inactive because it is an open ring from the position of the C-4 atom. Due to the presence of hydroxyl groups attached to the C-1′ and C-5′ atoms in the B ring of the cannabinol (CNB) molecule, it can bind to amino acids such as threonine, tyrosine, glutamic acid or glutamine via a hydrogen bond (Elmes et al., 2015).

Table 6. Comparison of Raman frequencies of Cannabinol (CNB) molecule from different calculated studies

Vibrational Motion	[a]Raman Shift (**cm^{-1}**) CBD	Raman Shift (**cm^{-1}**) CBD
Benzene Breathing /C-C Stretch	1605.8	1624.5
C=C Stretch	1674.2	1655.45
O-H Stretch	3719.4, 3724.5	3836.04, 3841.93
C-H Deformation	2921.2, 3038.5, 3119.4	3065.3, 3037.09, 3129.79

[a](Sigworth, 2020)

The C = C stretch for CBD at 1655.45 cm^{-1} is consistent with previous studies. It is important to determine the characteristic frequencies for CBD with Raman and to compare Raman data from the analysis of other cannabinoids.

The cannabidiol molecule is a difficult molecule to optimize because an alkene is very close to the phenol in its structure. Many optimization attempts either fail or lead to the removal of a phenol group from the cannabidiol molecule, which leads to a free water molecule, which also leads to the destruction of the terminal alkene. Optimizing the molecule resulted in an extremely strong repulsion between the terpene ring and both phenolic groups of CBD, as confirmed in previous studies. There are fewer resources for characterizing CBD vibrational modes. However, although many of the modes have been explored for the D-9 THC molecule, these modes agree well with the modes characterized for the CBD molecule. The GaussView program was used to calculate these modes (Sigworth, 2020).

Some Raman vibration values for the CBD Molecule in Table 6, it is compared to another study of this molecule in the literature. Although there are few studies on this molecule, this is a downside to publication because it is a not fully explored molecule, there is a large area of study for scientists.

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