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ACADEMIC RESEARCH
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CONTENTS

Foreword

Throughout history, human being has seen the world as an inexhaustible resource. Especially after the industrial revolution, mankind went into a consumption frenzy and all resources were ruthlessly plundered for the sake of development, until the balance of the world was disturbed. After these warnings, which are seen as climate change, various environmental disasters and diseases, development models that are compatible with the world started to emerge. The most important of these is the sustainable development model. According to this model, any invention or development should be in harmony with the environment, society and economy. Exactly related to this issue, studies that gave us hope were presented at the "3rd International Academic Researches for Sustainability 2021" conference held with the support of Istanbul University-Cerrahpaşa. I am delighted to be the editor of this book, which presents some of these studies. I would like to express my sincere gratitude's to all my colleagues who contributed. I hope that the book will be a useful resource for anyone who is interested in the subject of sustainability and does research on this subject.

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1

THE RELATIONSHIP BETWEEN SUSTAINABILITY AND EDUCATION IN DEVELOPMENT PLANS IN TURKEY

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Abstract

The aim of this study is to examine the development plans implemented in Turkey within the framework of relations in the context of sustainability and education. The study employed qualitative research method and content analysis. According to the research findings, the concept of sustainability was included since the 7th Development Plan (1996-2000). The concepts of sustainability and education were associated in 16 places within the social dimension in the development plans. In the conclusion part of the research, suggestions are presented depending on the results of the content analysis.

***Keywords*:** *Sustainability, education, development plans*

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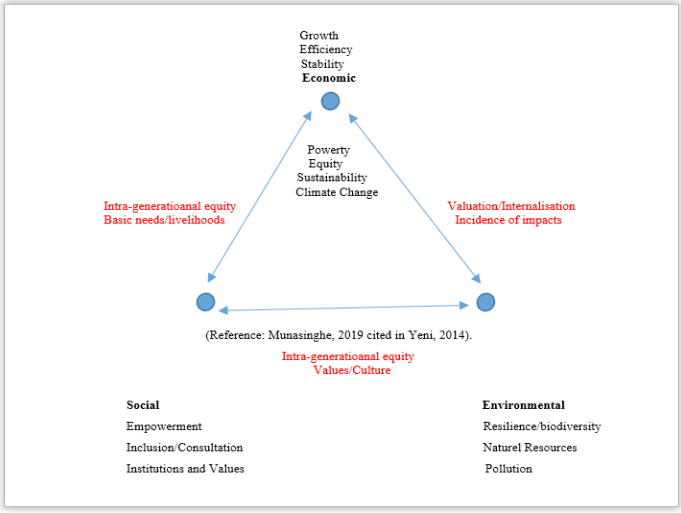
Introduction

In this study, the concept of sustainability was examined by revealing the sections in which it is associated with education. Today, the negative effects of development in the world are at the forefront. Societies are trying to minimize the damage that development causes to society and the environment, while creating a welfare society. In the literature part of this study, the concept of development, the emergence of sustainable development, the development plans implemented in Turkey, and how development and education are related in these plans are mentioned.

Context

Development can be considered as an important concept in shaping the future of societies (Yavilioğlu, 2002) in economic, social and environmental areas (Akgül, 2010). Since the concept includes different fields, it also affects education (Karakütük, 2019). Since 1963, 11 development plans were implemented in Turkey. The books "Sustainable Society" and "First Steps Toward a Sustainable Society: Repairs, Reuse, Recycling" written by Dennis Hayers are important in terms of explaining the sustainabale development as a concept (Karadayı, 2015). Also, World Environment and Development Commission Report (Brundtland Report) mentioned the concept for the first time in 1987. Sustainable development was defined as "meeting the needs of the present without compromising the ability of future generations to meet their own needs" (Marshall, 2009, p. 705). However, sustainable development is multidimensional. These dimensions can be seen in Figure 1.

Figure 1. Dimensions of Sustainable Development



(Reference: Munasinghe, 2019 cited in Yeni, 2014).

According to Figure 1, sustainable development can affect every area of concern to society. In addition, it can be understood from Figure 1 that social, economic and environmental dimensions should be considered together for the development of society. Within the framework of sustainable development, students are expected to participate in management. It is also important for students to be aware of environmental and economic values for the development of society. It is expected that the practices in the school will be implemented with a lifelong learning approach. Moreover, the family and the other environment will participate in education as well as the student, and an approach that takes into account universal values such as respect and love is expected. (United Nations Educational, Scientific and Cultural Organization [UNESCO], 2021).

In addition, the 4th goal, which is one of the goals set for sustainable development 2030 by the United Nations, is expressed as "qualified education" (United Nations [UN], 2016). Therefore, there is an important and undeniable link between education and sustainable development in the context of raising qualified people in the above-mentioned areas (Jikling & Wals, 2008). In addition, education provides the advantage of financial relaxation and benefiting from technology by providing social and ecological balance (Mochizuki, 2016).

Purpose

The purpose of this study is to examine the sustainability-education relationship in the context of the development plans made in Turkey (1st-11th development plans).

Method

In this study, qualitative research method was used. The aim of qualitative research is to express the new meanings of the research in a realistic way in a deep and detailed way (Merriam, 2015, p. 14; Patton, 2014, p. 14; Yıldırım, Şimşek, 2011, p. 48). In the study, all of the development plans (1.-11. development plans), which are available on the Presidency Strategy and Budget Presidency website (www.sbb.gov.tr) and whose full texts can be accessed were used as documents in order to gain new meanings in the context of sustainability-education. One of the data sources in qualitative research is official reports (Patton, 2014, p. 4; Christensen et al., 2015, p. 62). Content analysis is a type of analysis that includes the effort to systematically derive new meanings from existing documents and publications (Bilgin, 2014, p.1; Gökçe, 2019, p. 33; Krippendorff, 2004, p. 18). The content analysis performed in the study is shown in tables 1 and 2.

Findings

The concept of sustainability was included in development plans starting from the 7th five-year development plan. The inclusion of the concept in the plans is shown in table 1.

Table 1. The Concept of Sustainability in Development Plans

Development Plan (DP)	Period	Number of Articles/ Pages	Number of Articles/ Statements Containing the Concept of Sustainability
7	1996-2000	307	28
8	2001-2005	2088	42
9	2007-2013	738	36
10	2014-2018	1131	83
11	2019-2023	845	86

According to table 1, the concept of sustainability affected the plans made in the last 25 years. The concept of sustainability has economic, environmental and social dimensions. In the social dimension of the development plans, the concept Sustainability and Education (S/E) are evaluated together in a total of 16 places. This relationship is shown in Table 2.

Table 2. The Relationship between S/E in Development Plans in Turkey

Development Plans (DP)	Page/Article no on Sustainability-Education
7	Sayfa 19, 20, 22, 34, 36
8	640
9	581, 616
10	179, 625, 627, 1014
11	421, 842, 843, 843.1

According to Table 2, the concept is connected with education and included in the plans. However, the association with the concept of sustainability in the education dimension, as in the general of the plans, is new. Moreover, in the last development plan, the special handling of sustainability as a separate title -sustainable development goals, articles 842-845- can be considered as an indication that the relationship of the concept with all areas affecting the society is given importance.

S/E Relationship in the 7th Development Plan

In the 7th Development Plan, the relationship between sustainability and education was discussed in 5 places. It was observed that in the relevant plan, it was observed that the issue is tried to be clarified with policies that will be formed in the development of the country and that care about cooperation with different fields. This understanding emerged as "…the policies to be implemented in every field will be developed in a way that will respond to these aspirations, provide a structural transformation that increases productivity, and in a sustainable structure in the medium term without departing from economic realism" (TCCSBB, 2021, p. 19). In addition, it is seen that the importance of infrastructure, and especially the human element in terms of education, is given importance as "Policies related to physical infrastructure, manpower infrastructure and institution-

al infrastructure will be implemented in a way that will develop a sustainable growth environment" (TCCSBB, 2021, p. 20). The need and justification for the sustainability-education relationship is stated as "Turkey needs to implement serious Structural Change Projects in its economic and social life in order to maintain a sustainable growth environment and to reach the information society that emerged with the radical change and transformation process in production norms". On the other hand, education is mentioned in almost every area of development: "Rapid population growth prevents individuals from getting a larger share of the increase in welfare and the rapid realization of the economic change process, complicates sustainable development efforts, and increases the need for housing, health, education and infrastructure" (TCCSBB, 2021, p. 34). In these statements, it is understood that it is emphasized that a structural change should be experienced in all sectors in terms of the development of Turkey and that preparations should be made for these changes. While doing this, it is seen that the basic principle can be seen as follows: "Developing the human resources necessary for development, slowing down the population growth rate and creating a population structure consistent with sustainable development goals and policies are the basic principles" (TCCSBB, 2021, p. 36).

S/E Relationship in the 8th Development Plan

In the 8th Development Plan, the sustainability-education relationship is defined as a way to increase the quality of the population in various aspects and to increase the living standards by taking into account the whole society. improving the quality of life, increasing the quality of life and eliminating the differences between regions and settlements in these areas are the basic principles (TCCSBB, 2021).

S/E Relationship in the 9th Development Plan

In the 9th development plan, the sustainability-education relationship is revealed by drawing attention to the social dimension of human developments. It was observed that the fact that education is the main and priority service area was reflected in the policies to be followed. This understanding is stated in article 581 (TCCSBB, 2021). Considered as a basic service area, education has been given importance, especially its contribution to the economy. This was stated in article 616 (TCCSBB, 2021).

S/E Relationship in the 10th Development Plan

In the 10th development plan, the sustainability-education relationship is associated with medical education in article 179, with "...qualified medical education that will ensure the financial sustainability of hospitals..." (TCCSBB, 2021). In article 625, the necessity of increasing research and development capacities and meeting the researcher human needs is emphasized (TCCSBB, 2021). On the other hand, there is a need to provide qualified support to researchers and increase their employment in the private sector. In article 627, the establishment of a sustainable structure in universities for qualified manpower, which can be stated as a continuation of this approach (TCCSBB, 2021). Finally, in article 1014, the importance of the sustainability of the contribution of education to the economic structure is expressed (TCCSBB, 2021).

S/E Relationship in the 11th Development Plan

In the 11th development plan, the relationship between sustainability and education is expressed in connection with various fields in article 421: "Educational infrastructure will be strengthened in order to strengthen the defense industry ecosystem, ensure sustainability and meet the need for qualified manpower". The distinguishing aspect of the 11th development plan in terms of sustainability-education from other plans is the expression of the subject in a separate section, title. There are evaluations on sustainable development in 4 main and 3 sub-items in the plan (pp. 190-191). It is understood that the main purpose of these articles is to reflect the understanding of sustainability to all areas and to put forward the necessity of implementing the understanding of following the studies and reviewing them when necessary. The purpose of sustainable development is emphasized in article 842 and how this approach will be reflected is stated in article 843(TCCSBB, 2021).

Conclusion, Discussion and Recommendations

The following results were obtained in the research on the sustainability-education relationship, which are two important elements in development: *(i)* The concept of sustainability has been taken into account in planning studies, especially since the 7th Development Plan. *(ii)*The concept basically has an economic, environmental and social dimension. *(iii)*One of the fields that make up the social dimension is education. *(iv)* Sustainability-education relationship seems to take place in 16 places especially since the 7th Development Plan.*(v)* Although

the sustainability-education relationship is linked to the economy and the environment, the highlighted points include the effective and efficient use of resources, the development of the socio-economic level of the population, the need for researcher people and their training, and the development of inclusive policies for the specified needs. *(vi)* It is seen that the relationship between sustainability and education in terms of quantity and quality will be handled with increasing intensity and importance. In different studies, it is seen that sustainable development has an environmental and social dimension as well as its economic dimension (Bennett, Ylimaki, Dugan & Brunnerman, 2013). However, in one study conducted in terms of education, the level of understanding of the concept is 38%, which suggests that it should be explained to the public based on students (Başgelen, 2019). One of the ways to do this is to do enough research on the subject (Er Nas & Çoruhlu, 2017; Montebon, 2018). What is desired to be done is to find answers to the diversifying and increasing needs of the society in general. (Balakrishnan, Tochinai & Kanemitsu, 2020). In terms of education, it is to provide students with the necessary knowledge, skills, values and attitudes with a holistic approach (Ashokan, 2019). The mentioned researches reveal that there is a need for new studies on the comprehensive and inclusive nature of development plans, and the interaction between education and sustainability. For this reason, research on sustainability, which is a relatively new concept in development plans, can be conducted with different methods in the context of education. In addition, sustainability can be taken into consideration more in the context of concepts affecting education so that the education policies to be determined can achieve their goals.

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2

CERN-LARGE HADRON COLLIDER AND ITS IMPACT ON SUSTAINABILITY

İlknur HOŞ[3]

Abstract

CERN is a huge collaboration has the participants from all over the world including scientists and engineers mainly focusing on the particle physics experiments. LHC at CERN, is the largest particle accelerator that ever built, in Swiss-French border inside a tunnel of circumference of 27 km. LHC is designed to collide the proton and heavy ion beams for the search of matter at new scales and to test the Standard Model in details. There are four big experiments and several small experiments, not only for particle physics but also manner of whole physcics areas, are ongoing on the LHC accelerator. So, energy is one of the biggest issues for CERN society and it is been trying to find best energy management strategy. To provide environmental safety and protection, CERN monitor all parameters that might reveal any impact on the environment and obey the Host States regulations, European standards and directives. For a sustainable life, CERN aims to decrease energy need, to increase reuse of energy and reduce fleet of vehicles for limiting the impact from transport.

Key Words: *CERN, LHC, Sustainability*

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1. Introduction

Big bang, a cosmological model, tries to explain evolution of the universe. According to this model, 13.8 billion years ago universe started to expand from state of singularity at high density and temperature as illustrate in Figure 1. One can see how matter Physics actually dealing with all matter of scales, from the largest ones like astrophysics and to tiniest ones like particle physics. Particle physics is dealing with nature of the particles that constitute matter and radiation and are believed exists naturally in the time interval of first 10^{-6} second of big bang. As shown in Figure 1, Large Hadron Collider (LHC) helps scientist to provide the processes at the time of 10^{-12} s after big bang. LHC is the largest particle accelerator that ever built under the CERN collaboration (European Council for Nuclear Research-**C**onseil **E**uropéen pour la **R**echerche **N**ucléaire), has the participants from all over the world. CERN is established in 1954 by the 12 founding states and now has 23 member states, 3 pre-stage to membership and 7 associate member states.

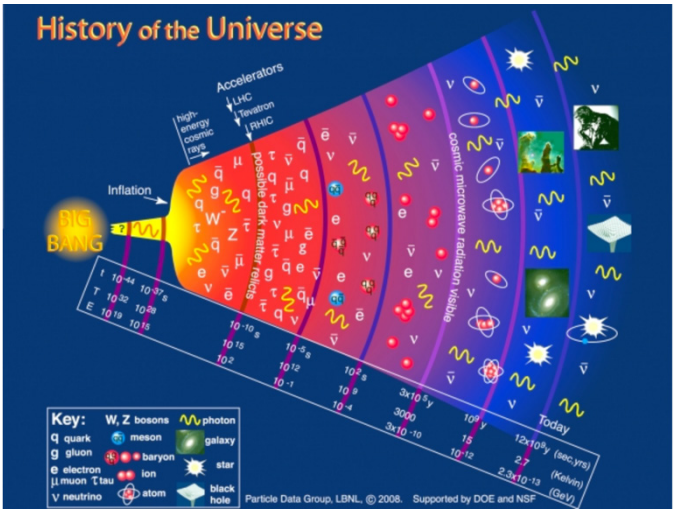

Figure 1. Illustration of history of the universe (PDG Group).

2. The Large Hadron Collider

LHC accelerator complex pictured in Figure 2, sits on the France-Switzerland border near Geneva, on the Large Electron-Positron (LEP at CERN) Collider ring with the circumference of 27 km. It is built to accelerate and collide protons

and ions ~100 m underground. Protons are obtained from Hydrogen atoms and accelerated step by step (Linac, Booster, Proton Synchrotron and Super Proton Synchrotron) before injected to the largest ring-LHC. Proton are accelerated to the energy of 6.5 TeV for each and then collided at four interaction points as named by the experiments; ATLAS, CMS, ALICE and LHCb. In the figure, there some fixed target experiments are also shown with some experiments use only proton beams for the medical purposes. The protons at these energies pass the 27-km ring more than 11 000 times in a second at the speed of 0.999999991 c (c is the speed of light). There are superconducting electromagnets to bend the beams, are operated under the -271.3 ºC (colder than outer space). Besides this very low temperature, in the heavy ion collisions the temperature exceeds 100 000 times that of the centre of the Sun.

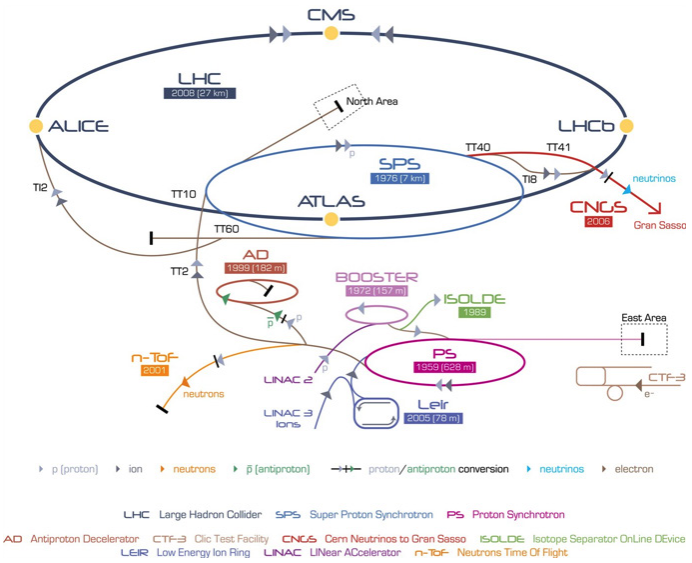

Figure 2. LHC accelerator complex.

Protons are accelerated in bunches of each contains around 1.2×10^{11} protons and LHC generates about 1 billion particle collisions per second. Collisions of these proton bunches for many hours produce very large data flow ~50 PB per year. CERN use a data centre to store data, to provide a grid for the analysis of data as well as for the e-mail or videoconferencing service shown in Figure 3. In this data centre, 230 000 processor cores and 15 000 servers run 24/7.

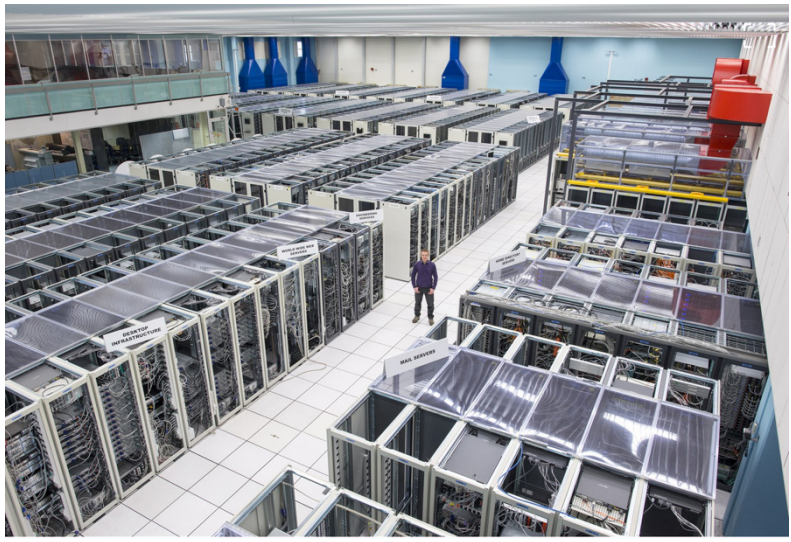

Figure 3. CERN data centre.

On the LHC ring there are four big experiments located at the interaction points: ATLAS, CMS, ALICE and LHCb. ATLAS (**A** **T**oroidal **L**HC **A**pparatu**S**) (Aad et al.) is a general-purpose detector dealing with wide range of physics at the LHC, from precision measurements of the Higgs boson to searches for new physics beyond the Standard Model. It is the largest-volume collider-detector with the size of 46 m long, 26 m high and 26 wide with the weight of 7000 tonnes (see Figure 4-up left). More than 3000 scientists and engineers are working in ATLAS experiment. CMS (The **C**ompact **M**uon **S**olenoid) (Chatrchyan et al.) is also a general-purpose detector with the different technical solutions and design (shown in Figure 4-up right). The most distinguish property of CMS is its huge solenoid magnet generates 4 tesla field (100 000 times the magnetic field of the Earth). CMS is the heaviest detector in LHC with the weight of 14000 tonnes and sizes of 21 m long, 15 m high and 15 m wide. There are more than 3500 scientists, engineers and students working in CMS collaboration. The third big experiment at LHC is ALICE (**A** **L**arge **I**on **C**ollider **E**xperiment) (Aamodt et al.) is built to measure and analyse lead-ion collisions and study the properties of quark-gluon plasma (see Figure 4-bottom left). It's 10 000 tonnes weight and 26 m long, 16 m high and 16 m wide. More than 1000 scientists are working for ALICE. The other experiment sits on the fourth interaction point is the LHCb (The **L**arge **H**adron

Collider-**b**eauty) (Augusto et al.) experiment shown in Figure 4-bottom right. Scientists study the slight asymmetry between matter and antimatter present in interactions of b-particles. The LHCb experiment uses a series of sub-detectors to detect and measure the decay of particles. The experiment has the sizes of 21 m long, 10 m high and 13 m wide and weight of 5600 tonnes. There are more than 1200 members of experiment.

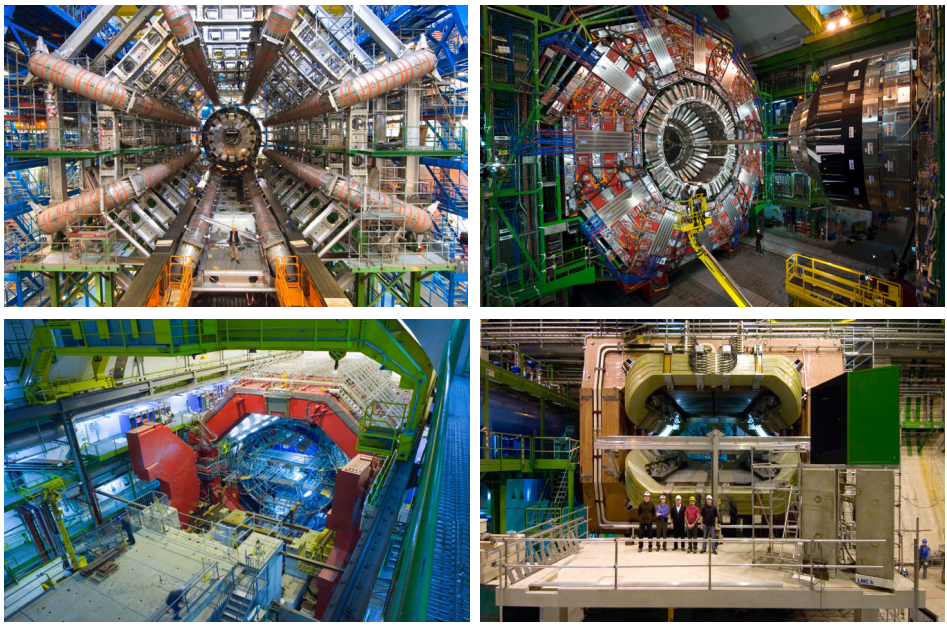

Figure 4. ATLAS, CMS, ALICE and LHCb experiments.

There are some other experiments on the LHC, too. TOTEM (Antchev et al.) and LHCf (Berti et al.) are the small experiments are built to study forward particles. MoEDAL and FASER are other small experiments uses LHC beams. Besides the experiments uses the collision of beams, CERN also hosts fixed target experiments like COMPASS, NA61/SHINE, NA62, CLOUD, etc. There are some facilities that help scientist about the research on medical physics; ISOLDE (The Isotope mass Separator On-Line facility – a unique source of low-energy beams of radioactive nuclides), MEDICIS (contributes to medical researches by producing novel radioisotopes). CERN Neutrino Platform and non-accelerator experiments are also developed with CERN collaboration. AMS and CAST are two of such experiments; while AMS is a particle-physics detector search for

signs of dark matte, antimatter and missing matter from a module attached to the outside of the International Space Station (ISS), CAST searches for hypothetical particles called 'axions' to understand the difference between matter and antimatter in processes involving the weak force, but not the strong force.

3. CERN and the Environment

Such big experiments, campuses and data centres need a great amount of energy, which is the one of biggest issues for the scientists. CERN tries develop best energy management strategy to limit its energy usage and in addition to its environmental awareness. To provide environmental safety and protection, CERN monitor all parameters that might elicit any impact on the environment and obey the Host States regulations, European standards and directives. CERN did commitment in 2019 to communicate on its environmental footprint every two years and in alignment with the internationally recognised GRI Sustainability Reporting Standards. Meanwhile CERN released its second public Environment Report on 24 Nov 2021, covers the years of 2019-2020 (CERN Col.). According to this report in 2019, CERN's energy consumption is 428 GWh (1541 TJ) of electricity and 68 GWh (246 TJ) of fossil fuel. A detailed energy consumption for the years of 2011-2020 can be seen in Figure 5.

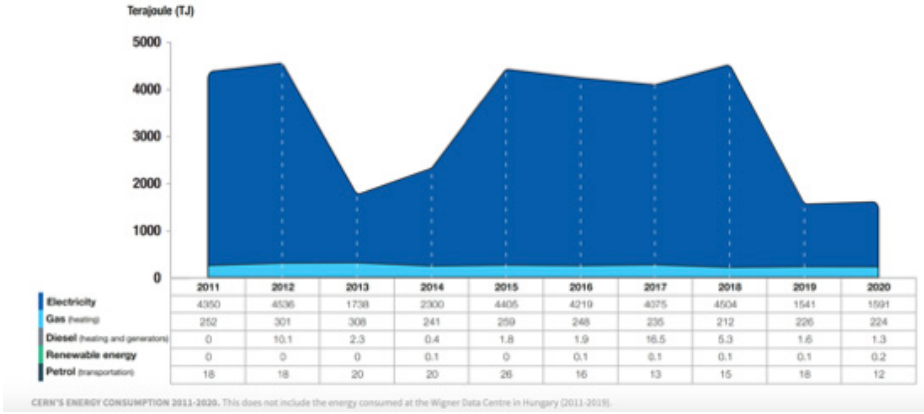

Figure 5. Energy Consumption at CERN.

During the years the LHC running, energy consumption is ~60% higher than 2019-2020. According to the commitment CERN will increase energy re-use and limit the rise in electricity consumption to 5% up to the end of 2024. In the latest report, CERN categorized its emission in 3 scopes and graphed contribution of

each for the years of 2017-2020 in Figure 6. First scope consists of direct greenhouse gas emissions were 78 169 tonnes of CO_2 in 2019. Indirect emissions, scope 2, arising from electricity consumption 10 672 tCO_2e. Scope 3, defined for the first time in the report of 2019-2020, contains indirect emissions from water purification, business travel, personnel commutes and catering and calculated as 12 098 tCO_2e.

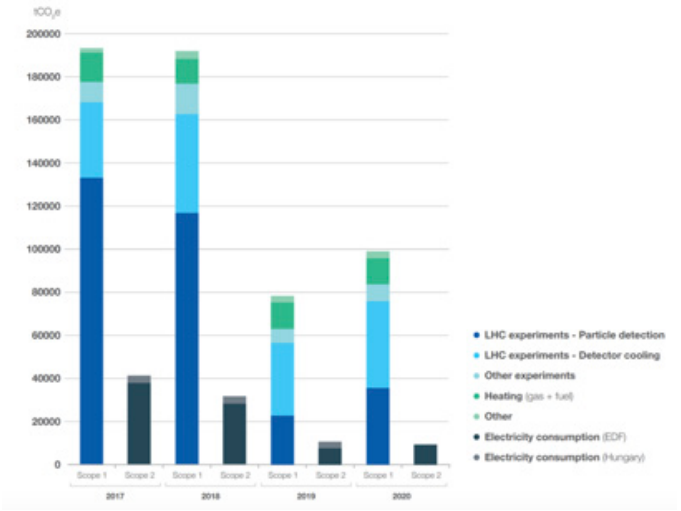

Figure 6. Emission due to accelerator and experiments at CERN.

Figure 7 shows the waste elimination for the years of 2017-2020. In 2019, 5589 tonnes of non-hazardous waste is eliminated and 57% of this waste was recycled. 1868 tonnes of hazardous waste also eliminated. Especially during the running of accelerators, CERN need water for cooling etc. In 2019 although the accelerator was not working during the whole year, 2006 megalitres of water drew.

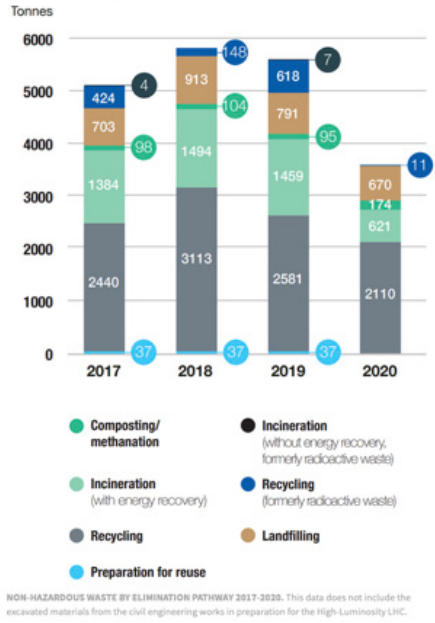

Figure 7. Non-hazardous waste elimination at CERN.

According to the agreement done with GRI Sustainability Reporting Standarts in 2019, laboratory will

- ✓ limit the rises in electricity consumption to 5% up to the end of 2024.
- ✓ increase energy re-use.
- ✓ replace fluorinated gases (F-gases) with carbon dioxide in the detector cooling systems, since it has less potential risk about the global warming than the F-gases.
- ✓ reduce direct emission by 28% by the end of 2024.
- ✓ to increase the current waste recycling rate.
- ✓ to keep its increase in water consumption below 5% by the end of 2024.

CERN also has an agreement with local French authorities to provide heating to the residential area obtained from its facilities. Houses will be heated with reduced CO_2 emissions at lower cost. Over the 8-10 years, CERN will increase the quantity of the heat reaching to 20 GWh in a year. CERN also aims to reduce fleet of vehicles for limiting the impact from transport. And also, all new buildings

are conceived to minimise their energy consumption, by systematically including solar panels, high-performance insulation materials, and more.

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3

NEUROSCIENCE FOR UNDERSTANDING AND DEVELOPING SUSTAINABILITY: NEUROSUSTAINABILITY

Fulya Şenbağcı ÖZER[4]

Abstract

Sustainability is a complex and innovative concept that inherently requires the new ways of thinking constantly for basic biological decision-making processes such as adaptation and collaboration. Neuroscience; is a scientific field that investigates the functions and responses of nervous systems and the brain, which are becoming more and more widely used with technological innovations in brain imaging. As an interdisciplinary field neuroscience is especially along with health, engineering, social sciences and art, has led to the emergence of new fields such as neuropsychology, neuroeconomics, neurosociology, neuroinformatics, neuropolitics, neuromarketing, etc. Although a vast majority of neural connections are operated for the continuity of an organism or an organization, unfortunately there have been no extensive studies in sustainability literature that use neuroscience findings, techniques and approaches. In this context, this study fill this gap with the concept of neurosustainability as a new interdisciplinary field that demonstrates its relationship with neuroscience in the field of sustain-

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ability and reveal strategic elements and implications for future research.

Key Words: *Sustainability, neuroscience, neurosustainability*

Introduction And Terminology

Sustainability is a complex and innovative concept that inherently requires the new ways of thinking constantly for basic biological decision-making processes such as adaptation and collaboration. Not just only for living species, these decision-making processes are nearly similar with continuity of an organization or a community at all.

Although sustainability is the basic aim for a creature from the beginning of life, first definitions were made on first decade of 20th century after economic and ecological crisis and also by the consequences of I-II World War and industrial revolutions. In 1987, the common and comprehensive definition of sustainability is provided by the World Commission on Environment and Development Report also known as Brundtland Report. By the view of economical development and sustainable economics, sustainability described as an economic and sociology based activity that *"meets the needs of the present without compromising the ability of future generations to meet their own needs"* (WCED, 1987, pp. 39). Brundtland Reports' sustainability definition indicates that environmental concerns are important, but the basic discussion is one of welfare, seen in a context of inter-generational equity (Kuhlman & Farrington, 2010) and people protect resources not for environment, for next generations. By the way, the social sustainability start to take attention and scientist began to investigate the importance of providing 'equity' and 'welfare' in community.

After the first definitions of sustainability were indicated three major dimensions of sustainability; European Commission called that a sustainable development triangle formed by 3P's: People, Planet, and Profit/Prosperity (European Commission, 2002) or 3E's: Environment, Economy and Equity (Purvis, Mao, & Robinson, 2019).

At the same time without collecting terms only just one letter, scientist called all pillars or dimensions of sustainability as "environment", "social" and "economy" and sustainability needs to be studied along with all these dimensions. (Scott Cato, 2009, s. 36-37)

Sustainable development defined as the practice of maintaining world processes of productivity by replacing resources used with resources of equal or greater value without degrading or endangering natural biotic systems (Kahle & Gürel-Atay, 2014). As Lawn described in 2000, sustainable development is a desirable human goal (Lawn, 2000). In 2015, 17 Sustainable Development Goals

(SDGs) were adopted by all United Nations Member States, with 169 targets to reach by 2030. These goals and targets are universal, meaning they apply to all countries around the world, not just poor countries (IISD, 2021). As a result, sustainable development goals could only carried out by simultaneously ensuring ecological, social and economic sustainability that is intertwined and balanced.

The more precise meaning of sustainability, however, depends on contexts and intellectual fields such as ecology, energy, engineering, environment, agriculture, social dynamics and anthropology (Murhy & McDonagh, 2016), population and demographics (Portney, 2015, pp. 53), politics, culture (UNESCO, 2021), justice (Seghezzo, 2009, pp. 549), public actions (Caradonna, 2014, pp. 251), technology and economics.

Although a vast majority of neural connections are operated for the continuity of an organism or an organization, unfortunately there have been no extensive studies in sustainability literature that use neuroscience findings, techniques and approaches. In this context, this study fill this gap with the concept of neurosustainability as a new interdisciplinary field that demonstrates its relationship with neuroscience in the field of sustainability and reveal strategic elements and implications for future research.

Neuroscience and Brain

Neuroscience is multidisciplinary sciences; that analyze the nervous system to understand the biological basis for behavior since the middle of the nineteenth century (Squire, ve diğerleri, 2008, pp. 3). In the last three decate, scientist take lots of answers by the developing brain stimulation and brain imaging (neuroimagening) technologies in neuroscientific practice (Boer, Molder, & Verbeek, 2020).

In decision making a number of cognitive processes come into play, among them the processing of the stimuli present in the task, the memory of previous experiences and the estimating of the possible outcomes of each option (Martínez-Selva, Sánchez-Navarro, Bechara, & Román, 2006, pp. 411). It is known that the decision-making process results from communication between the prefrontal cortex (working memory) and hippocampus (long-term memory) but there are other regions of the brain that play essential roles in making decisions, but their exact mechanisms of action still are unknown (Moghadam, Khodadad, & Khazaeinezhad, 2019). Also decision-making processes for a sustainable choice cannot be understand.

There are several branches that study with neuroscience (Squire, and etc., 2008; Akil, and etc., 2016; Pittenger & Duman, 2008; Jaak, 1990). These branches also have inter-relationship with each other while working on a specific field. As an interdisciplinary field neuroscience is especially along with health, engineering, social sciences and art, has led to the emergence of new fields such as neuropsychology, neuroeconomics, neurosociology, neuroinformatics, neuropolitics, neuromarketing, etc. But as told before in this study, there are very little studies in a very limited area of the sustainability studied with neuroscience.

Research Materials and Methods

Research method of this paper is literature review. In the study, primarily neuroscience and sustainability related resources were scanned together in English, Turkish, German and Spanish resources. Due to the limited number of results, sustainability and neuroscience topics in the literature were examined and the correlation of sustainability with other major neuroscience branches has been studied separately. The major branches selected due to the considered relations of sustainability. All recommendations have been made for future studies by subtracting the two way correlation between the ecological, social, economic dimensions of sustainability seperately with neuroscience.

Discussion and Conclusions

The literature on sustainability was examined but extensive research using neuroscience findings, techniques and approaches was not found. Very little research has addressed only by combining consumer expectations with sustainability issues. There have been studies in the field of environmental neuroscience, but these studies mostly indicates on the effects of environment on brain and do not support the effects and trends on social and economic phenomenon. Several studies have examined the effects of neuroscience in sustainable design in engineering science. When the branches of neuroscience are examined to study neuroscience of sustainability, that is understood that the branches of cognitive neuroscience, behavioral neuroscience, computational neuroscience, cultural neuroscience, affective neuroscience, social neuroscience, neuroeconomics, neurophysicology are necessary.

The central thesis of this study reveals many strategic elements and inferences by making multidimensional assumptions about the field of neuroscience of sustainability: neurosustainability.

Neurosustainability is a new field that try to clarify the effects and perceptions of sustainable conditions on brain and try to examine the neurological processes for the action of sustainable oriented thinking, sustainable design, sustainable innovation and also sustainability education with the all dimensions - ecological, social and economical- of sustainability. Neurosustainability field examines the three ways of correlation between neuroscience and sustainability:

1. Understanding of the brain networks that are involved in the adoption of sustainable habits, also thinking, acting and educating on sustainability,
2. Effects of sustainable conditions (for example clean energy, gender equity, urban sustainability or welfare) on brain,
3. Contributes of neuroscience, artificial intelligence and neuroplasticity on sustainability.

In sustainable thinking, the main processes are based on adaptation and collaboration, and these processes are also based on the most basic neurological processes, especially basic parts of the brain such as the amygdala, hippocampus and prefrontal cortex (PFC), which require the field of neurosustainability. By using neuroscience techniques, the concept of sustainability can be better understood and disseminated in all dimensions on field with new innovations. In addition, the effects of an ecologically, socially and economically sustainable environment on neurological processes in human brain can be examined.

What Is Expected From Field Neurosustainability?

Neurosustainability tries to fill the gap in sustainability science to understand sustainable thinking, sustainability action and sustainable learning on brain. Indeed, the field contains lots of new research area, summarized as:

- ✓ Define the effects of sustainable conditions (also the UN SDGs) on brain by neuroscience… For example; the effects of social gender equality /unequality on conscious brain development which points cerebral cortex. Or the effects of sustainable/unsustainable cities and communities on brain health.
- ✓ Understanding Society 5.0 (super smart society) dynamics with the approach of human development and antropology by using neuroscience.
- ✓ To define the PFC deep neural networks relations with lymbic system and hippocampus in decision-making processes for the option which is sustainable, ethic or humanistic one, instead of unsustainable or selfish one.

- ✓ Combine social and engineering sciences with sustainability by using artificial intelligence and neuroplasticity by using neuroscience technologies for sustainable oriented thinking, sustainable design, sustainable innovation and also sustainability education.

Avenues for Future Research

Researches from field of neuroscience indicate the sustainability learning and sustainable actions of community and organizations can be supported by neuroscience techniques, neuroplasticity and artificial intelligence. Also cognitive neuroscience, sustainability anthropology, sustainability biology, human development, brain development, neuroplasticity, prefrontal cortex, decision-making processes, behavioral changes must be worked together with the field and major indicators of sustainability, also SDGs. In addition, the effects of sustainability to neurological development in humans should be revealed.

Limitations Of Field

The limits for future research are as follows:

- ✓ Identifying deep neural connections related to sustainability as a whole, is still difficult in today's neuroscience technologies. For example: the blockage of amygdala cannot be understand for smoke while the person know the consequences of smoking. It cannot be understood that sometimes humans they sense that they are being forced to change their behaviour, they build up strong resistance (Schwartz, Gaito, & Lennick, 2021).
- ✓ Deep neural network related to the PFC are still being tried to explain.
- ✓ Because the decision-making mechanisms and behavioral changes on brain cannot be fully defined, it is difficult to explain the neural activity that drives the organism's orientation to what is sustainable.
- ✓ There are no researches on neuroplasticity or artificial intelligence that promote PFC for sustainable thinking, acting and learning.
- ✓ The difference on individual and collective results in researches on cognitive neuroscience; findings and reality.

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4

TREATMENT COMPLIANCE IN PATIENTS WITH FAMILIAL MEDITERRANEAN FEVER

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Abstract

Purpose: To determine compliance of adult patients with Familial Mediterranean Fever to treatment and addressing factors affecting them in depth.

Design: An interpretative phenomenological design.

Methods: The study group was determined using the purposive sampling

method and consisted of 14 patients with Familial Mediterranean Fever. Data collection included in-depth interviews conducted between September 2020 and December 2020. Interviews were audio recorded, transcribed verbatim, and analysed using the Interpretative Phenomenological Analysis. The Consolidated Criteria for Reporting Qualitative Studies were followed in the writing of the article.

Findings: Three main themes emerged from the analysis of the interviews: (a) experience of having attacks; (b) insufficient knowledge about the treatment; and (c) irregular use of colchicine.

Conclusions: Patients cannot fully comply with treatment due to the lack of knowledge. While the experience of having attacks may positively affect to patients' compliance with the treatment, their insufficient knowledge about the treatment and their irregular use of colchicine affect them negatively.

Clinical Relevance: The main goal in nursing interventions is to strengthen the interaction of patient with his/her environment and to increase his/her compliance. There is a need to increasing their compliance with treatment while planning patient education programs for adult patients with Familial Mediterranean Fever.

Key words: Colchicine, compliance, familial mediterranean fever, patient

Introduction

Familial Mediterranean Fever (FMF) is an autosomal recessive, autoinflammatory genetic disease caused by mutations in the MEFV gene located on chromosome 16 (Tufan & Lachmann, 2020). FMF is frequently observed in Turkish, Armenian and Arab communities living in Eastern Mediterranean Region. Nevertheless, it is also reported from different parts of the world (Ben-Chetrit and Yazici 2019; Chan, Sun, and Chen 2018; Kishida et al. 2014; Manna and Rigante 2019; Tufan and Lachmann 2020). Although the prevalence of FMF in Turkey varies between 0.03 and 0.81%, it has significant geographical differences (Alparslan et al. 2020; Çobankara et al. 2004; Kisacik et al. 2009; Onen et al. 2004; Tunca et al. 2005).

The clinical phenotype is usually more severe and the presence of the M694V mutation is more common in patients with an onset of attack at an early age (Sönmez et al., 2019; Yaçinkaya, Özçakar, Tanyildiz, & Elhan, 2011). Patients have recurrent clinical attacks consisting of fever, serositis, arthritis, and high levels of inflammatory reactants. The attacks resolve spontaneously within 1-3 days (Tufan & Lachmann, 2020). FMF attacks can be triggered by infection, stress, menstruation, exposure to cold, eating foods rich in fat, and the use of certain medications (Maggio and Corsello 2020).

The purposes of FMF treatment are to reduce the frequency, severity and duration of attacks, to prevent amyloidosis by minimizing chronic or subclinical inflammation, and to improve the patient's quality of life. Colchicine is the first choice in the treatment of patients with FMF (Kavrul Kayaalp et al., 2021) and is considered as an effective treatment for most patients (over 90%) (Ben-Chetrit & Yazici, 2019). Colchicine may often lead to side effects such as vomiting, diarrhea, and transient elevations in transaminases (Migita et al., 2018; Sönmez et al., 2019). Interleukin (IL)-1 antagonists are preferred in cases resistant or intolerant to colchicine (Tufan and Lachmann 2020).

Background

The patient's with treatment is of great importance in the prevention of attacks and amyloidosis (Migita et al. 2018; Sönmez et al. 2019). It is essential for the patient to fully comply with the use of colchicine to achieve success in treatment. Adolescents may have some difficulties in complying with treatment completely. Various factors such as parental problems, low socioeconomic status, and the

adolescent's refusal to take medication to avoid side effects may lead to non-compliance with treatment (Sönmez et al. 2019).

No qualitative study examining the compliance with treatment of adult patients with FMF was found in the literature. Determining adult patients' with Familial Mediterranean Fever compliance with their own treatment and addressing the factors affecting them in depth can make significant contributions to clinical evaluations.

The aim of this study was to determine adult patients' with FMF compliance with their own treatment and addressing the factors affecting them in depth. In the study, answers to the questions of "What do patients know about medications (colchicine)?", "How do patients reflect their knowledge of compliance with treatment in their behaviors?", "What are the situations in which patients have difficulty or non-compliance with medication?", and "What do patients know about health problems that may develop when they do not regularly use colchicine?" were sought.

Methods

The study was a qualitative study planned based on Heidegger's interpretive phenomenological research approach (Erdogan et al., 2020; Horrigan-Kelly et al., 2016; Patton 2015). Phenomenology is a philosophical approach to the study of experience. The aim is to explore a lived experience (Charlick et al., 2016). This approach was put forward by Husserl and developed by Heidegger, Sartre, and Merleau-Ponty (Patton, 2015). Heidegger's phenomenological approach conceptualizes the understanding within ourselves determined by what is important to us in the form of existence (Erdogan, 2015; Horrigan-Kelly, Millar, & Dowling, 2016). Experience is everything that happens in a person's daily life, and everything that happens is a phenomenon. The interpretative phenomenological approach is used in nursing studies to reveal the phenomena and understand the subjectivity of care through the transfer of experience (Guerrero-Castañeda, Menezes, and Prado 2019).

When the phenomenon is started to be explained by the researchers, prejudices and theories are suspended, events are not objected or approved, what is done is to put the experienced reality in parentheses. The external world in which the phenomenon to be investigated lives in is examined. In this respect, phenomenology is trying to find what is indicated as life speaks of itself by providing

understanding and interpretation of each specific situation of experience (Guerrero-Castañeda et al. 2019). The interpretative phenomenological research design was preferred in this study by considering that it is a method that can contribute to an in-depth examination of the experience of the patients with FMF on their treatment.

Sample and Setting

The study was conducted in a university hospital in Istanbul. 14 patients who were followed in the Rheumatology Outpatient Clinic with the diagnosis of FMF, determined using the purposive sampling method, and agreed to participate in the study constituted the study group. The inclusion criteria for the study were (a) being a patient with FMF for at least 5 years, (b) being a patient with at least 1 M694V mutation from the exon 10 mutation, (c) using colchicine for at least 5 years, (d) being open to communication and (e) being over the age of 18. Using a drug other than colchicine for the treatment of FMF was determined as an exclusion criterion from the study.

The data analysis was performed along with the inclusion of patients in the study in order to determine data saturation. Data saturation was achieved since new codes or themes did not emerge as a result of the interviews, and the study was completed with 14 patients.

Data Collection

One of the most frequently used data collection tools in qualitative research is face-to-face interviews. The Patient Information Form and Interview Guide prepared by the researchers using the literature were used in data collection (DiMatteo 2004; Yesilkaya, S., Acikel, C., Fidanci, B.E., Polat, A., Sozeri, B., Ayaz, N.A., Makay, B.B., Simsek, D., Akinci, N., Ozcelik, G., Kavukcu, S., Emre, S., Donmez, O., Delibas, A., Yuksel, S., Berdeli, A., Poyrazoglu, H., Saldir, M., Fidanci, K., Ca 2015). Patient Information Form consisted of 17 questions related to patients' socio-demographic characteristics, health status and drug use. The Interview Guide was a semi-structured form consisting of 4 open-ended main questions and 9 auxiliary questions about the knowledge, attitudes and behaviors of patients about their compliance with medication (Table 1).

TABLE 1. Interview Guide

1. What do you know about your medication (colchicine)?
a. How does colchicine affect your attacks?
b. Is it necessary to use colchicine regularly? Why?
c. Do you think that the use of colchicine may have negative effects? Why?
d. What can happen if colchicine is not used regularly?
e. Do you need to pay attention to your food consumption while using colchicine? Why?
2. How do you reflect your knowledge about compliance with treatment on your behaviors?
a. What kind of behavior change have you developed to comply with your treatment?
b. Is there any case where you do not comply with your treatment although you know? Why?
3. What are the situations in which you have difficulty in complying with your medication or you do not comply even though you know it?
a. Is there any case where you do not take your medications at the same time every day? Why?
b. Have you had periods when you skipped taking medication for a long time? Why?
4. What do you know about the health problems that can develop if you do not use colchicine regularly throughout your life?

Two of the researchers were female academic nurses with a doctorate who had took qualitative research courses, attended courses in this field, had experience in qualitative interviews and conducted various qualitative studies. Other researchers were male academic physicians with a doctorate who conducted the outpatient follow-up of the patients with FMF.

The Patient Information Form and the Interview Guide were tested by interviewing two patients who were not included in the study. The content was rearranged and the Interview Guide was finalized.

The interviews were conducted by the researchers in a room belonging to the Rheumatology Outpatient Clinic of the hospital between September 15, 2020 and December 15, 2020. One of the researchers identified the patients to be included in the study group and directed them to the interview room. Another researcher interviewed these patients face-to-face respectively by following the Covid-19 measures and collected data using the individual in-depth interview method. Before starting the interview, information was given about the aim of the study, the principle of voluntary participation and the process.

Attention was paid to the absence of anyone in the room in order to ensure the comfort and privacy of patients during the interview. After obtaining informed consent from the patients, interviews were conducted and digital audio recordings were taken. Furthermore, pedigree drawing was performed and the presence of other healthy/sick individuals in the family was determined. The questions included in the Interview Guide were asked. Each interview took approximately 30-45 minutes. The audio recordings of the interviews were transcribed by the researchers, and the absence of missing or erroneous information was checked by comparing the audio recordings with the minutes. Patients' non-verbal expressions such as smiles, silences and sighs were recorded on the relevant forms.

Ethical Issues

Ethical approval was obtained from a university, which follows international standards and the principles adopted by the World Medical Association of Declaration of Helsinki. Before the individual interviews, the patients were assured that their voices would be recorded on a voice recorder and that the information would not be used for any other purpose, and their consent was obtained. The principle of autonomy was followed by telling the patients that they were free to decide to participate or not to participate in the study from the first stage, and that they could terminate their participation at any point. The names of the patients were symbolized in alphabetical letters within the framework of respect for individuality and human dignity.

Data Analysis

The data were analyzed according to the principles of Interpretive Phenomenological Analysis (Smith, Flowers, and Larkin 2009). The data were read independently by two researchers. A coding scheme was created by identifying important phrases and their formulated meanings. All recorded data were exam-

ined again according to this scheme. The categories and themes were determined according to the codes by researchers manually. A consensus was reached as a result of the meetings held with the participation of all researchers.

The Consolidated Criteria for Reporting Qualitative Studies (COREQ) were followed in the writing of the article.

Rigour

Various measures were taken by the researchers to minimize or eliminate the factors that affected or threatened validity and reliability in the study (Oral and Çoban 2020; Rose and Johnson 2020). The interview guide was prepared by taking expert opinion and tested with two patients from outside the study in order to ensure the internal validity of the study. The design of the study, the study group, data collection tools, data collection, data analysis and findings were explained in detail in order to ensure the external validity. Data loss was prevented by using a voice recorder during the interviews. The results of the study were presented to the reader without commenting, which had a significant effect on increasing the internal reliability (consistency) of the study.

Findings

Participant Characteristics

The mean age of the patients was 30.9±8.7 years (min. - max.: 21 - 55), 66.7% of them were female, 53% of them were single, and 60% of them were not working in any job. It was determined that 47% (n = 7) of the patients did not have an attack in the last three months, and that 71% of these patients complied with the colchicine treatments using exact dose for the last six months. It was determined that most of the patients (64%) stopped using colchicine in a period of the FMF treatment process in accordance with their own decision. When the patients' FMF disease severity was examined according to the PRAS Score, it was determined that 40% of them had mild disease, 40% of them had mild-severe disease and 20% of them had severe disease.

Themes

Three themes were identified in the interviews and presented in Figure 1.

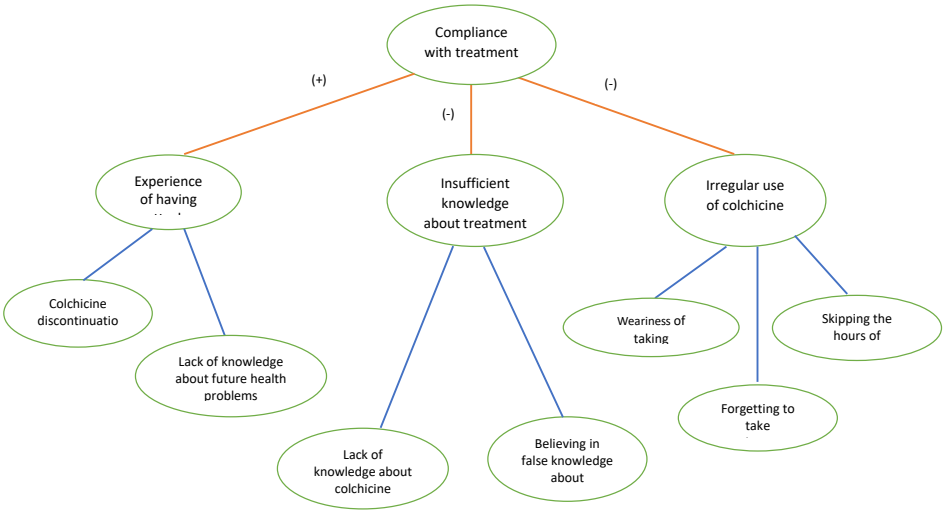

FIGURE 1. Data categories and themes

Themes 1. Experience of having attacks. The theme of experience of having attacks was examined in two categories: colchicine discontinuation, and lack of knowledge about health problems caused by non-compliance with treatment. The remarkable statements of the patients regarding the colchicine discontinuation category are presented below:

I did not use medication for six months and my attacks became more frequent, at that time I became unable to walk. [M]

I wanted to discontinue medication since I had not had an attack for 20 years. … . I know that I will not have attacks when I use this drug. I suffered a lot when I did not take medication. It was a very difficult period. [B]

My complaints increase when I do not use medication. I try to use the drug by saying I have to, but there are periods when I stop taking the drug. [D]

The remarkable statements of the patients regarding the category of lack of knowledge about health problems caused by non-compliance with treatment are present below:

I have no knowledge. [N]

I do not know. [J]

I know it can damage my kidneys if I do not use it. I do not know anything else. [B]

Themes 2. Insufficient knowledge about the treatment. The theme of insufficient knowledge about the treatment was examined in two categories, including lack of knowledge about colchicine treatment, and belief in false knowledge. The remarkable statements of the patients regarding the category of lack of knowledge about colchicine treatment are presented below:

I think that when I use colchicine regularly, it delays my attacks and alleviates my problems. It was beneficial for me. However, I do not know anything else about the drug. [E]

I guess this drug is used in psoriasis or Behçet's disease. I know it affects kidney, liver, stomach. I do not have any other information. [G]

I know that I need to use it to a degree that will reduce my future discomfort and terminate my painful attacks. I think there is a problem in cases such as having children in the future. The kidneys may also be severely affected. Stomach disorders. [L]

The remarkable statements of the patients regarding the category of belief in false knowledge are presented below:

Every drug already causes damage to the body. It is both beneficial and harmful. But what will it be? I think it will appear in the future. [A]

Since I have been taking medicine for 10 years, it does not feel like I am using any medicine at all, I got used to it. I know the drug is harmful to the kidneys. [F]

I stopped taking the medicine because I thought that I was forgetful. [L]

Themes 3. Irregular use of colchicine. The theme of irregular use of colchicine was analyzed in three categories, including weariness of taking medications, forgetting to take medications, and skipping the hours of medication. The remarkable statements of the patients regarding the category of weariness of taking medications are presented below:

It is very difficult for people to take medications. I mean, I am tired of taking it, however, I have never stopped taking it. [A]

…. I mean, it is enough, I'm bored, I don't use it. Frankly, I am bored because I have been taking it since my childhood. [M]

… I have weariness. I forget, I say never mind. But I always have to carry the medicine with me. Because, when I didn't use medicine, I had big complaints. I use it by saying I have to. [D]

The remarkable statements of the patients regarding the category of forgetting to take medications are presented below:

I do not have any problems other than forgetting. I do not like to use drugs unless I have to. I take them because they are useful for me. [F]

I may forget to take my medications. I do not think about taking later. [K]

Sometimes I forget to take my medications, sometimes I don't have time to take. [I]

The remarkable statements of the patients regarding the category of skipping the hours of medication are presented below:

I do not take my medications at the same time every day, but I certainly take them. [G]

It happens when I don't take my medications, even if I can't take at that time, I take my medications even in the evening or late. [H]

Discussion

In this study, the experiences of the patients with FMF regarding their compliance with were analyzed with a phenomenological approach. The results were summarized in 3 themes consisting of 7 categories. The themes were named as (a) experience of having attacks, (b) insufficient knowledge about the treatment and (c) irregular use of colchicine. The study is of great importance in terms of the experiences of the patients with FMF during their treatment and making sense of these experiences.

Callista Roy, one of the nursing theorists, defines the individual as a system that is in constant interaction with its environment and tries to adapt to the changes it experiences. According to Roy, the aim of the nurse is to help the individual to exhibit compatible behaviors during the health and disease process. The main goal in nursing interventions is to strengthen the interaction of the patient with his/her environment and to increase his/her compliance. Therefore, the patient's behaviors and the stimuli that cause these behaviors and affect compliance should be first evaluated (Fawcett and Desanto-Madeya 2013).

Compliance with treatment refers to the patient's use of the prescribed drug at the recommended dose and time. Furthermore, it is also defined as the patient's compliance with the recommendations of the healthcare professionals and the development of a positive behavioral change for his/her treatment (Coskun & Bagcivan, 2021; Yılmaz, 2016). In case of failure to comply with the treatment, patients cannot optimally benefit from the clinical benefits of their treatment (Choudhry et al., 2017). Full compliance with colchicine treatment is generally 40% and decreases to 20% especially in adults (Corsia et al., 2017). It was observed that the compliance with treatment of the patients examined in this study was not at the desired level. Their insufficient knowledge about their treatment and their irregular use of colchicine constituted the basis of their non-compliance, which may be considered to be effective in not achieving the predicted clinical benefit in patients. On the other hand, some patients' experiences of having attacks appeared as a triggering factor in increasing their compliance with treatment. Patients had attacks since they stopped using colchicine for a while and did not have enough knowledge about the health problems to be caused by not taking medications. Although this is an undesirable problem, the experience of an attack affects the compliance with treatment positively. It was considered that the patients continued to use colchicine in order not to experience the attack symptoms again and they were trying to comply better with the treatment.

Experience of having attacks. FMF disease is characterized by recurrent polyserositis and fever attacks. Patients may have 2.5±5.3 attacks per year during childhood (Sen et al., 2021). The use of colchicine is a basic treatment option in reducing the frequency and severity of recurrent attacks and even the risk of developing amyloidosis (Ben-Chetrit & Yazici, 2019). Patients' non-compliance with colchicine treatment is an important factor in the development of subclinical inflammation and amyloidosis. Patients may stop using the drug due to the side effects of colchicine (Satiş et al., 2020). In this study, it was remarkable that patients who came to their routine outpatient follow-ups stopped using colchicine from time to time. Furthermore, it was also remarkable that patients had a lack of advanced knowledge about the health problems that would occur if they did not comply with their drug treatments. It was observed that discontinuation of medications reflected on them as a bad experience in the form of re-development of FMF attacks or an increase in the frequency and severity of attacks. It was considered that patients continued to use colchicine because they avoided experiencing

these experiences again. In this context, it can be said that patients' experiences of having attacks had a positive effect on compliance with medication.

Insufficient knowledge about the treatment. The shortcomings in compliance with treatment reduce treatment efficiency and worsen the clinical outcomes. It is largely needed to focus on patients for the interventions to improve compliance with treatment, however, the strategies that have been shown to be effective are difficult to implement in practice (Zaugg, Korb-Savoldelli, Durieux, & Sabatier, 2018). Nevertheless, many patients may have problems continuing their colchicine treatment due to the side effects of the drug (Salimov et al. 2019) first time in the literature, we aimed to evaluate and compare auditory functions in pediatric FMF patients during the attack periods and attack-free periods to see the possible effects of autoinflammation that was caused by FMF attacks. Methods: 40 pediatric FMF patients (80 ears.

In the treatment of FMF, the main objective is to reduce the frequency, severity and duration of attacks, to prevent long-term organ damage and to improve the patient's quality of life (Ben-Chetrit & Yazici, 2019). Colchicine treatment is a standard treatment that has been long used in FMF disease (Indraratna, Virk, Gurram, & Day, 2017). When patients comply with colchicine treatments, repetitions of attacks can be prevented (Ben-Chetrit & Yazici, 2019). Non-compliance with treatment leads to the repetitions of attacks. Recurrent attacks of peritonitis may lead to peritoneal adhesions and fallopian tube obstruction. The development of infertility is associated with severe FMF disease and inadequate colchicine treatment (Sotskiy et al., 2021). Cochlear involvement may also be observed in patients due to FMF attacks and inflammation processes, and they may have hearing problems. However, it has been demonstrated that long-term colchicine treatment also protects the patient against cochlear involvement and has a positive effect on hearing (Salimov et al., 2019). In this study, it was observed that patients had an intense lack of knowledge about their treatment. With regard to colchicine treatment, patients had limited knowledge that colchicine was effective in having milder or preventing FMF attacks. It was determined that almost all of the patients did not have enough knowledge about how their regular colchicine treatment affects FMF attacks, why regular colchicine use is important, what they can experience if it is not used regularly, and what to pay attention to while using colchicine. No qualitative study that provides similar and detailed information regarding the lack of knowledge of patients on colchicine treatments was found in the literature.

When regular colchicine use is maintained and kept under control, FMF does not negatively affect the reproductive system and pregnancy outcomes. It has even been reported that the non-application of colchicine treatment may lead to infertility and poor pregnancy outcomes (Sotskiy et al., 2021). Furthermore, it is recommended that the incidence of abortion is lower in women who use colchicine during pregnancy and that colchicine treatment should not be discontinued during pregnancy (Indraratna et al., 2017). Nevertheless, it is reported that FMF disease does not have a significant negative effect during pregnancy (Iskender et al., 2020). In this study, it was remarkable that some patients had false knowledge about the treatment, which may negatively affect their compliance with treatment. There were patients who had false knowledge that long-term use of colchicine would harm their body, damage their kidneys, would cause forgetfulness and infertility. Major side effects limiting the optimal doses of colchicine treatment are diarrhea by 10.8%, increase in transaminase by 5.9%, and leukopenia by 1.1%. Colchicine intolerance is a major problem mainly due to diarrhea and liver toxicity (Satiş et al., 2020). Therefore, it is of great importance that patients are followed up regularly in the outpatient clinic. Apart from the side effects due to optimal dose, no study indicating that colchicine causes damage to the body or causes an organ damage and causes forgetfulness was found. Moreover, no information indicating that colchicine would cause infertility was found in the literature. However, it is written on the drug package inserts that "When COLSIN is used during pregnancy, it has harmful effects on the baby (chromosomal damage). COLSIN should not be used during pregnancy." (https://kt.ilacprospektusu.com/ilac/5829-kolsin-draje-kt). It is predicted that patients follow the information written in the drug package inserts. In this respect, updating the package insert information by drug manufacturers may positively affect patients' compliance with treatment. On the other hand, deficiencies in patient education may be the basis of insufficient knowledge about treatment in patients. Patient education significantly increases the compliance with treatment (Taibanguay, Chaiamnuay, Asavatanabodee, & Narongroeknawin, 2019). The absence of a planned patient education program for adult patients with FMF and an improved FMF treatment compliance scale can be considered as a significant disadvantage in patients' having sufficient knowledge about treatment and monitoring their compliance with treatment. In the literature, there are studies examining the effect of patient education on compliance with treatment for various chronic diseases (Krishnakumar, Govindarajulu, Vishwanath, Nagasubramanian, & Palani, 2020; Oláh et al., 2020;

Taibanguay et al., 2019), however, no study on patient education for adult patients with FMF and their compliance with treatment was found.

Irregular use of colchicine. In chronic diseases, patients' non-compliance with treatment is 22-50% and varies according to the disease (Corsia et al., 2017; Llorca et al., 2021; Prabahar, Albalawi, Almani, & Alenizy, 2021). Patients may have non-compliance with the treatment due to forgetfulness or inability to access the drug despite planning to take medications. Moreover, they may stop the treatment voluntarily by considering that they do not need the drug due to its side effects, the lack of knowledge, and not benefiting from the treatment in a short time (Unni, Sternbach, & Goren, 2019). Patients' perceptions of the drug can be effective in compliance with medication (Sezgin & Mert, 2010). In this study, patients' weariness of taking medications, forgetting to take medications, and skipping the hours of medication constituted the reasons for the irregular use of colchicine. The diagnosis of FMF in the adolescence period and long-term use of colchicine may have led to the weariness of taking medications. The fact that the use of medication is essential for the relapse or mild recovery of the attack symptoms may be lead to a negative pressure on the patients. In this respect, it is important to know the psychological responses to the disease. Nurses have a key role in evaluating the psychosocial status of the patients they care for and supporting them to find solutions to their problems (Aydemir & Çetin, 2019). It is obvious that defining the psychological responses to the disease before the patient is given education and then making a suitable planning for the education is of great importance in learning. Furthermore, it should not be overlooked that the learning competencies of the patients may differ according to their age periods. In the study, it was determined that most of the patients had a lack of knowledge about their treatment, which may have developed due to the fact that patient education was provided after the patients were diagnosed with FMF before their psychological responses to the disease were defined. Patients' forgetting to take medication or skipping the hours of medication may have developed because the disease was not taken seriously. The fact that the importance of treatment was not well understood can also be considered as an important factor. The patient's failure to comprehend the importance of the treatment may cause non-compliance with the treatment. It was considered that patient education was needed in almost all patients.

Limitations

The fact that the study was conducted among adult patients and in a single-centre are the limitations of the study.

Conclusions

Patients cannot fully comply with the treatment due to the lack of knowledge. While the experience of having attacks may positively affect the patients' compliance with the treatment, their insufficient knowledge about the treatment and their irregular use of colchicine affect them negatively. Planning patient education programs with a holistic and integrated perspective can make significant contributions to increasing or completely ensuring their compliance with treatment.

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5

CASTOR OIL-BASED DRUG DELIVERY SYSTEMS

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Abstract

Increasing carbon dioxide emission and environmental pollution have caused scientists to search for alternative raw materials for polymer fabrication instead of fossil feedstocks. Plant oils are useful for renewable raw materials in the polymer industry. Polymer-based drug delivery systems require biocompatible starting materials as well. Plant oil-based monomers attract more and more attention every day due to their biodegradability character, low price, good availability, and environmentally friendly chemistry. Castor oil is one of the common plant oils used for polymer synthesis. Castor oil comprises ricinoleic acid with functionalizable hydroxyl, vinyl, and ester groups. This study focuses on recent biomedical applications of castor oil, primarily plant oil-based nano/microemulsion systems developed for effective drug delivery systems. The topics discussed here include formulation and preparation methods and synthesis and characterization of castor oil-based drug delivery systems. The present research has underlined lipid-based nano/microcarriers containing vegetable oils compared to the chemical systems, highlighting their use in biotechnology to provide eco-friendly and more effective biomedical applications. In the presented study, our motivation is to provide a perspective on using castor oil in drug delivery systems.

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Keywords: *Castor oil, drug delivery systems, plant oil, renewable resources*

Introduction

Synthetic polymers derived from petroleum hydrocarbons decompose into small pieces when exposed to UV radiation in sunlight. However, these small polymer pieces are hard to degrade, so they cause the accumulation of plastic objects in the environment (Li, 2019). Due to non-biodegradability, high cost, and the scarcity problem of petroleum resources, the need for alternative raw materials has arisen. Renewable resources are raw materials obtained from animals, plants, microorganisms, and derivatives (Li, 2019). There are numerous studies in the literature concerning the production of monomers, polymers, or resins from vegetable raw materials (Li et al., 2005; Wool and Sun, 2011; Petrović, 2008; Isikci et al., 2020; Cayli et al., 2019)

Plant oil-derived raw materials and plant oils are widely used to produce polymer composites containing synthetic and natural micro/nanoparticles or fibers. The main component of plant oil is a triglyceride, an ester of glycerol that includes three fatty acids (Meier et al., 2007; Miao et al., 2014). The glycerol is the same in almost all vegetable oils, whereas the fatty acid components vary by plant. The functionality of plant oils is determined according to the chain lengths of the fatty acids and the positions of reactive double bonds and hydroxyl groups (Güner et al., 2006). The reactive groups can be functionalized with various reagents (Khot et al., 2001). These molecules can be modified by hydrolysis, transesterification, or reacting/replacing the unsaturated sites in fatty acid chains (Mosiewicki and Aranguren, 2013).

Plant oils are becoming more and more interesting for biomedical applications because of their biocompatibility (Lligadas et al., 2013). Moreover, plant oil-based materials are popular due to their eco-friendly and biodegradable characters. Double bonds and other functional groups can be defined as reactive sites of plant oils. Besides, ester groups and allylic positions are also essential for plant oils used to develop a variety of monomers and polymers (Miao et al., 2014).

Castor oil (CO), a natural derivative of the *Ricinus communis* plant, is obtained from natural sources in large quantities and high purity (Fig.1). Castor oil contains cis-double bonds that can be hydrogenated, oxidized, halogenated, and polymerized. Furthermore, it is safe and biocompatible (Sandford et al., 2021). Castor oil is the source of ricinoleic acid, which is a fatty acid with chain lengths between 18 and 20 carbon atoms and a hydroxyl group. The most important advantage of CO is that it can be used in polyol synthesis without any modification

because it has natural hydroxyl groups in its structure (Das et al., 2017). This review mainly focuses on castor oil-based materials developed for biomedical applications, particularly drug delivery systems, to further contribute to this area.

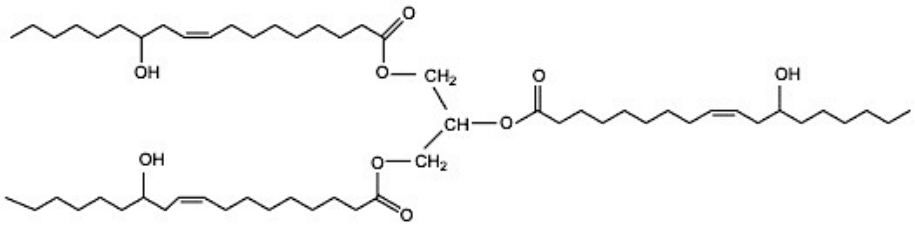

Figure 1. Structure of castor oil

Castor oil-based bulk systems for drug delivery

Gallon's study is an example of CO-based drug delivery systems. They used CO with 3-isocyanatopropyltriethoxysilane (IPTES) to have a bulk system, and then these materials were functionalized to have microparticles by oil cross-linking. In this study, the sizes and shapes of microparticles were investigated. Moreover, these particles were evaluated in drug release capacities for 10h. It was reported that these microparticles showed good cytocompatibility results. Their results proved that CO-based systems have sufficient and feasible characters for pharmaceutical applications (Gallon et al., 2017). On the other hand, in Cardoso's study, CO derivatives, 10-undecenoic acid, and 1,3-propanediol were used as raw materials for renewable α,ω-diene monomer. Then this monomer was used in the synthesis of poly (thioether-ester) nanoparticles. Biocompatibility and cytotoxicity tests were applied for these nanoparticles. According to their results, high cell viability was obtained. Furthermore, no hemolytic capacity was observed towards normal red blood cells. These promising results show that the obtained nanostructures can successfully be used for drug release studies (Cardoso et al., 2017). In another study, Gulmez et al. (2021) investigated a series of CO/polyethylene glycol (PEG)-based polyurethane samples as controlled drug release systems. CO/PEG-based polyurethane samples, prepared at various molar ratios, were loaded with the drug molecules at pH 9.1, and their release profiles were determined at different pH values (pH 2.3, 5.8, 6.4). As a result of the study, it was concluded that the CO/PEG ratio in the polymer structure should be 70:30 by weight for an effective drug release. Shelke (2007) prepared two different polyurethane films with CO-ethylene glycol and CO-polyethylene glycol.

The impacts of CO on the formation of the film and the diffusion properties of the CO-based PU-mediated indomethacin drug molecule were investigated. The drug permeability of the films loaded with different amounts of indomethacin was examined. As a result, it was observed that sustained drug release occurred for a long time. Nerantzaki (2018) prepared thioether-containing ω-hydroxy acid (TEHA)-based microparticles by various hydrophobic/hydrophilic ratios derived from CO for effective release of the naltrexone (NLX). Saeedi (2021) produced CO-based redox and pH-sensitive amphiphilic copolymers for controlled doxorubicin delivery in another study.

In our research group, novel thermoresponsive hydrogels were synthesized using bacterial cellulose and castor oil. The effects of CO on the physical and thermal behaviors of hydrogels were investigated. In this study, we concluded that the hydrogels with CO are more sensitive to temperature changes than the one without CO (Isikci et al., 2020). In another study reported from our research group, acrylated methyl ricinoleate, derived from castor oil, was used as the crosslinker instead of plant oil. Photopolymerization was realized with a varied molar ratio of initiators, and thermoresponsive hydrogels were obtained on the glass surface (Cayli et al., 2019).

Self-emulsifying drug delivery systems

The oral route is one of the most common techniques for treating diseases. However, the drug solubility is generally the rate-limiting step of absorption processes for hydrophobic drugs with poor aqueous solubility. Self-emulsifying drug-loaded capsules are beneficial for drug molecules with poor aqueous solubility and highly metabolized by the liver. There are different types of oil-based formulations like emulsions, self-emulsified drug-carrier formulations, self-micro and self-nano emulsifying drug carrier systems, and drug solutions in oil media.

Self-emulsifying drug delivery systems are defined as oil-based formulations described like isotropic mediums including natural oils/synthetic forms, surfactants, single/multiple hydrophilic solvents, and co-solvent/surfactant. The unique property of the system is the formation of *in situ* oil-in-water emulsions upon mild agitation in aqueous body fluids. Self-emulsifying drug delivery systems are defined as stable media that improve the aqueous solubility of drug molecules in oral administration by providing a large surface area (Arslan et al., 2005). The advantages of these systems are that they increase oral bioavailability, reduce the

required dose, provide controlled drug release, allow selective drug release, and improve the intestinal lymphatic passage of drugs to reduce the first-pass effect.

Micro and nanoemulsion systems are preferred to enhance drug release performances. Indeed, nanosystems have a high surface area to volume ratio, which provides a higher loading capacity. The emulsion systems create many opportunities for drug release systems because they can be used instead of liposomes and polymeric vesicles (Masiero et al., 2021).

Micro and nanoemulsion systems

The term "microemulsion" describes the thermodynamically stable solutions obtained using different concentrations of oil, surfactants, and water. The proportion of water, oil, surfactants, and physical conditions can affect the stability of microemulsion systems. It is possible to have various separate phases having all the components in equilibrium. The morphology of obtained emulsion systems can be cylinder-like, spheroid, micelles, or plane/sponge-like, depending on the separate phases. Nonpolar tails of surfactants are assembled to form a hydrophobic core in the microemulsion system. Thanks to this configuration, contact surfaces created thermodynamically between water and nonpolar molecules can be reduced. Hydrophilic groups of the surfactant become active and protrude in the aqueous media (McClements, 2012). Lipid molecules can react with the hydrophobic interior of a micelle or between the tails of the hydrophilic group.

"Nanoemulsion" can be defined as a conventional emulsion method containing tiny particles. There are two standard methods: water in oil and oil in water. There are two immiscible liquids with thermodynamically unstable colloidal dispersion. The procedure depends on dispersing one type of liquid like little spherically droplets ($r < 100$ nm) in other kinds of liquid. Indeed, nanoemulsion systems can be obtained without using a surfactant; they generally need oil and water. But sometimes, nanoemulsions can be created with oil and water, various ratios of surfactant, and a co-surfactant as in the microemulsion technique (McClements, 2012). However, thermodynamic stability is the most distinct difference between micro and nanoemulsion models; nanoemulsions are defined as thermodynamically unstable, while microemulsions are known as thermodynamically stable systems.

Drug-loaded capsules and nano/microemulsions attract attention in the design of drug release systems. Many researchers have been focusing on oil-based cap-

sules for an effective drug release. For example, in Fardous's study, CO was used as a stabilizer, polyoxyethylene hydrogenated CO (HCO-60) was used for a novel nanoemulsion system for effective drug delivery. High drug loading efficiency was achieved with this nanoemulsion system. Therefore, they proved that organogel based nanoemulsion plays an essential role in drug delivery (Fardous et al., 2021). A new nanoemulsion system for the sustained release of brinzolamide, a medicine that treats high pressure inside the eye related to ocular hypertension, was developed. For this purpose, CO, polyoxyl 35 CO, and polysorbate 80, and gellan gum were used to prepare the nanoemulsions. In vitro drug release performances of the systems were evaluated, and globule size, zeta potential, and stabilities were investigated. The findings exhibited that the quantity of CO was essential for determining the drug loading capacities of nanoemulsions. In addition, it was proved that globule size and stability of nanoemulsion were affected directly by the quantity of CO. Additionally, Bhalerao's team showed that concentration of CO affects the formulation of their systems (Bhalerao et al., 2019). Their results demonstrated that CO with Polyoxyl 35 CO caused the increase of brinzolamide in the oil phase. In conclusion, they achieved a nanoemulsion formulation system that gives opportunities for controlled drug release.

Gunarto and coworkers reported the applications of microemulsion systems in the biomedical field, especially drug delivery. They developed systems containing plant oil, water, surfactant, and cosurfactant (Gunarto et al., 2020). In their study, CO was used as the lipid phase, while Tween 80/20 was applied as the surfactant group and glycerol/ethanol as the cosurfactant for microemulsion preparation. After mixing of 5 wt.% CO and 85 wt.% surfactants, 10 wt.% water was added for microemulsion. In this study, particle size distributions of the particles were evaluated. Also, physical measurements such as zeta potential and polydispersity index were the other parameters examined with these particles. Drug loading performances were measured with astaxanthin, the model drug, a lipophilic substance. This study showed that CO was successfully used in the development of microemulsion-based drug release systems. Similar to Gunarto's study, Boddu's team examined the microemulsion-based systems, including CO, for the solubility of diazepam (Boddu et al., 2020). In their research, CO, ethanol, and polyethylene glycol monostearate were selected as solvents because all of the reagents were approved by the FDA to prepare nasal applications. In the optimization studies, the solubility was examined, and other factors such as pH, refractive index, particle size, morphology, and thermal properties were evaluated.

A novel microemulsion system with 3% w/w diazepam was designed to treat an acute repetitive seizure. Different mediums such as CO, Tween 80, and propylene glycol were used to evaluate diazepam's solubility. Their outcomes displayed that CO could dissolve diazepam; thus, they chose CO as the oil phase.

Nguyen's research is another example of the studies concerning the oil-oil suspension loaded with nano/microparticles. Granisetron hydrochloride, a medication used to prevent nausea and vomiting due to chemotherapy, was examined for effective sustained-release parenteral formulation to increase pharmacokinetic properties. Following this purpose, Nguyen (2019) designed a new formulation that included an oil suspension loaded with microparticles. Release performances of granisetron hydrochloride were investigated. In the study, methylcellulose was used as the polymer material to have microparticles. The suspension containing methylcellulose and granisetron hydrochloride was evaluated. This research proved that microparticles containing methylcellulose could be applied to encapsulate the drug molecules with hydrophilic characters. Furthermore, supporting these systems with oil-based materials ensures a novel approach to improve controlled and sustainable drug release mechanisms (Nguyen et al., 2019).

Nanostructured lipid carriers were developed by Jawahar (2018). In this study, olanzapine was used as the model drug molecule. It has poor dissolvability in aqueous solvents and low oral bioavailability. Nanostructured lipid-based carriers were believed to overcome these solubility problems. CO, glyceryl tripalmitate, and Pluronic F-68, Soylecithin were used as liquid lipid, solid lipid, and surfactants, respectively. Lipid-based nanostructured drug carries were successfully synthesized, and the systems were loaded with the lipophilic model drug, olanzapine, effectively. This study evidenced that this drug carries increased the bioavailability of the hydrophobic drug molecules. Ewonkem (2015) synthesized bolaamphiphilic molecules from CO. When self-assembled in aqueous media, the new bolaamphiphilic compounds can be obtained like nano-sized vesicles that demonstrated stable spherical cationic characters. Water-soluble drugs were expected to be an effective drug carrier when loaded into vesicles prepared in this systematic way.

Conclusion

This review focused on the studies concerning the usage of CO for drug delivery and controlled release systems. The studies involving castor oil-based bulk systems, self-emulsifying drug delivery systems, and micro and nanoemulsion

systems were evaluated. Micro and nanoemulsion systems are mostly preferred for the enhancement of drug delivery systems. Nanoemulsion systems have some advantages over microemulsion systems, such as a high surface area to volume ratio. They can successfully be used instead of liposomes and vesicles. Microsystems need thermodynamically stable isotropic liquids, and these liquids can be obtained by mixing oil, water, and surfactants. The studies regarding castor oil-based drug delivery systems underline the significance of the proportion of water, oil, and surfactants for the formation of nano or microemulsions. Moreover, the morphology of the emulsion systems can be varied, such as cylinder-like, spheroid, micelles, plane/sponge-like according to the reactions between nonpolar groups and water molecules. In summary, this review demonstrates that novel and eco-friendly drug delivery systems based on castor oil can be successfully developed for biomedical applications.

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6

SYNTHESIS AND CHARACTERIZATION OF BIO-BASED POLYALLYL AMIDES

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ABSTRACT

Polyamides one of the most used engineering plastics. They are widely used for many industrial applications from construction to medical industries. Especially, nylon 66, nylon 6 and nylon 11 are produced in large quantities. The main route is the condensation reaction between free amino and carboxyl groups. For this purpose, equimolar amine compound and carboxyl compounds mixed and heated at elevated temperature. Except nylon 11, other polyamides are unfortunately petroleum based compounds. Due to the depletion of the petroleum reserves, environmental concerns and sustainable development, there is great need to find bio derived polyamides. In this work, synthesis of polyallyl amide is demonstrated. For this purpose, methyl oleate was synthesized first and then this compound was converted to congruent allylic bromides. After amination of this brominated compound, the resultant amino ester was polymerized at 240 °C. FTIR and ^{1}H NMR techniques were used for characterization of the monomer. Thermal properties of the polyamide were also evaluated.

Keywords: *Renewable resources, fatty acid methyl esters, polyamides, es-*

ter-amide interchange, olive oil

INTRODUCTION

Polyamides are one of the most used engineering plastics. They are mainly used in textiles an automotive industry. Additionally many goods such as, kitchen utensils, sportswear and carpets can be made of polyamides due to their high strength and resistivity. Polyamides can be natural or synthetic. Proteins are the example of the naturally occurring polyamides [1-3].

In ındustry polyamides can be synthesized via condensation reactions. Carboxylic acid or their derivatives can be used with a diamines. At the end of the reaction water produced as a side product and should be removed from the medium. Beside this reaction ε-caprolactam can be produced via ring opening polymerization [4-5].

The main source of synthetic polyamides is petroleum. Due to the depletion of petroleum reserves and environmental concerns, new routes and sources for polyamide production is getting great attention. For the sake of sustainable development, it would be vital to find an alternative renewable resource in order to produce polyamides in industrial scales. Plant oil triglycerides would seem to be an alternative resource for the synthesis of many compounds and polymers. Plant oil triglycerides are complex compounds that poses many reactive sites. They can be produce vast amount and with a high purity. In this work starting from olive oil and soybean oil, fatty acid methyl esters are synthesized first and allylic bromination occurs on those fatty acid methyl ester. When these bromide derivatives are reacted with excess ammonia, they readily turn to reactive amino ester. If this ester is heated to high temperature such as 240 ^{o}C, it would be converted to congruent polyamide [6-12] (Figure 1-2).

MATERIALS AND METHODS

Soybean oil and olive oil used were food grade and they were dried in vacuum oven before use. NBS (N-bromo succinimide), CC_{l4}, and $CHCl_3$ were purchased from Merck and they were used as received. 7 N NH_3 in methanol was purchased from Aldrich and used as received.

IR characterization of compounds was performed by Perkin-Elmer FT-IR 1600 series spectrometer using KBr windows. The ^{1}H NMR spectra were recorded on a Varian 400-MHz NMR instrument (Varian Associates, Palo Alto, CA) operating at a frequency of 399.986 MHz for proton and 100.587 MHz for carbon.

The spectra were recorded as ppm (δ) with CDCl3 as a solvent. DSC characterizations were performed by "Thermal Analyses" Q 200 instrument (New Castle, DE, USA) with a heating rate 10 °C / min under nitrogen atmosphere from -70 to 120 oC. TGA characterizations were performed by "Thermal Analyses" Q 50 instrument (New Castle, DE, USA) with a heating rate 10 °C / min under nitrogen atmosphere from room temperature to 600 °C.

RESULTS AND DISCUSSIONS

The simplest amine that one can use in the substitution of a halide is ammonia. Reaction of simple alkyl halides with ammonia often gives a mixture of the primary secondary, and tertiary amines, and quaternary ammonium salts. To prevent obtaining a mixture of amines ammonia should be used as high as 70-100 per cent excess of alkyl halide.

Ammoniac solutions unfortunately, cannot be used for the preparation of allylic amines of methyl oleate and soy oil methyl esters because of the hydrolysis of the methyl esters in the basic solutions. For this reason, it was preferred to use 7N NH_3 in dry CH_3OH solution. In the literature, it was found that some benzylic halides which contained 12 or more carbon were reacted with this reagent at room temperature for two hours and gave primary amines at 85 percent yields. Rest of the product composed of secondary amines [13].

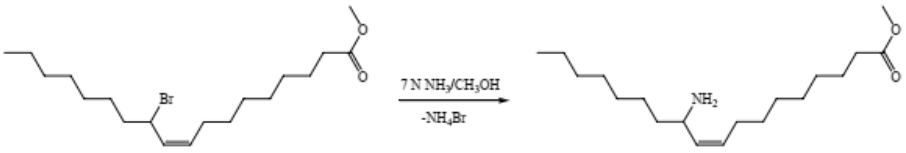

Figure 1. Synthesis of allyl amine of methyl oleate

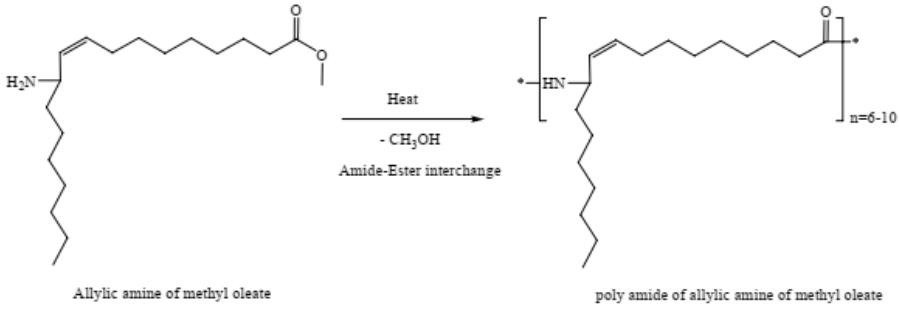

Figure 2. Polymerization of allylic amine of methyl oleate.

This procedure was also suitable for the amination of the methyl oleate. Methyl oleate was also soluble in this reagent and this would facilitate the reaction. In the first trial, methyl oleate was stirred with this reagent at room temperature for 24 hours and it was observed that just 5 percent of methyl oleate underwent ester amide exchange and the rest was aminated as desired. In other reactions, ABMO and ABSOME were stirred with 7 N NH_3/CH_3OH different time intervals such as 2, 4, 6, and 24 hours. ^1H NMR showed that when the reaction time were increased, yields of the reaction also increased. After 24 hours approximately 100 per cent yields could reach.

Allylic amines of the methyl oleate and soy oil methyl esters were characterized by IR and 1H NMR. In the IR spectrum at 1670 cm^{-1} a weak peak was observed due to the NH deformation vibration. At 1090 a medium peak was observed due to the C-N vibrations of the allylic amines. Peak at 963 cm^{-1} was shifted to 970 cm^{-1} because of the change of the environment of the allylic positions. Peak at 650 cm^{-1} disappeared due to the lost of bromine atom in the molecule. There is new peak observed at 845 and 880 cm-1 probably due to the out of plane bending of the N-H bonds. Peaks at 784 and 767 cm^{-1} disappeared also (Figure 3).

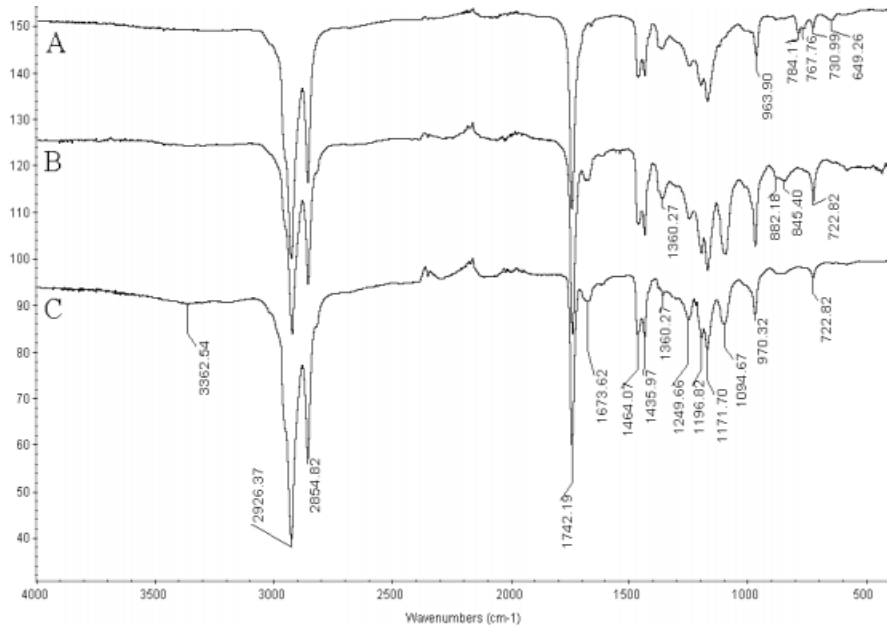

Figure 3. IR spectra of (A) allylic brominated methyl oleate, (B) allylic amine of methyl oleate, and (C) allylic amines of soy oil methyl esters.

In the ^{1}H NMR spectra it was easier to follow the changes. Few changes were observed. Peak at 4.4 ppm disappeared and new peaks were observed at 3.4 ppm (-C**H**-NH_2) and 3.2 ppm (-CH_2-N**H**$_2$). The shape of allylic protons in the NMR spectrum also changed. A new peak was observed in the ^{1}H NMR spectrum of the allylic amine of the ABSOME at 4.2 due to the amine at the double allylic position. The ratio of the peak intensities at 3.2 to 3.4 gave 2 which was the proof of the structure (Figure 4)

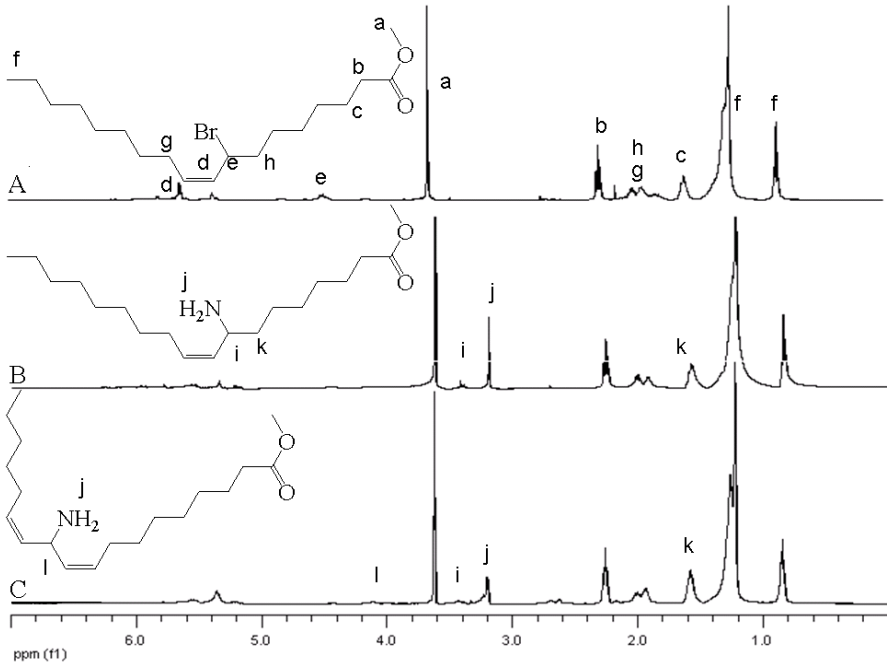

Figure 4. 1H NMR's of (A) allylic brominated methyl oleate, (B) allylic amine of methyl oleate, and (C) allylic amines of soy oil methyl esters

Allylic amines were also polymerized without catalyst. Monomers were heated to 120 under N_2 for 4 hours than temperature was raised to 180 ^{o}C for 12 hours. Then materials heated to 200 ^{o}C. For 10 hours. After the process slightly cross linked polyamides were obtained. TGA of the reaction mixture was also strengthening these findings. In the TGA graphs, the weight lost peaks of the materials shifted to higher temperatures (Figure 5). Five per cent weight lost temperatures were also shifted from 137 ^{o}C to 187 ^{o}C.

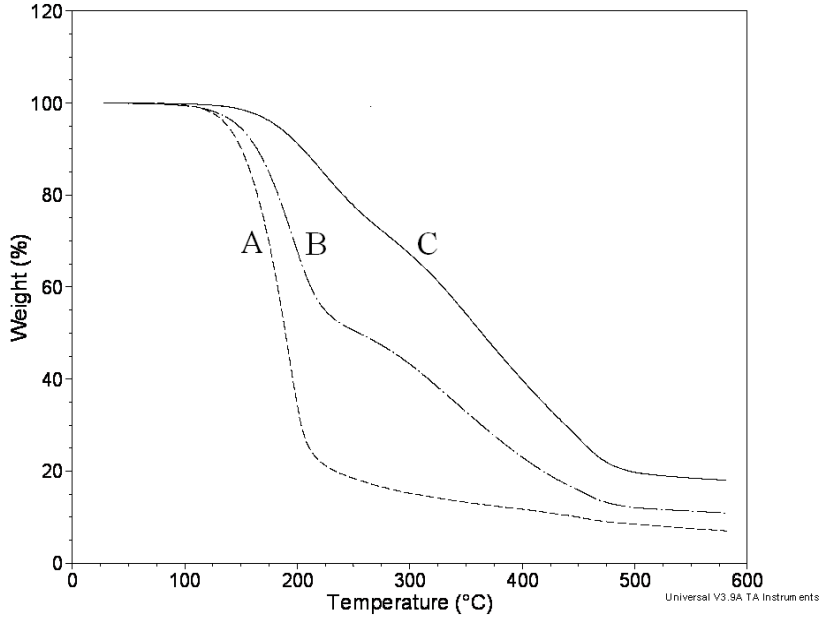

Figure 5. TGA traces of (A) AAMO (B) PAAMO at 180 °C and (C) PAAMO at 200 °C

In the IR spectra the characteristic peaks for the polyamides were observed. At 3470 cm^{-1} a peak observed due to the NH stretching. Peaks were detected at 1662 and 1625 cm-1 due to the CO absorption (Amide I band). A weak peak was observed at 1535 and 1380 cm^{-1} due to 125 the NH deformation (Amide II band). Peak at 1090 cm^{-1} of the allylic amines disappeared. A new peak was also seen at 1005 cm^{-1} probably due to the NH stretching of secondary amides (Figure 6)

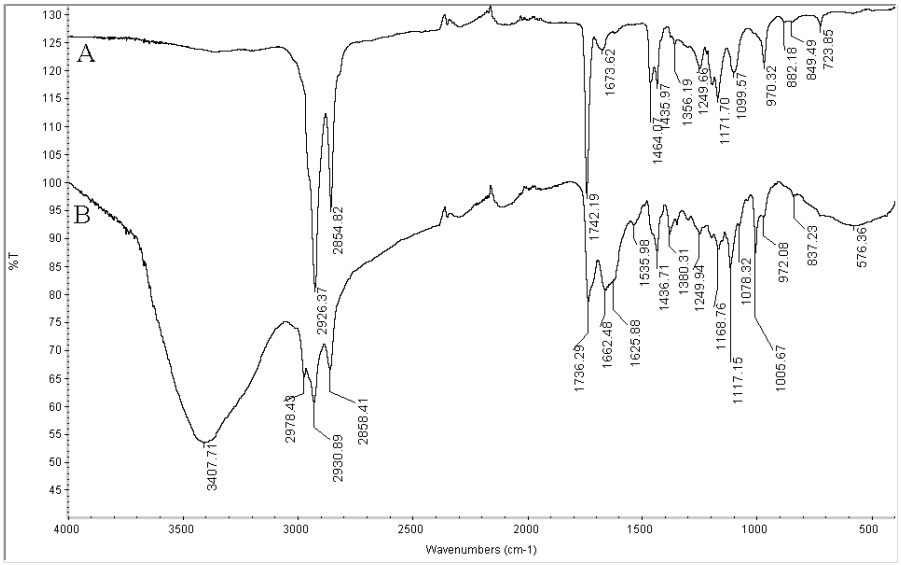

Figure 6. IR spectra of (A) Allylic amine of methyl oleate (AAME) and (B) polyamide of AAME

The polyamides produced did not show any good mechanical and thermal properties. They are gummy materials which molecular weights around 2000 Dalton with a 2, 5 PDI. This molecular weight indicated that the number of the repeating unit is around 7.

CONCLUSSIONS:

This work reveals an alternative way for polyamide synthesis. As a renewable resources, fatty acid methyl ester were used. Although soybean oil and olive oil was used in this study, used cooking oils can also be used for the polyamide synthesis. Synthesized polymers do not show the same mechanical or thermal properties of the industrial polyamides but with the aid of future modifications, those properties of the synthesized bio based polyamides can be improved. Additionally, the polyamides synthesized contain double bonds and further modifications can be performed by using these bonds.

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7

SYNTHESIS AND CHARACTERIZATION OF MONOGLYCERIDES VIA BORIC ACID ESTER ACIDOLYSIS

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ABSTRACT

Monoglycerides are mono esters of glycerol and a fatty acids. They are used for vast varietyy of applications. Monoglycerides are actually natural surface active agents. They have polar head and non-polar tail. They can be obtained by transesterification reaction according to the conventional synthesis. Desired triglyceride is mixed with excess glycerol in the presence of a catalyst then heat is applied to the mixture during the synthesis. After a certain period of time, monoglycerides are obtained. Reaction temperature is usually 220 °Cor higher and reaction time is generally 24 hours. Yields of the reaction is usually 60 %. In this work a novel method is introduced. Synthesis of monoglycerides composed of two

steps. Synthesis of boric acid esterof glycerol is the first step. Reaction is carried at 125 °C and completed in 2 hours. Acidolysis of triglyceryl borate with free fatty acid is the second step of the synthesis. Second step is also completed in 2 hours and reaction temperature is between 150-170 °C. Overall yield of the reaction is 90% or more. When considering the environmental impact, energy conservation and purity of the materials. The last method seems as a better method than the conventional methods. According to our knowledge this is the first report in the literature.

Keywords: Glycerol, fatty acids, boric acid, acidolysis, renewable resources

INTRODUCTION

Plant oil triglycerides are one of the important renewable resources. Triglycerides are tri ester of fatty acids and glycerol. Structure of a typical triglyceride is shown in figure 1. Most common fatty acids that can be found in the triglycerides are shown in figure 2. Fatty acids have even numbered carbon chain and carbon number generally varies between 10-20. Especially 18 carbon containing fatty acids are dominant. Sometimes plant oil triglycerides may contain unsaturated fatty acids. Oleic, linoleic and linolenic acids are three of the most common unsaturated fatty acids. Structure of those acids are shown in figure 1.

They are readily available, easily modified and many types of reactive materials can be produced by using simple reactions [1-4]. In a typical triglycerides 4 main reactive positions can be observed. Those are i-double bonds ii-allylic positions iii-ester groups and iv- **α methylene groups to ester carbonyls** (Figure 1). By manipulating of these groups, one can synthesize many types of valuable compounds and polymers [5-7]. It should be taken into account that during the functionalization of those reactive parts, some side reactions may appear and that would cause disruption of other reactive parts. Among to those reactive parts, modification of double bonds and ester groups have drawn special attention. It is relatively easy to produce any monomer or polymer by using those groups.

Alkyd resins that are obtained with the modification of ester groups are mainly used for painting industries. Although ester moiety is changed in alkyd resins, other reactive parts still remain in the product.

Other industrially important groups of compounds are mono and diglycerides. They are generally obtained via acidic or basic transesterification reactions [8-9]. For a typical industrially acceptable reaction, plant oil triglycerides are trans esterified with excess glycerol via base or acid catalysts. Reaction times are usually 24 hours and reaction temperature are usually 220 ^{o}C or above. Typical yields are low and varies between 40 % and 60 %. Although some enzyme catalyzed reaction are reported. They are not readily applicable to industrial scale. With the aid of the work explained in this chapter, a new and relatively simple method is introduced. The synthesis is consists of two steps. Synthesis of boric acid glycerol ester is the first step and the reaction of this ester with free fatty acids is the second step. When compared with the conventional methods, the new method requires shorter reaction time and lower temperatures. According to the best of our knowledge this work is the first example in the literature.

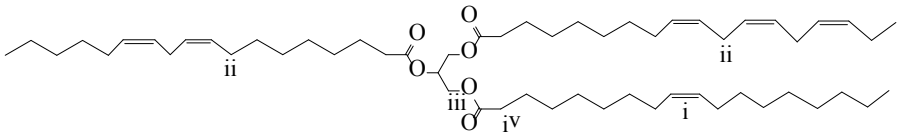

Figure 1. Reactive parts of a typical plant oil triglyceride

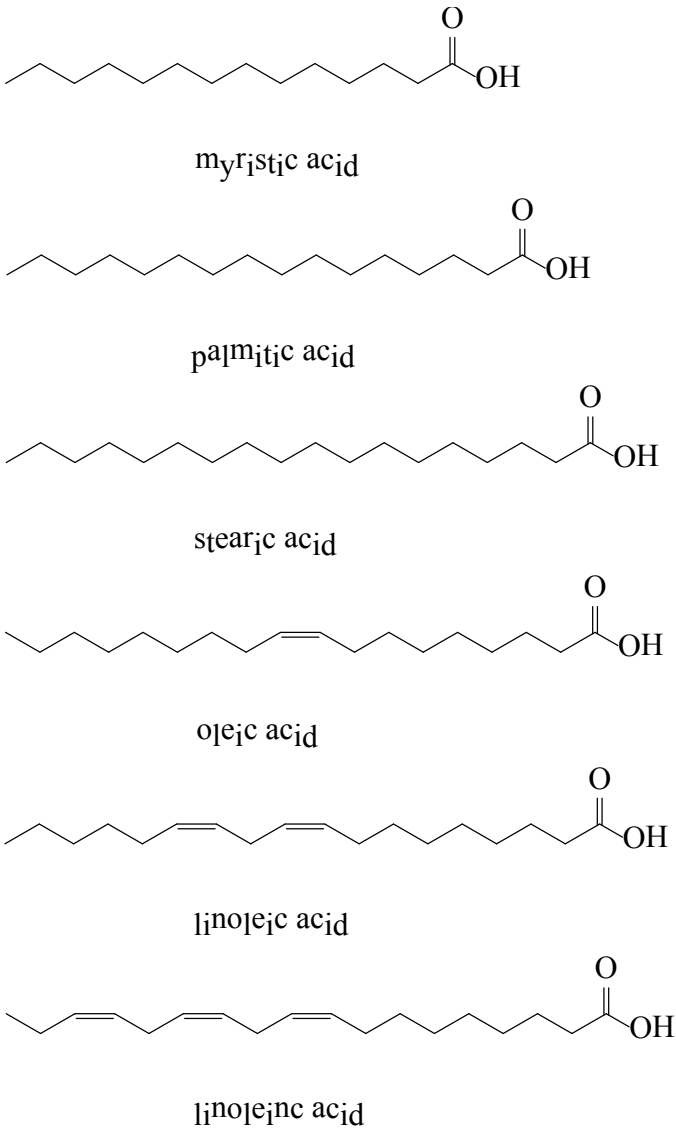

Figure 2. Structure of common fatty acids found in triglycerides

MATERIALS AND METHODS

Stearic acid, boric acid, glycerol, sodium sulfate (dry), dichloromethane and toluene were purchased from Merck and they were used as received. Glycerol is dried in an vacuum oven before using.

IR characterization of compounds was performed by Perkin-Elmer FT-IR 1600 series spectrometer using KBr windows. The ^{1}H NMR spectra were recorded on a Varian 400-MHz NMR instrument (Varian Associates, Palo Alto, CA) operating at a frequency of 399.986 MHz for proton and 100.587 MHz for carbon. The spectra were recorded as ppm (δ) with $CDCl_3$ as a solvent. DSC characterizations were performed by "Thermal Analyses" Q 200 instrument (New Castle, DE, USA) with a heating rate 10 °C / min under nitrogen atmosphere from -70 to 120 °C. TGA characterizations were performed by "Thermal Analyses" Q 50 instrument (New Castle, DE, USA) with a heating rate 10 °C / min under nitrogen atmosphere from room temperature to 600 °C.

Synthesis of Triglyceryl Borate

For a typical procedure, 0,1 mol of boric acid (6,183 g) and 0,3 mol of dried glycerol (27,63 g) are mixed 250 ml round bottom flask. 150 ml toluene is added to the mixture and reflux condenser and dean stark apparatus are attached to round bottom flask. System refluxed and refluxing is continued till all water is collected at dean stark apparatus. Reaction stopped and toluene is removed by simple distillation.

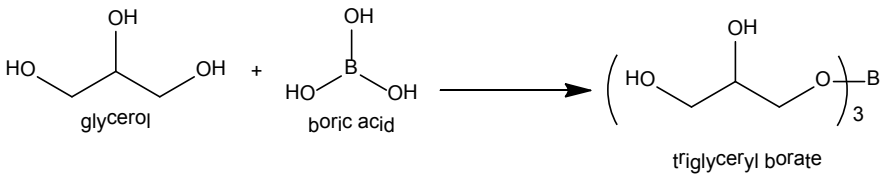

Figure 3. Synthesis of triglyceryl borate

Acidolysis of Triglyceryl Borate

For a typical reaction, 0,1 mol triglyceryl borate (10 g) and 0,15 mol stearic acid (42,67 g) and 1,28 g p-toluenesulfonic acid are mixed and temperature is

raised to 170 °C and the mixture is kept for 2 hours. Then the cooled mixture was dissolved in 100 ml dichloromethane. Washed with several times with 100 ml tap water. Then the mixture dried over anhydrous sodium sulfate and dichloromethane evaporated. The yield is 97 % according to fatty acid.

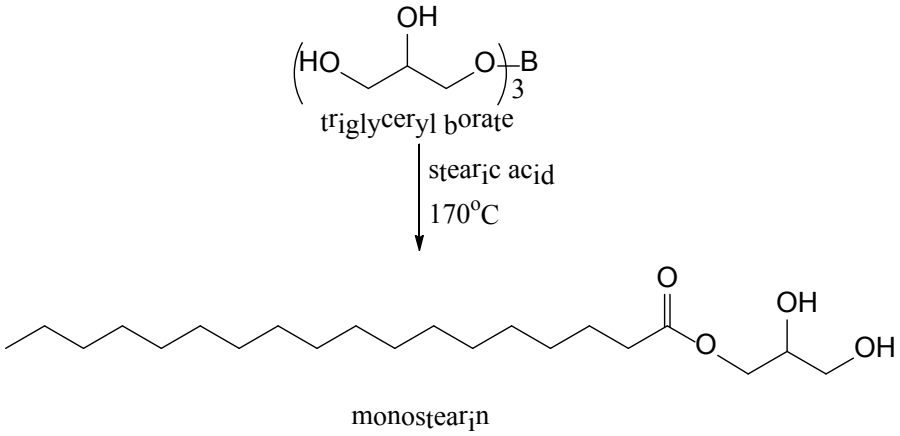

Figure 4. Acidolysis reaction of triglyceryl borate with stearic acid

RESULTS AND DISCUSSIONS

When boric acid mixed with glycerol in toluene, congruent triglyceryl borate ester forms easily. The reaction can be monitored just measuring the amount of evolved water. At the boiling point of toluene water makes azeotrope and this azeotrope swipe the produced water at the end of the reaction. While the azeotrope is condensed water droplets separates and deposited at the end of the side arm of the dean- stark apparatus. That reaction usually takes 2 hours. After reaction completed, boric acid ester layer seen as a viscous and colorless liquid. Due to the viscosity difference toluene is easily separated. Boric acid esters are actually a Lewis acids and when they mixed with a suitable reagents such as carboxylic acids, they readily turn to boric acid and an ester of carboxylic acid. Niyazi Bıçak and et al had many works about this reaction [10-11]. When triglyceryl borate mixed with stearic acid and p-toluene sulfonic acid, monostearin is obtained in 2 hours and the yield reaches to 97%. The material synthesized easily characterized with FTIR, ^{1}H NMR, DSC and TGA technique. According to results the

obtained monostearin is a mixture of α and β monostearin. The structures of α and β monostearin is shown in figure 5

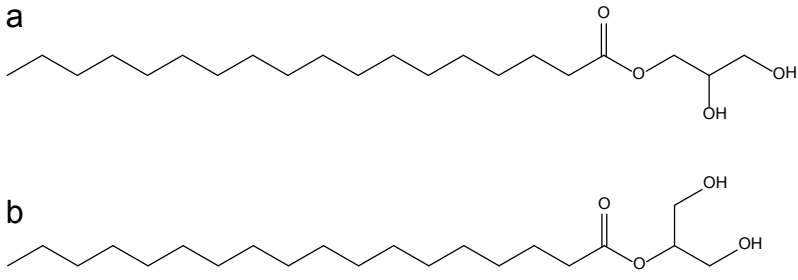

Figure5. Structures of a-α monostearin and b- β monostearin

When FTIR spectrum analyzed some characteristic peaks are observed a relatively small –OH peak observed at 3000-3500 cm^{-1} region and a peak at 1740 cm^{-1} belongs to an ester carbonyl that peak cannot be observed in the stearic acid or glycerol spectrum

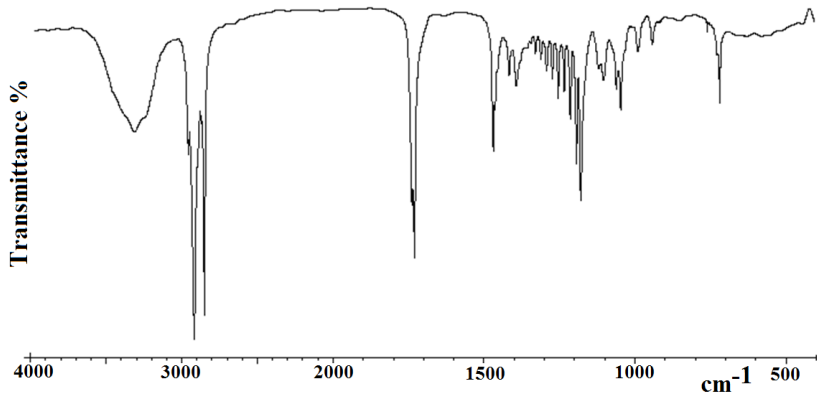

Figure 6. FTIR spectrum of monostearin

Additionally, ^{1}H NMR spectrum of monostearin gives valuable information, hydrogen of glycerol moiety appear at 3,5-3,7 and 4,0 ppm. Hydrogen of ester group appear at 4,2 ppm. The peak of α methylene hydrogens appear at 2,3 ppm. If there are free hydroxyl groups a peak should be observed around 3 ppm. If un-

reacted stearic acid would be present, a small peak should be observed at 10 ppm. Those peaks did not observed.

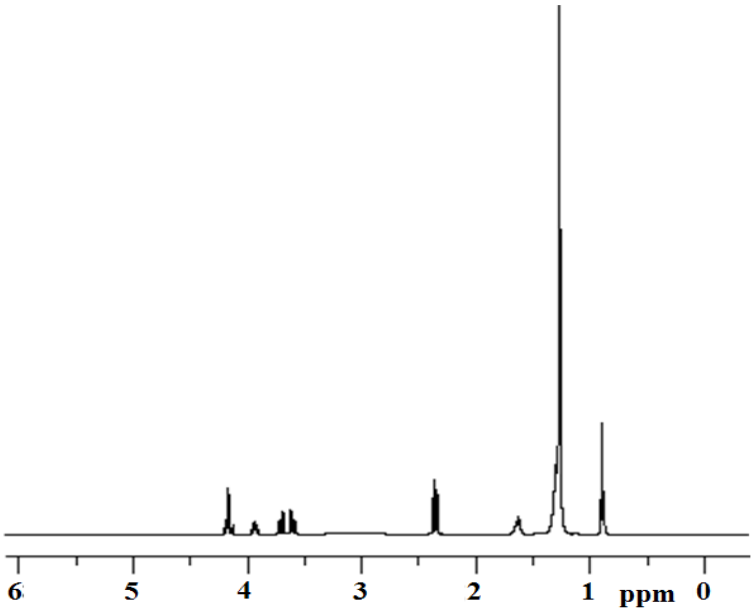

Figure 6. ^{1}H NMR spectrum of monostearin

Thermal properties of the synthesized monostearin also evaluated for this purpose TGA (thermogravimetric analysis) and DSC (Differential scanning calorimetry) techniques were used. DSC traces were congruent with literature and a melting peak was observed at 60 ^{o}C. When material cooled a two closed peak was also observed due to the presence of a-α and β monostearin. TGA trace was also suitable with literature a smooth degradation curve was observed and after 400 ^{o}C all material vaporized.

CONCLUSIONS

This study reveals an alternative and better monostearin synthesis methodology. The synthesis contains two steps. At the first step, boric acid glycerol ester synthesized. The reaction completed in 2 hours. Acidolysis of the triglyceryl borate with stearic acid also completed in 2 hours. Compared to conventional methods this method showed better results. In a typical conventional method,

monoglycerides are synthesized at 220-240 ^{o}C and 24 hours reaction time is required. When considering environmental concern and sustainable development. Synthesis of monoglycerides via boric acid ester acidolysis reaction seems advantageous method when compared to conventional synthesis. Moreover, pure monoglycerides can be synthesized with new method. Triglycerides contain mixture of fatty acid, thus pure monoglycerides of a one fatty acid cannot be considered when they prepared via conventional techniques.

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8

OPTIMIZATION OF EXTRACTION OF PHENOLICS FROM *MORINGA OLEIFERA* LEAVES USING RESPONSE SURFACE METHODOLOGY

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Keywords: *Phenolics, optimization, Moringa oleifera; Homogenizer-assisted extraction*

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Abstract

Today's Green Chemistry is pushing for the search and application of novel methods for the extraction of bioactive ingredients from biomass, which are directly related to human health such as food, pharmaceutics and cosmetics. Therefore, the purpose of this study is to propose a green extraction method by application of automated solvent extraction (HAE) from the leaves of *Moringa oleifera.* Central composite design (CCD) together with Response Surface Approach (RSA) was exploited for designing of experimental work, modelling, and optimization reasons. Extraction time (30-90 sec), mixing speed 5000-9000 rpm and ethanol (solvent) concentration (30-90 %, v/v) were the process factors, which were analysed statistically by means of CCD. The best conditions to attain the greatest total phenolic (TPC) and flavonoid (TFC) contents (17.51 mg-GAE and 271.3 mg-CE per g dried sample) are 77 sec of extraction time, 9000 rpm of mixing speed and 30% (v/v) ethanol solution. The generated second-order models were satisfactory, which was confirmed by validation study (< 2%). In order

to verify the findings of the antioxidant activity of the extracts, 2.2'-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid diammonium salt (ABTS), and 2,2-diphenyl-1-picrylhydrazil (DPPH) tests were also conducted. A positive and satisfactory correlation (> 0.90) between test results tests the accuracy of the results.

Introduction

Background

Moringa Oleifera tree grows in tropical and semi-tropical areas of the world where it is found in India, which is the homeland of this tree. Moringa can withstand the fluctuations of different weather conditions from hot dry to frost. Moringa plant is called the Miracle tree as well.(Ezejindu D N, 2014)

The plant is characterized by the fact that each part of it has a high nutritional value. Moringa is said to provide seven times more vitamin C than oranges, ten times more vitamin A than carrots, seventeen times more calcium than milk, nine times more protein than yoghurt, fifteen times more potassium than bananas and twenty five times more iron than spinach(J. L. A. Rockwood, B.G.,Casamatta, D.A., 2013), where the leaves are low in fats and carbohydrates and rich in minerals, iron and vitamin such as vitamins B(Foidl N., 2001).

A wide range of pharmacological properties such as anti-inflammatory, antitumor, antimicrobial, hypotension, hypoglycemia and hypoglycemic acid were reported for Moringa Oleifera leaves in scientific literature(Jed W. Fahey, 2005a). In another study, the scientific use of Moringa Oleifera leaves as an ethnic drug for the treatment of diabetes was scientifically verified("Effect of Moringa oleifera Lam. leaves aqueous extract therapy on hyperglycemic rats," 2009). Moringa seeds have been reported to have potential asthma activity, possibly due to their gastrointestinal expansion, mastectomy, and anti-inflammatory and antimicrobial properties("Investigation into the mechanism of action of Moringa oleifera for its anti-asthmatic activity," 2008).

Particularly, leaf of this plant is considered one of the most important parts due to its nutrients and bioactive materials such as phenolic compounds and flavonoids that give it anti-disease properties. Therefore, it has been used in the treatment of many diseases like cancer, type two of diabetes, chronic diseases,

and in the treatment of viral and bacterial diseases. Furthermore, it has been also used in the manufacture of cosmetic products(Anwar, Latif, Ashraf, & Gilani, 2007).

There are many factors that affect the qualitative and quantitative composition of Moringa Oleifera leaves such as the growing area, climatic factor, planting season, fertilizer, harvest time and method, leaf cleaning method, drying method and extraction methods. Physical factors such as humidity, temperature, light, storage method, storage time can influence the concentration of phenolic compounds, flavonoids and antioxidants extracted from the Moringa Oleifera leaves. In addition, various physical factors such as solvent type, solvent concentration, ratio of solvent to solute, particle size, extraction temperature, extraction time, and pH can affect the efficiency of the extraction process of bioactive compounds from Moringa oleifera leaves. Therefore, extraction methods and conditions were studied to improve, evaluate and stabilize phenolic compounds and flavonoids in *Moringa oleifera* leaves(Nweze & Nwafor, 2014; Shin, Ryu, Liu, Nock, & Watkins, 2008).

Problem Statement

At recent days, there are many diseases and health problems that need to be treated. Major part of people resorts to the use of chemically synthesized medicines. To the most of their problems they find the answer in using medicinal natural herbs. It is believed that in Moringa, huge number of people will find the resort to solve a lot of medicinal and malnutrition problems.

Therefore, the aims of this thesis:

- ✓ extract the bioactive compound such as phenolic compounds and flavonoid from Moringa Oleifera leaves by using environmentally friendly and economically solvents.
- ✓ using different modern extraction methods to extract the bioactive compounds from Moringa Oleifera leaves.
- ✓ Determined and selected the good extraction conditions that important to recovery the bioactive compounds.
- ✓ Determined the optimum conditions to obtain optimal extracts of bioactive compounds.

Literature Review

Phytochemical

Phytochemicals are physiologically active plant components found in fruits, vegetables, cereals, and other plant foods that aid to lessen the risk of chronic illnesses(Craig, 1997). The term "phyto-" comes from the Greek word "phyto," which means "plant". Phytochemicals, plant components with various bioactivities for biochemistry and metabolism in human life, are employed as nutritionally active components in meals. Carotenoids, phenolic compounds, organosulfur compounds, nitrogen-containing compounds, and alkaloid compounds are examples of phytochemicals. Phytochemistry is a branch of chemistry that studies the chemistry of plants, with a focus on the biosynthesis, extraction, separation, structural identification, and bioactivities of several classes of secondary metabolites(Boyer & Liu, 2004).

Moringa Oleifera

Moringa oleifera grows in tropical and subtropical regions. India is the original home of these plants. Recently, the presence of this plant has spread in many countries of the world. This plant is called as the miracle tree (Abdull Razis, Ibrahim, & Kntayya, 2014) . All parts of this plant can be used, since it contains very important nutrients such as vitamins, proteins, calcium, fats, zinc, copper, magnesium, sodium and iron (Moyo, Masika, Hugo, & Muchenje, 2011).

Particularly, leaf of this plant is considered one of the most important parts due to its nutrients and bioactive materials (Abdull Razis et al., 2014). Therefore, it has been used in the treatment of many diseases like cancer (Tiloke, Anand, Gengan, & Chuturgoon, 2018), type two of diabetes (Kumari, 2010), chronic diseases (Vergara-Jimenez, Almatrafi, & Fernandez, 2017), and in the treatment of viral and bacterial diseases (Anwar et al., 2007). Furthermore, it has been also used in the manufacture of cosmetic products.

Cultivation and Planting of Moringa Seeds

In Sudan(Samia Al Azharia Jahn), traditional cultivation of Moringa Oleifera focused on seed propagation, whereas vegetation propagation is very common in India, Indonesia and parts of West Africa.

In the northern Sudan sowing was tested during the cool dry, the hot dry sea-

son and the rainy season. The pretreatment of Moringa Oleifera seeds has no advantage at any time of the year. On the contrary, cooling to 8°Ccaused slight delay in the onset of germination and minor reduction of germination frequency, but there was evidence that cooling to 8°Cis good for germination of Moringa Long tuba seeds.(Samia Al Azharia Jahn)

The optimum light condition for germination of Moringa Oleifera was half shade, germination frequencies of Moringa Oleifera amounted to only 40 and 52 percent in full light as compared with 92 and 94 percent in half shade also the average and maximum heights of Moringa Oleifera were 1.7-2.2 times higher in half shade than in full light. In addition, the growth of seeds in half shade takes place in the dry cool season faster than in the hot dry season. Moreover, the seedlings should not be transplanted too early. The first flowers appeared after two and half year.

Adequate watering is important for example: under watering is harmful as leaving the young plants for days in pools of stagnating water. Satisfactory growth of the transplanted seeds depends on suitable spacing and adequate watering.

In India and tropics with rainfall of between 250mm and 3000mm. It is drought resistant; through in drought condition it may lose its leaves. This does not mean it is dead and it should recover when the rains arrive. It grows best at altitudes up to 600 m but it will grow at altitude of 1000 m. Moreover, Moringa need a temperature range of 25°C to 40°C but can be tolerate temperature of 48°C and light frosts. (Thurber MD, 2009)

Moringa was grown in India has slightly different nutritional values than it grown in Nigeria. Many studies were made on the nutritional differences in the leaves from the ecological locations semi-deciduous and Savannah regions. It was found that in Savannah regions the nutrition's were less this is due to high temperatures in this region. The proteins and enzymes get denatured and will be affected by high temperatures(William Jasper Asante, 2014).

In addition, soil is an important factor that defines the nutrient content and strength of the plant(S. O. Dania, 2014). Moringa Oleifera prefers neutral to slightly acidic soils and grows best in well-drained loam-to clay-loam. It tolerates clay soils but does not grow well if water logged. Moringa prefers this tropical climate(S. O. Dania, 2014).

In Senegal(Caroline Olivier, 2013), at the present time and since September

2001, a Moringa farm is cultivated at a site located 300 kilometers north of Dakar, department of louga. Apiece of land situated 200 m from an irrigation canal. A soil analysis showed that the land was free of salt and has satisfactory pH balance (between 7 and 8).

In order to determine the optimal spacing of the Moringa trees, so as to obtain the highest possible quantity of green matter, a study was analyzed comparing production rates of plant densities, ranging from one-by-one meters 10,000 plants per hectare, spacing of 2.5 x 2.5 cm, 16 million plants are hectare. Also, the optimal planting density in soil that is sandy, fertile and well-drained is 10 x 10 cm, one million plants per hectare.(Foidl N., 2001)

Selection of planting density depends on the production objectives. For example, if the objective is production of green fodder with a maximum protein and minimum lignin content, then harvesting should be done every 33 to 40 days. If the objective is to produce a maximum lignocelluloses fibers paper pulp, the harvesting would ideally be done after six to eight months growth.(Caroline Olivier, 2013)

Before planting, plowing of land to a depth of 50 cm, then manure application (60 T/ha), installation of drip irrigation system (Dip holes distanced at 20 cm), purchase of gasoline powered motor pump, seeding seeds spaced at intervals of 10 cm. According to fertilization it was found that best performing results with water soluble fertilizer 21/7/20(NPK), enriched with minerals and trace elements. The amount of fertilizer applied to the hectare was between 32 and 48 kg per week, depending on the visible conditions of the plants. In addition to NPK fertilizer, urea was used at the rate of 8 kg to the hectare every two weeks.(Caroline Olivier, 2013)

Quantity of water used according to season:(Caroline Olivier, 2013)

* Rainy season: 72,000liters/ha/day, at the rate of one hour with one bar of pressure.

*Dry season: 108,000liters/ha/day, at the rate of an hour and a half watering with the same pressure.

It requires 8 kg of fresh leaves (with stems removed) to produce one kg of dried leaves. Harvesting is every two months during the year until the plants reach a height of 1 m.

Technical document (Caroline Olivier, 2013)

Irrigation method: Drip tubes

Soil: 95% sand

Number of plants/ha: 1,000,000 plants. After 2 years, about 600,000 surviving

Planting distance: 10cm

Distance between drip holes: 20cm

Harvesting method: Manual (hand)

Drying method: 250 drying shelves protected from sunlight in drying room

Irrigation: Rainy season: 72,000liters/ha/day

Dry season: 108,000liters/ha/day

Fertilization: 16-48 kg/ha/week of NPK (21-7-20) and 8 kg year/2 weeks.

General Nutritional and Medicinal Values

The general uses of Moringa Oleifera:

Based on many experiments and studies, the research shows the uses of *Moringa Oleifera* in many applications, including simple and other complex starts from the use of this plant as a food source enters the food and ends in pharmaceutical and other industries.

All parts of the *Moringa Oleifera* tree (from seeds, fruits, flowers, leaves and pods) are edible and have long been consumed by humans. There many uses of *Moringa Oleifera* tree such as: medicines, human food, water purification, animal fodder, alley cropping, fertilizer, living fence, domestic cleaning agent, fuel wood and other uses. The *Moringa Oleifera* plant increases the energy of the human body because it contains many nutritional elements of vitamins, protein and others, where seeds and leaves are the most used parts in many applications because they contain many important nutrients. Leaves and seeds are used as a useful source of materials food, medicine, sterilization, cleaning dirty water, planting the alley and other applications.(Jed W. Fahey, 2005b)

Natural medicines:

Moringa plant is a plant that treats and prevents many diseases such as ane-

mia, cancer and others, where more than 300 kinds of diseases are treated, because they contain many nutrients(Gedefaw, 2015).

Various parts of the Moringa plant are used as circulatory stimulants, anti-heart disease, antiepileptic, antipyretic, antiulcer, anti-inflammatory, antispasmodic, anti-hypertensive, diuretic, antioxidant, anti-diabetic, cholesterol lowering, antibacterial, hepatoprotective and antifungal activities. Table 1 shows the diseases treated by each part of the Moringa plant.

Moringa leaves are one of the most widely used parts of the medical field for treatment of diseases after being converted into a powder called Moringa powder contains many vitamins , nutrients and chemicals(Gedefaw, 2015).

❖ **Leaves:**

The leaves are used either fresh or dried, where they are dried and grinded in a manner suitable for the manufacture of tablets or capsules used to treat many diseases, including hyperglycemia, asthma, flu, heart burn, Dyslipidemia, malaria, syphilis, diarrhea, pneumonia, scurvy, headaches, bronchitis, skin diseases, eye and ear infections. Also reduces, blood pressure and cholesterol and acts as an anticancer, Antioxidant, antimicrobial, anti-atherosclerotic and ant diabetic agents, neuro protectant.(Lakshmipriya Gopalakrishnan, 2016)

❖ **Seeds:**

Seeds of Moringa help in treating Chrohn's disease, hyperthyroidism, rheumatism, anti-herpes simplex virus arthritis, cramp, epilepsy gout, and sexually transmitted diseases, can act as antimicrobial and anti-inflammatory agents.(J. L. Rockwood, Anderson, B.G.Casamatta, D.A., 2013)

❖ **Roots:**

Root bark acts as an anti-ulcer, anti-inflammatory and cardiac stimulant agent. (Choudhary MK, 2013)

❖ **Flowers:**

Moringa flowers act as anti-arthritic, hypocholesterolemia agents can cure urinary and cold problems(Lowell J. Fuglie, 2005) .The Moringa flower juice improves the quality and flow of mothers' milk when breastfeeding(L.J Fuglie, 2001).

❖ **Pods:**

Moringa pods use to treat liver, diarrhea and spleen problems, and joint pain. (Josephine N. Kasolo, 2010). The table 1 will summarize the medicinal properties of each part of the Moringa Oleifera (Anwar F, 2007).

Table 1: Summary ofthe medicinal uses of different plant parts of Moringa oleifera.

Part of Moringa Oleifera	Medical properties
Roots, bark, fresh leaves and defatted and shell free seeds	Antimicrobial
Leaves, roots, bark and seeds	Anticancer
Leaves, flowers and roots	Anti-hepatotoxic
Leaves and flower buds	Antiulcer
Roots, dried seeds,	Anti-inflammatory
Flowers, seeds, leaves, bark and roots	Anti-inflammatory
Dried leaves and oil from dried seeds	Antioxidant
Leaves and fruits	Anti-hyperlipidemia
Roots and bark	Antifertility
Roots	CNS depressant

Phytochemistry

Phytochemicals are chemicals produced by plants. The word refers to only those chemicals which may have an impact on health, or on flavor, texture, smell, or color of the plants, but are not required by humans as essential nutrients. An examination of the phytochemicals of Moringa species affords the opportunity to examine a range of fairly unique compounds. In particular, this plant family is rich in compounds containing the simple sugar, rhamnose, and it is rich in a fairly unique group of compounds called glucosinolates and isothiocyanates. For example, specific components of Moringa preparations that have been reported to have hypotensive, anticancer, and antibacterial activity include 4-(4'-O-acetyl-a-L-rhamnopyranosyloxy)benzyl isothiocyanate, 4-(a-L-rhamnopyranosy-

loxy)benzyl isothiocyanate , niazimicin , pterygospermin , benzyl isothiocyanate , and 4-(a-L-rhamnopyranosyloxy)benzyl glucosinolate . While these compounds are relatively unique to the Moringa family, it is also rich in a number of vitamins and minerals as well as other more commonly recognized phytochemicals such as the carotenoids (including β-carotene or pro-vitamin A). (Jed W. Fahey, 2005b)

Cholesterol lowering

The crude extract of Moringa leaves has a significant cholesterol lowering action in the serum of high fat diet fed rats which might be attributed to the presence of a bioactive Phytoconstituents, i.e. β- sitosterol. Moringa fruit has been found to lower the serum cholesterol, phospholipids, triglycerides, low density lipoprotein (LDL), very low-density lipoprotein (VLDL) cholesterol to phospholipid ratio, atherogenic index lipid and reduced the lipid profile of liver, heart and aorta in hypercholesteremic rabbits and increased the excretion of fecal cholesterol. (Durgesh Kumar Dubey, 2013)

In blindness and eye infections

Though there are many causes of blindness Vitamin A deficiency causes impaired dark adaption and night blindness. Eating Moringa leaves, pods and leaf powder which contain high proportion of Vitamin A can help to prevent night blindness and eye problems in children. Ingesting drumstick leaves (Bcarotene and leutin) with oil helps in improving Vitamin A nutrition and perhaps delays the onset of cataract. Also, the juice can be instilled into eyes in cases of conjunctivitis.(Durgesh Kumar Dubey, 2013)

Cardiac and circulatory stimulant

All parts of the tree are reported to be used as Cardiac and circulatory stimulant. The Root bark of the moringa tree contains the alkaloid compounds moringine and moringinine, which act as a cardiac stimulant and acts on the sympathetic nervous system.(Durgesh Kumar Dubey, 2013)

Antioxidant activity

Antioxidant activity in oil from the dried seeds of Moringa plant is higher than BHT and alpha Tocopheryl. Aqueous methanol (80%) and ethanol (70%) extracts of freeze dried leaves showed radical scavenging and antioxidant activities. The drumstick leaves are found to be a potential source of natural antioxidants.

(Durgesh Kumar Dubey, 2013)

Human food

All Moringa food products have a very high nutritional value. The parts of the tree can be represented in human's food, especially young shoots, young pods, flowers, roots, and in some species even the bark. Humans can cook the parts and included in their meals as the way they love to.

❖ Leaves

Of all the products of the tree the leaves are used the most because there are low in fats and carbohydrates and rich in minerals, iron and vitamin B. They become tougher as they get older so it is best to pick the growing tips and young leaves. Remove the leaves from the woody stem, as this will not soften during cooking. The leaves can be used in the same way as spinach.

A leaf powder can be produced by drying the leaves and crushing or pounding them. You can sift the powder to remove leaf stems. This powder can then be added to sauces at the same time as other condiments or vegetables are added(Sánchez., 2015). Also, dried or fresh leaves can be added to salad (Monica Premi*, 2010).

❖ Flowers

The flowers can be cooked and mixed with other foods or fried in batter. They can also be placed in hot water for five minutes to make a kind of tea. They are also a good source of nectar for honey producing bees.(L.J Fuglie, 2001)

❖ Pods

The pods can be eaten from when they first appear to when they become too woody to snap easily (up to 30cm long). They are cooked like other green beans and have a similar flavor to asparagus. Beware as some bitter varieties are poisonous if too many are eaten. Even the pods that have become too woody can be boiled until they are tender. They are opened and the white flesh is scraped out and returned to the boiling water. This can be used in soups and stews.(L.J Fuglie, 2001)

❖ Seeds

The seeds are often referred to as peas and can be used from the time they appear until they turn yellow and their shells begin to harden. Seeds are used in cooking.

When the seeds are mature, their coating hardens and becomes bitter. This can be pressed for oil extraction. If a press is not available the seeds can be browned or roasted, ground, added to boiling water and the oil floats to the surface. The seeds contain 35% oil and this is used for cooking purposes. The oil does not turn rancid and also burns without smoke.(L.J Fuglie, 2001)

- **Roots**

A sauce similar to horseradish sauce can be made from the roots when the seedling is only 60cm tall. The root bark should be completely removed as it contains harmful substances, then the root is ground up and vinegar and salt are added. However, it should not be eaten in excess. It is best to store the sauce in a refrigerator.(L.J Fuglie, 2001)

Animal fodder:

Moringa is used in animal feed. Studies have shown that the presence of Moringa in animal feed can lead to an increase in weight by up to 32% and can also be supplemented with fresh Moringa leaves with 15 to 17 kg of daily feed for livestock. 43%. Milk production can also be increased by 58% by supplementing the leaves of fresh Moringa with 2 kg of dry fodder and increasing by 65% when supplementing fresh Moringa leaves with 3 kg of dry fodder. (Foidl N., 2001)

Water purifying:

The seed powder is used to purify the dirty water where it is considered it is easy, simple and inexpensive and replaces chemicals such as aluminum sulphate and other chemicals that pose a danger to people and the environment. The powder is associated with solid materials and drowns down and this way removes (90-99) % of the bacteria present in the water.The water is purified by adding 2 g of Moringa seed powder to 20 liters of water and shaking for 5 minutes. Dirty water that is to be treated can be filtered through a clean cloth into the container. Until the water becomes clear and the impurities have sunk to the bottom leave the bucket undisturbed for one hour then filter the water through a clean cloth boil the water before drinking.(Prof. Dr. M. Ashfaq, 2011)

Fertilizer

The seed cake, which is produced by pressing the seeds to extract oil, cannot be eaten as it contains harmful substances. However, it contains high levels of protein and makes a good fertilizer for use in agriculture.(L.J Fuglie, 2001)

Natural pesticide

By digging Moringa leaves into the soil before planting, damping off disease (Pythium debaryanum) can be prevented among seedlings.(“Moringa oleifera A multi-purpose tree,” 2002)

Homogenizer Assisted Extraction Method

A small gap between the rotor (spinning shaft) and the stator (fixed portion) is characteristic of rotor-stator homogenizers, as well as a high rotor tip speed, which results in extremely high shear speeds in the gap and extremely high rates of energy loss in the stator area. Rotor-stator homogenizers are well-known for being an efficient mixing instrument that speeds up solid-liquid phase transitions.

Because of the rapid speed and close proximity of the rotary shaft to the stationary component, highly strong shear forces are generated, resulting in particle size reduction and vigorous mixing. These characteristics of rotor-stator homogenizers make them ideal for phase-transfer processes involving solid materials. These devices offer various benefits over traditional homogenization procedures, including: relative ease of setup and operation; cheap investment costs; high yield; compatibility with viscous systems; and the ability to generate large-volume emulsions, among others. Inertia and shear forces in turbulent flow are responsible for the effective droplet separation energy in homogenizers. A rotor (moving part) with two or more blades and a stator (fixed portion) with vertical or inclined gaps around the homogenizer cell’s wall make up the rotor-stator assembly. The rotor is concentrically positioned within the stator(Scholz & Keck, 2015).

As the moving portion rotates, it generates a vacuum that draws the liquid in and out of the assembly, causing the environment to circulate. The mechanical impact force on the wall caused by rapid fluid acceleration is one of the two major factors that can diminish the size of scattered droplets. The shear force, which is mostly found in the space between the rotor and the stator, is another force. The flow in this area is very turbulent at high rotational speeds and comprises eddies of various sizes(Gazolu-Rusanova, Lesov, Tcholakova, Denkov, & Ahtchi, 2020).

The leaves of olive trees are among the most famous plants in most countries of the world. Olive leaves treat many diseases for this reason, there are many scientific researches related to the biologically active compounds extracted from olive leaves. Selin Şahin et al . In their study of olive tree (Olea europaea)

leaf extraction with methanol by homogenizing-assisted extraction (HAE) and ultrasound-assisted extraction methods (UAE), which are new techniques used in this field. The results were presented by extract yield and total phenol contents expressed as gallic acid equivalent (GAE) per gram of dried leaves. The yield of HAE extract ranged from 102.27 to 443.16 mg/g of dried leaves. The total polyphenol content in the leaves ranged from 10.11 to 61.66 mg-GAE/g dried leaves. For the UAE, the extract production changed between 88.75 and 350.82 mg/g dried leaves, while the total phenolic content ranged from 7.35 to 38.66 mg-GAE/g dried leaves.(Bilgin & Şahin, 2013). Chanioti, S et al. In their study on the extraction and protection of phenolic compounds by encapsulation or incorporation into nano-emulsions from olive pomace. Innovative auxiliary extraction methods such as microwave (MAE), homogenization (HAE), ultrasound (UAE) and high hydrostatic pressure (HHPAE) have been evaluated using various solvent systems including ethanol, methanol, and natural deep eutectic solvents (NADESs). By HAE at 60 °C/12,000 rpm and UAE at 60 °C, the total phenolic content (TPC) of the extracts was 34.08 mg gallic acid (GAE)/g bw and 20.14 mg GAE/g dry weight for chromated copper arsenate. , and by MAE at 60 °C and HHPAE at 600 MPa/10 min, the TPC was 29.57 mg GAE/g dry weight and 25.96 mg GAE/g dry weight for CLA. HAE has proven to be the best method for extracting phenolic compounds from olive pomace (Chanioti & Tzia, 2018).

Spectrophotometric analysis

Spectrophotometric methods are one of the oldest methods in analytical chemistry. Absorption of visible light by certain chemicals has been used for many years to visually determine their concentrations. The term colorimetry/colorimetric is used for analytical methods in which chemical elements are determined by comparing the color of unknown samples, either on graduated cylinders or on visual comparators. The use of photoelectric instruments led to the measurement of the absorption of radiation as it passed through the analyzed samples, thereby extending the useful range of radiation outside the visible region(Sommer, 2012).

Today, spectrophotometry is considered an instrumental technique based on measuring the absorption of electromagnetic radiation in the ultraviolet (UV, 200 - 380 nm), visible (VIS, 380 - 780 nm) and near infrared region. Inorganic analysis uses UV VIS spectrophotometry. Spectrophotometric methods have also been used in basic studies, regardless of their usefulness in quantitative analysis. For example, it is applied to determine the composition of chemical compounds, the

dissociation constants of acids and bases, or the stability constants of complex compounds (Marczenko & Balcerzak, 2000).

Spectrophotometry as a measurement technique has evolved greatly as a result of advances in technology and the development of new materials and data processing methods. The development of special optics and spectrophotometers combined with microprocessors that control spectrophotometer operation has significantly expanded the possibilities of using these instruments, recording absorption spectra and processing of collected data.

Spectrophotometric methods have proven particularly suitable for automation in both analytical procedures and data processing. It belongs to the most commonly used detection techniques in automatic flow injection analysis(Marczenko & Balcerzak, 2000; Sommer, 2012)

Methodology

Material and methods

Raw material and reagents:

Plants material: *Moringa oleifera* leaves were brought from an agricultural engineer interested in cultivating rich plants in Palestine. The damaged leaves were removed, then washed well using distilled water. They were sieved using a sieve, and dried from water using a clean cotton cloth without too much pressure on the leaves. Then, they were laid on a clean piece of cloth in a room, where the sun's rays did not enter directly at the room temperature. They were dried with continuous flipping daily to ventilate them, and prevented from the formation of mold. *Moringa oleifera* leaves were crushed by a household mixer, and then sieved using a different size strainer (2000, 1000, 710, 500, 355, and 250 μm) to obtain the required size of particles. The average size of the particles was adopted as (2000, 1500, 855 , 605 , 427.5 and 302.5 μm) depending on the preliminary experiments.

Chemical materials: In this studies, the ethanol was chosen as a polar, non-toxic and inexpensive solvent to extract the bioactive materials from *Moringa oleifera* leaves.

Folin-Ciocalteu reagent, sodium carbonate (≥ 99.0%), (+)-catechin, 2.2'-az-

ino-bis (3-ethylbenzothiazoline)-6-sulfonic acid diammonium salt (ABTS), hydrochloric acid, sodium hydroxide, 2,2-diphenyl-1-picrylhydrazil (DPPH) and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) and gallic acid monohydrate (≥ 98.0%) were from Sigma-Aldrich (St. Louis, MO, USA), ethanol (≥ 99.8%) , methanol (≥ 99.9%) , Aluminum chloride, sodium nitrite and potassium persulfate were purchased from Merck (Darmstadt, Germany). Distilled water and deionized Millipore Milli-Q treatment system was used to prepare the mixture analyses for the study.

Methods of work

Calibration study.

calibration is a laboratory chemical process using a substance of known concentration to obtain a calibration equation by which the concentration of a solution is calculated by adding a solution of known concentration.

The values of the calibration equations of the spectrophotometric measurements of the total phenolic content, total flavonoid content and antioxidant capacity in pure solutions of Gallic acid, Catechin and trolox at different ethanol concentrations were determined by adding 0.01g of gallic acid to 100 ml distilled water then the concentration was diluted from 100 ppm to 6.25 ppm to obtain the calibration equation for total phenolic content by UV- spectrophometer analysis of the sample at 765nm, adding 0.05g of Catechin hydrate to 50 ml distilled water then the concentration was diluted from 1000 ppm to 62.5ppm to obtain the calibration equation for flavonoid content by UV- spectrophometer analysis of the sample at 510nm, addıng 0,0025 g Trolox to 50 ml distilled water then the concentration was diluted from 50 ppm to 6.25ppm to obtain the calibration equation for antioxidant capacity (DPPH and ABTS) by UV- spectrophometer analysis of the sample at 517nm for DPPH and 734nm for ABTS. Furthermore, calibration equations were determined at different concentration of ethanol (30, 60 and 90%).

Antioxidant properties of extracts of Moringa Olefirea leaves powder by using homogenizer extraction methods.

Homogenizer Assisted Extraction (HAE) : In this method the extraction was done by taken a constant amount of Moringa Oliefra leaves (0.5±0.0005) g with particle size 2000 μm. on another hand three factors were studied by using ho-

mogenizer extractor (speed (rpm) , solvent concentration %(v/v) and extraction time (sec).

(0.5±0.0005) g of moringa oleifera leaves powder were weghited and extracted with 35 ml of solvent. Ethanol and water mixture at (30/70), (60/40) and (90/10) were selected as extraction solvents. The moringa Olefira extracts were prepared using Homogenizer Assisted Extraction (IKA T25 ULTRA TURRAX). A 0.45 µm syringe filter was used to filter the extracts. Extract samples obtained were stored at 4°C. in centrifuge plastic test tubes with a capacity of 14 ml. The experimental was done by used following factors:

- ✓ extraction time (30, 60 and 90 second)
- ✓ Mixing speed (5000, 7000 ve 9000 rpm)
- ✓ Solvent concentration (%30 %60,%90, v/v)
- ✓ Solvent volume (35 mL)
- ✓ Weight of moringa oleifera leaves powder (0.5 g)

Analyze method

Antioxidant activity by applying spectrophotometric methods.

Determination the total Phenolic Contents by the Folin Ciocalteu method.

TPC was determined by UV-spectrophotometer (PG Instruments, T60/ Leicestershire, England) at 765 nm. Briefly, 350 µL of distilled water was added to 20 µL of extracted samples. Then, 2000 µL of Folin-Ciocalteu reagent (10%, v/v) and 1600 µL of sodium carbonate (7.5%, w/v) were added to the mixture. After mixing carefully, the mixtures were incubated for 30 min. These recorded results were calculated in terms of gallic acid equivalent per liter Moringa oleifera extract (mg-GAE L-1). Total phenol contents were obtained from the calibration curves, and expressed in mg-GAE g-1 dried Moringa oleifera.

Determination the total Flavonoid Contents by the aluminum chloride method.

TFC was determined by UV-spectrophotometer (PG Instruments, T60/ Leicestershire, England) at 510 nm. Briefly, 2225 µL of distilled water was added to 25 µL of extracted samples. Then, 113 µL of NaNO- (5%, w/v) after six minute in dark 225 µL of AlCl- (10%, w/v) were added to the mixture and the

mixture stand in dark for 5 minute. The 750 µl of NaOH (%4, w/v) was added to the mixture in the last 412 µl of distilled water was added. These recorded results were calculated in terms of (+) Catechin equivalent per liter Moringa oleifera extract (mg-CE L-1). Total Flavonoid contents were obtained from the calibration curves, and expressed in mg-CE g-1 dried Moringa oleifera.

Determination of antioxidant activity (AA) to scavenging the free radicals by DPPH method:

DPPH was determined by UV-spectrophotometer (PG Instruments, T60/ Leicestershire, England) at 517 nm. At the first step the DPPH solution was prepared , 0.0079 g of (2,2-Diphenyl-1-Picrylhydrazyl) mixed with 40 ml Methanol (%80 v/v), then DPPH solution was diluted by adding 40 ml of methanol (%80, v/v) for each 10 ml of DPPH solution. 600µL Methanol (%80, v/v) added to 100µl of extracted samples. 3000 µl of diluted DPPH was added to the mixture. The mixture was left in dark for 30 min at room temperature. The absorbance of samples was determined at 517 nm. The radical scavenging capacity was determined and expressed as a percentage of the effect (E%) by using the following equation, where this equation depended on $A_{control}$ (absorbance of ferrozine complex) which prepared by adding 600 µl of Methanol(%80, v/v) to 100 µl of Ethanol (%60, v/v), then 3000µl added to mixture(Chen, Bertin, & Froldi, 2013).

$$AA\%\ (inhibitions) = \frac{Acontol - Asample}{Acontrol} \times 100\% \quad \text{.....(1)}$$

These recorded results were calculated in terms of pure trolox (6-hydroxy-2,5,7,8- teramethyl chromane-2-carboxylic acid) equivalent per liter Moringa oleifera extract (mg-TEAC L^{-1}). Total DPPH contents were obtained from the calibration curves, and expressed in mg-TEAC g^{-1} dried Moringa oleifera.

Determination of antioxidant activity (AA) to scavenging the free radicals by ABTS method

ABTS was determined by UV-spectrophotometer (PG Instruments, T60/ Leicestershire, England) at 734 nm. At the first step the ABTS solution was prepared, 0.129 g of (2,2-azino-bis (3-ethyl benz-thiazoline-6-suIfonic acid) and 0.0331 g of Potassium persulfate mixed with 50 ml distilled water, Then the conical flask containing the solution was wrapped in aluminum foil, and the solution

was placed in the refrigerator at 4 °C for 16 hours. ABTS solution was diluted by adding 800 µL of ABTS solution to 80 ml methanol (%80, v/v). ABTS solution corresponding to 0.7 absorbance value was used in photometric mode and UV device zeroed with methanol (%80, v/v) at 734 nm. 3000µL ABTS solution added to 30 µl of extracted samples. The mixture was left in dark for 5 min at room temperature. The absorbance of samples was determined at 734 nm. The radical scavenging capacity was determined and expressed as a percentage of the effect (AA%) by using the previously equation, where this equation depended on A control (absorbance of ferrozine complex) which prepared by adding 3000 µl of ABTS solution to 30 µl of methanol (%100, v/v)(Ozgen, Reese, Tulio, Scheerens, & Miller, 2006). These recorded results were calculated in terms of pure trolox (6-hydroxy-2,5,7,8- teramethyl chromane-2-carboxylic acid) equivalent per liter Moringa oleifera extract (mg-TEAC L^{-1}). Total ABTS contents were obtained from the calibration curves, and expressed in mg-TEAC g^{-1} dried Moringa oleifera.

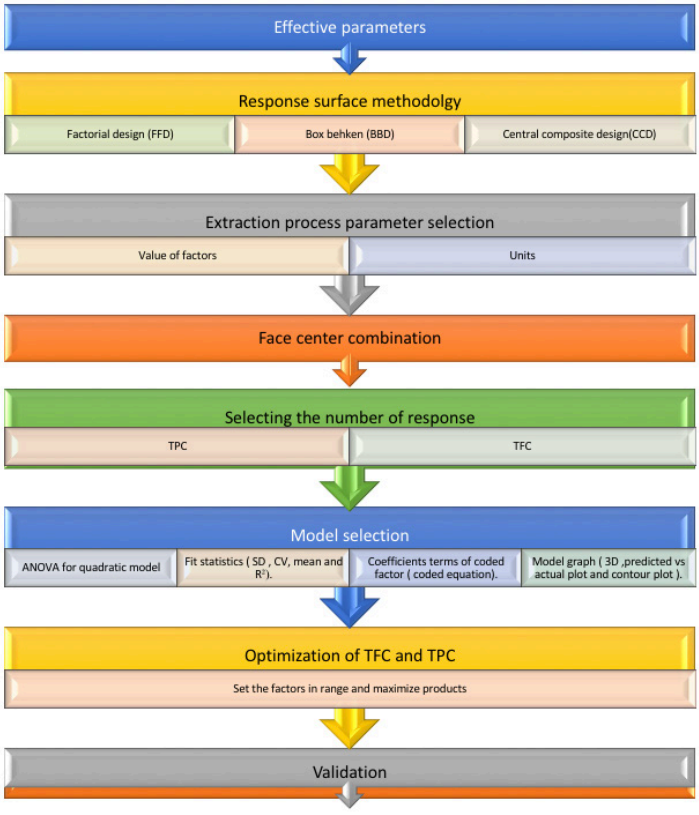
Modeling of extraction methods

The extraction methods were modeled by using response surface method (RSM) and Design expert program (12^{th} edition). where the RSM is a combination between statistics and mathematical techniques that's important to improvement, developing and optimizing the processes. The response is the term for the performance indicators for quality qualities. in this research the RSM used to optimizing the amount of bioactive martials by using different extraction methods. On the another hand, these surfaces were used to describe how the independent variables affect the response, provide information on the interrelationships between the independent variables, in addition, describe the combined effect of all the independent variables on the response. Dependent variables are the parameters that influence the process, while dependent variables are the responses. For example, the amount of phenolic, Flavonoid and antioxidants affecting by extraction time, solvent concentration, extraction power and ect. These parameters are called independent variables. Preliminary tests were used to determine the parameters required for the extraction process to take place, as well as the independent variables for each extraction method. Table 2 shows the independent parameters, units, variable symbols, and level values used in the extraction of moringa oleifera leaves using the HAE.

Table 2: p independent parameters, units, variable symbols, and level values used in the extraction of moringa oleifera leaves using the HAE.

Methods	Independent factors	Unit of factors	Sign of variables	level code		
				-1	0	1
HAE	Solvent concentration	%v/v	X_1	30	60	90
	Mixing speed	Rpm	X_2	5000	7000	9000
	Extraction time	sec	X_3	30	60	90

In this research the RSM used to optimizing the amount of total phenolic and flavonoid by using Design expert program (12th edition) by following the steps outlined in the following diagram:



RESULTS AND CALCULATION

The phenolic content, flavonoid content and antioxidant capacity determination tests were carried out and samples were analyzed using a UV- spectrophotometer. The principle of this device is based on the amount of light absorbed. The sample absorbs light, and the photodetector converts the amount of light absorbed by the sample into a number that appears on the device screen.

Table3 shows the absorbance values for spectrophotometric measurements of pure solutions of Gallic acid, Catechin, and Trolox with different concentrations of ethanol. The figure 1 shows the relationship between the concentration of the calibrated samples and the amount of light absorbed.

The table4 describes the calibration equation values for spectrophotometric measurements of total phenolic content, total flavonoid content, and antioxidant capacity in pure solutions of Gallic acid, Catechin, and Trolox with varying ethanol concentrations. Additionally, by the amount of light absorbed for samples, the concentration of phenolic, Flavonoid and antioxidant can be calculated using the obtained calibration equations.

Table 3: the absorbance values for spectrophotometric measurements of pure solutions of Gallic acid, Catechin, and Trolox with different concentrations of ethanol.

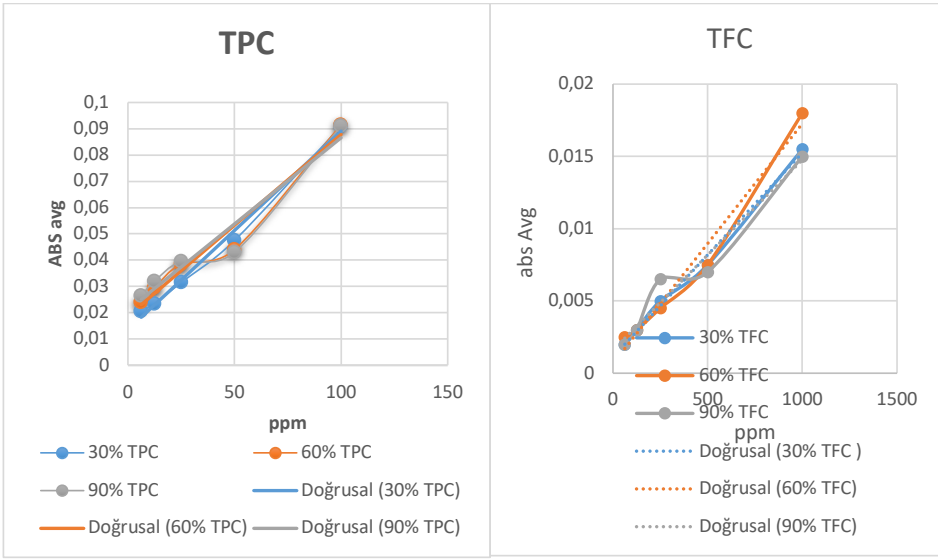
Compound	Spectrophotometric analysis method	ppm	EtOH Concentration (%,v/v)	ABS(1)	ABS(2)	ABS Average
		100		0.098	0.084	0.0910±0.009
		50		0.047	0.048	0.0475±0.001
		25	30	0.031	0.032	0.0315±0.001
		12.5		0.024	0.023	0.0235±0.001
		6.25		0.020	0.021	0.0205±0.001
		100		0.083	0.100	0.0915±0.012
Gallic acid	TPC – Folin	50		0.043	0.045	0.0440±0.001
		25	60	0.037	0.040	0.0385±0.002
		12.5		0.029	0.030	0.0295±0.001
		6.25		0.024	0.024	0.0240±0.000

		100		0.087	0.095	0.0910±0.005
		50		0.046	0.040	0.0430±0.004
		25	90	0.038	0.041	0.0395±0.002
		12.5		0.031	0.033	0.0320±0.001
		6.25		0.027	0.026	0.0265±0.001
		1000		0.016	0.015	0.0155±0.001
		500		0.008	0.007	0.0075±0.001
		250	30	0.005	0.005	0.005±0.0000
		125		0.003	0.003	0.003±0.0000
		62.5		0.002	0.002	0.002±0.0000
		1000		0.017	0.019	0.018±0.0020
Catechin	TFC- AlCl□	500		0.007	0.008	0.0075±0.001
		250	60	0.004	0.005	0.0045±0.001
		125		0.003	0.003	0.003±0.0000
		62.5		0.003	0.002	0.0025±0.001
		1000		0.013	0.017	0.015±0.002
		500		0.007	0.007	0.007±0.000
		250	90	0.004	0.009	0.0065±0.003
		125		0.003	0.003	0.003±0.0000
		62.5		0.002	0.002	0.002±0.0000
		50		0.344	0.346	0.345±0.001
		25	30	0.439	0.435	0.437±0.002
		12.5		0.48	0.48	0.480±0.0000
		6.25		0.503	0.496	0.4995±0.004
Trolox	AA- DPPH	50		0.409	0.393	0.401±0.011
		25	60	0.469	0.465	0.467±0.002
		12.5		0.493	0.491	0.492±0.001
		6.25		0.513	0.696	0.6045±0.13
		50		0.591	0.585	0.588±0.004
		25	90	0.666	0.662	0.664±0.002
		12.5		0.693	0.687	0.69±0.004
		6.25		0.704	0.703	0.7035±0.000
		50		0.601	0.622	0.6115±0.014
		25	30	0.656	0.656	0.656±0.0000
		12.5		0.676	0.673	0.6745±0.002

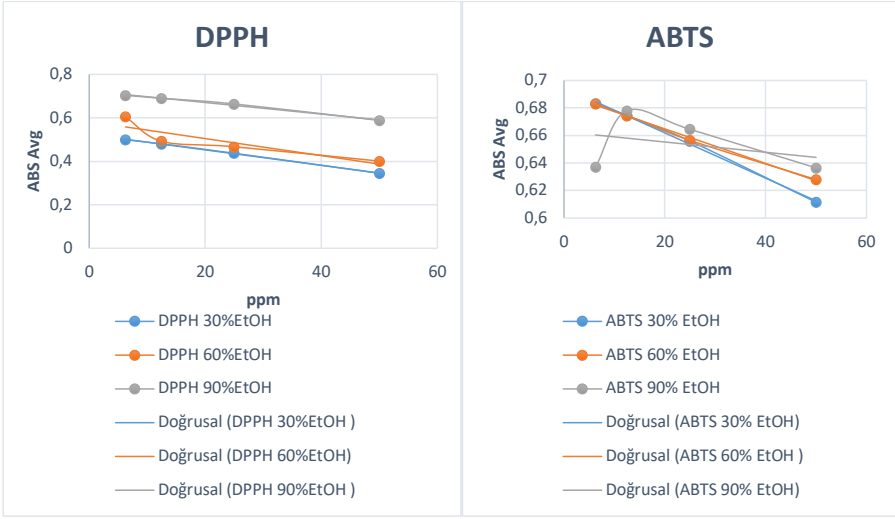
		6.25		0.684	0.682	0.683±0.001
Trolox	AA- ABTS	50		0.634	0.622	0.628±0.008
		25	60	0.655	0.658	0.6565±0.002
		12.5		0.677	0.672	0.6745±0.003
		6.25		0.684	0.682	0.683±0.001
		50		0.639	0.634	0.6365±0.003
		25	90	0.667	0.662	0.6645±0.003
		12.5		0.679	0.677	0.678±0.001
		6.25		0.638	0.636	0.637±0.001

Table 4: Calibration equation parameters developed for various analysis methods of gallic acid, catechin, and trolox solutions in ethanol at various concentrations.

Compound	Spectrophotometric analysis method	Calibration equation Y=aX±b		Ethanol (%, v/v)	
			30	60	90
Gallic acid	TPC- Folin	A	0.0008	0.0007	0.0007
		B	0.0135	0.0187	0.021
		R^2	0.9927	0.9614	0.9439
Catechin	TFC-AlCl-	A	0.00001	0.00002	0.00001
		B	0.0011	0.0007	0.0016
		R^2	0.9943	0.9787	0.9592
Trolox	AA- DPPH	A	-0.0036	-0.0039	-0.0027
		B	0.5236	0.5827	0.7236
		R^2	0.9993	0.9959	0.9917
	AA- ABTS	A	-0.0016	-0.0013	-0.0004
		B	0.6948	0.6899	0.6627
		R^2	0.9971	0.9967	0.9218



(a) (b)



(c) (d)

Figure 1: the relationship between the concentration of the calibrated samples and the amount of light absorbed (a) TPC ; (b) TFC ; (c) DPPH , (d) ABTS.

Table 5 show the independent parameters of HAE that selected by primarily experiments for the extraction of bioactive substances from Moringa Oleifera. Whereas, table 6 compares the experimental design of the moringa oleifera leaves extract prepared by the HAE method, the TPM and TFM yields, and the results calculated using the applicable model equations.

Table 5: Process parameters of HAE for the extraction of bioactive substances from Moringa Oleifera.

Parameter	Symbol	Code with level		
		−1	0	1
Solvent concentration (%, v/v)	A	30	60	90
Speed (rpm)	B	5000	7000	9000
Time (sec)	C	30	60	90

Table 6: Experimental results formed by face centered central composite design*

	A: Solvent concentration (%, v/v)	B: Speed (rpm)	C: Time (sec)	Response 1 TPC (mg-GAE/ g-DP)	Predicted TPC (mg-GAE/ g-DP)	Response 2 TFC (mg-CE/g-DP)	Predicted TFC (mg-CE/g-DP)
1	90	5000	30	0.233±0.002	0.178±0.002	49.400±0.000	53.990±0.000
2	30	5000	90	17.09±0.014	16.75±0.014	275.31±0.002	274.80±0.002
3	60	7000	60	15.68±0.011	17.17±0.011	119.95±0.002	132.38±0.002
4	60	7000	90	16.85±0.014	17.53±0.014	134.87±0.003	146.97±0.003
5	60	7000	60	16.79±0.014	17.17±0.014	125.83±0.003	132.38±0.003
6	60	7000	60	17.44±0.015	17.17±0.015	126.87±0.001	132.38±0.001
7	30	5000	30	14.65±0.010	15.13±0.010	230.16±0.001	240.73±0.001
8	60	7000	60	16.64±0.011	17.17±0.011	130.39±0.003	132.38±0.003
9	30	9000	90	17.35±0.013	17.46±0.013	272.08±0.002	269.31±0.002
10	60	9000	60	18.35±0.016	18.21±0.016	138.33±0.005	145.62±0.005
11	60	7000	60	18.43±0.007	17.17±0.007	137.30±0.001	132.38±0.001
12	30	9000	30	15.93±0.017	15.99±0.017	253.43±0.002	255.32±0.002
13	60	5000	60	16.95±0.015	16.87±0.015	135.08±0.002	120.50±0.002
14	90	7000	60	2.220±0.004	2.340±0.004	118.99±0.001	120.86±0.001
15	90	9000	30	1.760±0.004	2.150±0.004	107.37±0.003	109.71±0.003
16	60	7000	30	16.63±0.014	15.75±0.014	122.16±0.003	102.77±0.003
17	30	7000	60	16.82±0.018	16.50±0.018	276.03±0.003	266.86±0.003
18	90	5000	90	2.290±0.002	2.280±0.002	128.47±0.002	128.40±0.002
19	60	7000	60	17.63±0.013	17.17±0.013	139.33±0.002	132.38±0.002
20	90	9000	90	4.520±0.002	4.090±0.002	172.79±0.001	164.04±0.001

* Data are given as the mean (3 replicates) ± standard deviation

On the other hand, table 7 covers the antioxidant capacity results of *Moringa Oleifera* leaf extract produced by HAE method, which were examined using two different methods (DPPH and ABTS).

Table 7: Independent variables of the HAE method and experimental results of DPPH and ABTS capacity *

	A: Solvent concentration (%, v/v)	B: Speed (rpm)	C: Time (sec)	AA DPPH (mg-TEAC/g-DP)	ABTS (mg-TEAC/g-DP)
1	90	5000	30	5.04±0.003	18.42±0.004
2	30	5000	90	5.96±0.000	27.88±0.040
3	60	7000	60	7.40±0.003	23.96±0.010
4	60	7000	90	7.68±0.001	21.50±0.030
5	60	7000	60	7.40±0.001	21.75±0.040
6	60	7000	60	7.40±0.001	23.68±0.007
7	30	5000	30	5.43±0.001	29.25±0.020
8	60	7000	60	7.42±0.002	23.46±0.009
9	30	9000	90	9.90±0.001	26.49±0.020
10	60	9000	60	9.02±0.001	23.66±0.020
11	60	7000	60	7.42±0.000	25.28±0.020
12	30	9000	30	9.26±0.000	27.51±0.020
13	60	5000	60	5.40±0.001	24.08±0.020
14	90	7000	60	7.22±0.006	17.32±0.010
15	90	9000	30	8.84±0.010	19.91±0.010
16	60	7000	30	7.10±0.001	27.26±0.030
17	30	7000	60	7.60±0.001	25.76±0.020
18	90	5000	90	5.57±0.090	17.03±0.007
19	60	7000	60	7.42±0.007	25.47±0.010
20	90	9000	90	9.27±0.010	16.39±0.020

* Data are given as the mean (3 replicates) ± standard deviation

The model was analyzed where the quadratic model equations for the respective systems (TPC and TFC) extracted by Homogenizer assisted extraction method were expressed by the following equations:

$$\textbf{HAE-TPC (mg-GAE/g-DP)} = 17.17 - 7.08A + 0.67B + 0.8916C + 0.2769AB + 0.1191AC - 0.0388BC - 7.76A^2 + 0.3706B^2 - 0.5321C^2 \quad \ldots\ldots\ldots\ldots\ldots\ldots 2$$

HAE-TFC (mg-CE/ g-DP) = 132.38 – 73A + 12.56B +22.10C + 10.28AB + 10.08AC -5.02BC + 61.49A² + 0.6822B² – 7.51C²……. ……..3

Statistical analysis (ANOVA) findings produced from CCD utilizing the Design Expert program are reported in table 8 for each response one by one, along with the appropriate statistical variables.

Table 8: Analysis of variance test results.

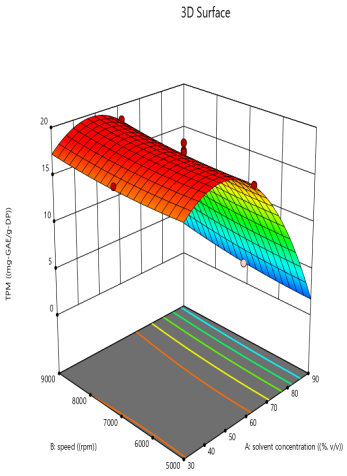
	Source	**Sum of Squares**	**Df**	**Mean Square**	**F Value**	***p*-value Prob > F**	
HAE-TPC (mg-GAE/ g-DP)	Model	823.61	9	91.51	138.96	< 0.0001	significant
	A-solvent concentration	501.29	1	501.29	761.20	> 0.0001	
	B-speed	4.49	1	4.49	6.82	0.0260	
	C-time	7.95	1	7.95	12.07	0.0060	
	AB	0.6135	1	0.6135	0.9315	0.3572	
	AC	0.1134	1	0.1134	0.1722	0.6869	
	BC	0.0121	1	0.0121	0.0183	0.8950	
	A^2	165.39	1	165.39	251.14	>0.0001	
	B^2	0.3777	1	0.3777	0.5735	0.4664	
	C^2	0.7787	1	0.7787	1.18	0.3024	
	Residual	6.59	10	0.6586			
	Lack of Fit	2.12	5	0.4231	0.4732	0.7845	not significant
	Pure Error	4.47	5	0.8941			
	Cor Total	830.20	19				

C.V.: 6.14% R^2=0.9921 Adjusted R^2=0.9849 Predicted R^2=0.9668

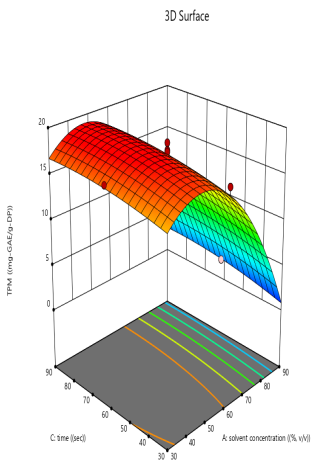
HAE-TFC (mg-CE/g-DP)	Model	78250.52	9	8694.50	61.84	< 0.0001	significant
	A-solvent concentration	53290.80	1	53290.80	379.03	> 0.0001	
	B-speed	1577.02	1	1577.02	11.22	0.0074	
	C-time	4883.77	1	4883.77	34.74	0.0002	
	AB	845.79	1	845.79	6.02	0.0341	
	AC	813.65	1	813.65	5.79	0.0370	
	BC	201.52	1	201.52	1.43	0.2588	
	A^2	10396.52	1	10396.52	73.95	> 0.0001	
	B^2	1.28	1	1.28	0.0091	0.9259	
	C^2	155.03	1	155.03	1.10	0.3184	
	Residual	1405.97	10	140.60			
	Lack of Fit	1137.47	5	227.49	4.24	0.0695	not significant
	Pure Error	268.50	5	53.70			
	Cor Total	79656.48	19				

C.V.: 7.42%	R^2=0.9823	Adjusted R^2=0.9665	Predicted R^2=0.8863

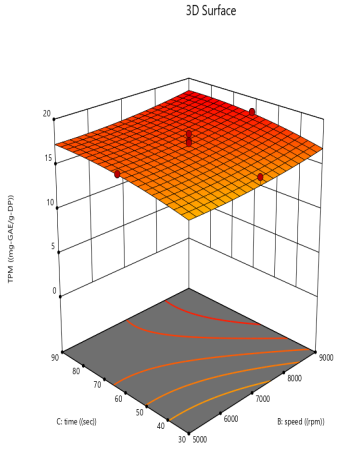
In addition, the effect of visually independent treatments on response values in extracting the active substances from Moringa leaves by Homogenizer assisted extraction method was studied. The adjacent figures[2-3] shows a three-dimensional graph to understand how the independent factors affect the phenolic and flavonoid content extract by HAE. On the other hand, the figure 4 shows the actual results versus the expected results (predicted results) for TPC and TFC.



a

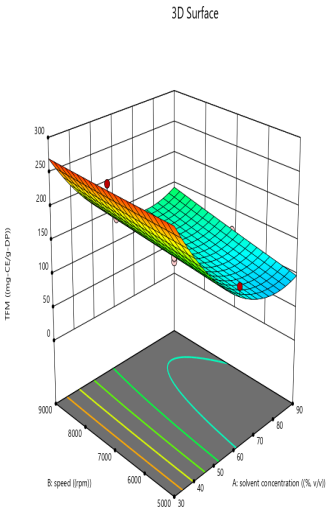


b

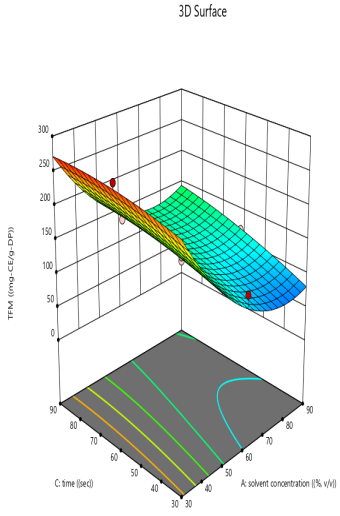


c

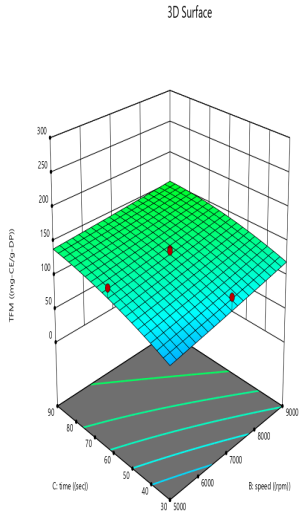
Figure 2 : Response surface plot for the TPC (a) as a function of solvent concentration to speed (time = 60 sec); (b) as a function of solvent concentration to time (speed = 7000 rpm); (c) time to speed (solvent concentration = 60 %



a2

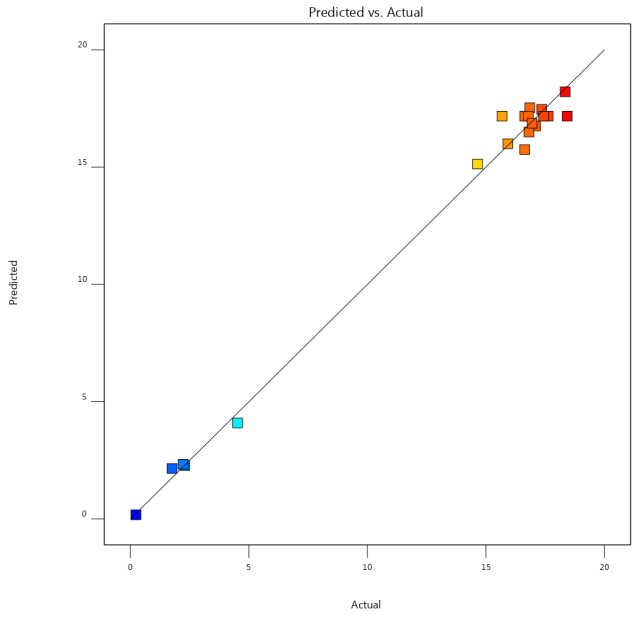


b2

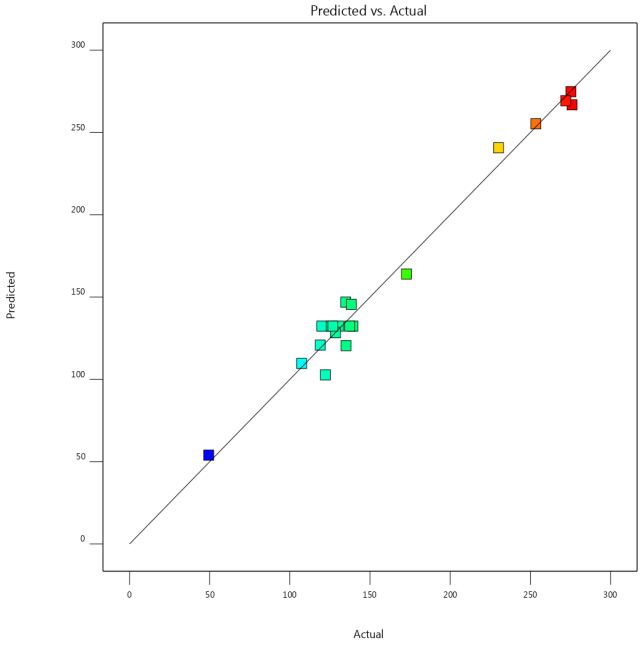


c2

Figure 3: Response surface plot for the TFC (a2) as a function of solvent concentration to speed (time = 60 sec); (b2) as a function of solvent concentration to time (speed = 7000 rpm); (c2) time to speed (solvent concentration = 60 %)



a



b

Figure 4: Actual findings versus predicted findings, (a) TFC; (b) TFC.

Optimization analysis was performed in order to optimally optimize TPC and TFC. The table 9 shows the optimum conditions and maximum response values calculated under these optimum conditions. where, extraction was done by using HAE.

Table 9: Verification results of the optimum condition for HAE.

Optimum Extraction Conditions			Response	Predicted	ExperimentaL		SD	Error
A (%,v/v)	B (rpm)	C (sec)			CV%			
			TPC	17.51	17.20	0.812	4.64	
77	9000	30	TFC	271.37	266.24	11.86	4.37	<2

* Data are given as the mean (n=3) ± standard deviation

DISCUSSION AND CONCLUSION

In this study, the stability of phenolics, flavonoid and antioxidants compounds extracted by different extraction methods were studied. Dried Moringa leaves were extracted using ethanol as a solvent. Ethanol is one of the best and safest chemical solvents. HAE was used in this study. The phenolics , flavonoids and antioxidants compounds were examined using different screening methods.

The phenolics compounds were quantified from the four methods on the basis of electron exchange was measured spectrophotometrically by using Folin Ciocalteu reagent, and the results were presented in equivalents of gallic acid. The amount of flavonoids comounds were also measured using $AlCl_3$ colorimetric spectrophotometer method and the results were expressed in terms of catechin equivalents . Also, the amount of antioxidant compounds were measured using two different methods ABTS and DPPH, by using 2,2-Diphenyl-1-Picrylhydrazyl as a reagent for DPPH and 2,2-azino-bis 3-ethyl benz-thiazoline-6-suIfonic acid as a regent for ABTS. where the results were given in terms of Trolox equivalents.

Independent coefficients were investigated based on preliminary experiments by determining the amount of phenol for each extraction process. The most significant parameters for HAE was predicated according to previous scientific researchs that have been studied. Selin şahin , Ezgi Sayım and Mehmet Bilgin (2017) reported the independent parameters for HAE method, 30 to 90 (second) for extraction time and 4000 to 10000 (rpm) for mixing speed(Şahin, Sayım, & Bilgin, 2017).Consequently, 30 to 90 (sec) for extraction time, 5000 to 9000 (rpm) for mixing speed and 30 to 90 (%, v/v) for ethanol concentration were selected for HAE method as shows in table 5.

In order to optimize the HAE process, central composite design (CCD) of Response Surface Approach (RSA) was used. The system was optimized to maximize the total phenolic (TPC) and flavonoid (TFC) contents in the extract. This statistical approach also provided the design of experimental study, modelling the HAE, ASE, MAE, UAE and analyzing the experimental data. Furthermore, antioxidant activity of the extracts was evaluated by several in vitro measurements to produce more precise and reliable findings.

TPC and TFC measurements under various conditions designed by CCD are given in Table6 . TPC values measured in the samples extracted by HAE

changed between 0.233 and 18.43 mg-GAE/g-DP, whereas TFC varied from 49.4 to 275.03 mg-CE/g-DP as shown in table 6. Pereira, J.M., et al. (2021) reported the TPC values of the *Moringa oleifera* leaf extracts obtained by HAE through 1:1 ethanol: water as 1.06±0.07 and 2.75 mg-GAE/g-DP. the TFC varied from 0.86 to 2.52 mg-CE/g-DP depending on solvent composition and solid/solvent ratio(Pereira et al., 2021). Similarly, Rocchetti, G., et al. also reported TPC and TFC values, which are in agreement with those of the current study(Rocchetti et al., 2020). Samples extracted by pure methanol gave 10.60 mg-GAE/g-DP, 6.23 mg-GAE/g-DP by methanol: water (50:50 v/v), while 5.77 mg-GAE/g-DP was determined by pure ethanol and the TFC varied 2.4, 4.93 and 0.53 mg-CE/g-DP, respectively(Rocchetti et al., 2020).

Furthermore, two antioxidant activity tests (DPPH and ABTS) were used to investigate the antioxidant activity of Moringa oleifera leaf extracts. The free radical scavenging activity of Moringa oleifera leaf extracts produced under different conditions is shown in table 7.

As shown in table 7 the scavenging activity of the samples against DPPH free radicals ranged from 5.04 to 9.90 mg-TEAC per gram dried samples. on the other hand, activity against ABTS free radical was measured between 16.39 and 29.25 mg-TEAC/g-DP. The results of both tests were positively correlated ($r > 0.80$). The capacity of antioxidant activity by ABTS assay had been higher than the antioxidant activity finding by DPPH method. Angeloni, S., et al (2021) reported greater results with ABTS antioxidant activity test comparing to DPPH test for *Verbascum bombyciferum* plant extracted by HAE method, where reported the ABTS varied from 80.04 to 85.6 mg-TEAC/g-DP and the DPPH changed between 1.04 to 51.33 mg-TEAC/g-DP (Angeloni et al., 2021). Dall'Acqua, S., et al (2020), also reported the ABTS and DPPH values of the *Cochlospermum planchonii* extracts obtained by HAE as 766.27 and 525.51 mg-TEAC/g-DP, respectively(Dall'Acqua et al., 2020). From this results the antioxidant capacity of Moringa oliefera dry leaves was considerably greater when measured using the ABTS test than when measured using the DPPH assay. These results suggested that the ABTS test may be more effective than the DPPH assay for evaluating antioxidant capability in a wide range of foods and plants(Floegel, Kim, Chung, Koo, & Chun, 2011). This might be explained by the fact that ABTS provides to measure more antioxidants (both hydrophic and hydrophilic)(Martysiak-Żurowska & Wenta, 2012). Additionally, the antioxidant activity results produced by ABTS gave a higher positive correlation with TPC *($r = 0.6454$ vs 0.0265)* in

HAE method. This indicates that phenolics have more effect on the antioxidant activity of the Moringa oleifera leaf extract(Sridhar & Charles, 2019).

Table 6 presents the design matrix of the experimental work according to the CCD of RSA for the *Moringa oleifera* leaf extraction with HAE. The responses (measured values) are also given with the predicted values depending on the model equations (Eqs. 2 and 3) derived from CCD.

Table 8 gives the analysis of variance (ANOVA) test produced by Design expert program (12th edition). ANOVA test provides the verification about model fitting depending on the most famous statistical tools such as *F*-value, *P*-value, lack of fit value, coefficients of determination (R^2, adjusted R^2 and Predicted R^2), and coefficient of variation (C.V.). *P* values indicates that both of the quadratic models generated for TPC and TFC are statistically significant ($P < 0.05$) and convenient with the experimental findings. On the other hand, coefficients of determination of second-order equations seem satisfactory ($R^2 > 0.98$). R^2 of TPC and TFC show that meaning that 99.21% and 98.23% of the variabilities in the *Y* values can be explained by the proposed model equations. Adjusted R^2 of TPC and TFC was 98.49% and 96.65%. hence, the predicted R^2 of TPC and TPC was 96.68% and 88.63%. In this research, the high value of adjusted R^2 and its closeness to R^2 indicate the high importance of the model. Predicted R^2 describes how well the model predicts new data. The sufficiently high values of R^2 and predicted R^2 indicate the goodness-of-fit of the obtained statistical models(Ahmadi, Ghanbari, & Madihi-Bidgoli, 2016; Pollini et al., 2020). The predicted R^2 values in reasonable agreement with the adjusted R^2 values where the difference is less than 0.2. Adeq Precision measures the signal to noise ratio. A ratio greater than 4 is desirable. Adequate precision of 31.427 for TPC and 26.335 for TFC indicates an adequate signal. This model can be used to navigate the design space. Additionally, the CV is an important coefficient that demonstrates the models repeatability if it is less than 10. It was discovered that the resulting CV value was 6.14 for TPC and 7.42 for TFC, implying that the model is repeatable. According to the results, the model is a good model that may be utilized for optimization and prediction.

Additionally, As shown in table 8 , the lack of fit values for TPC and TFC derived from CCD-based model have been found non-significant ($P > 0.05$), demonstrating that the system is desirable that the model fits. The Lack of Fit F-value of 0.47 for TPC implies the Lack of Fit is not significant relative to the pure error. There is a 78.45% chance that a Lack of Fit F-value this large could

occur due to noise. Non-significant lack of fit is good. Also, The Lack of Fit F-value of 4.24 for TFC implies there is a 6.95% chance that a Lack of Fit F-value this large could occur due to noise. Lack of fit is bad.

Considering the HAE process parameters for the yield of TPC, solvent concentration was the most effective parameter ($P < 0.05$), followed by the mixing speed and extraction time. Quadratic effects of solvent concentration were also statistically significant ($P < 0.05$). As for TFC, the solely ethanol concentration and second order of ethanol concentration were the most significant parameter ($P < 0.0001$), followed by mixing speed and extraction time were also statistically ($P < 0.05$). followed by the interaction of ethanol concentration and mixing speed was also statistically ($P < 0.05$). Also the interaction of ethanol concentration and mixing speed was also statistically ($P < 0.05$) influential for the recovery of TFC from *Moringa oleifera* leaf extraction with HAE.

On the another hand, the effects of independent variables on responses can be explained by The three-dimensional response surface plots were produced by model equations. as show in figure 2 and Figure 3 the effects of HAE independent variables on TPC and TFC when one of independent factor was constant. the color of area changing from blue to red demonstrates an increase of responses. As shown in Figure 2 and 3the mixing speed and extraction time have a positive effect on the systems. Hence, the TPC and TFC increased slightly with the increase in mixing speed (rpm) and extraction time (sec). Also as show in figure 2a, the amount of TPC gradually increased with the increase in the concentration of the ethanol until it reached the highest value at approximately 50% of ethanol concentration. after that the phenol content began to decrease gradually. Additionally, as show in figure 3a the TFC decreased significantly with the increase in the ethanol concentration. Similarly, Kaur et al observed an increase in the phenolic content of Stevia rebaudiana Bertoni when the ethanol concentration increased from 55% to 70%, followed by a decrease after 70% of ethanol solution (Kaur, Manchanda, & Sidhu, 2021). A similar situation was also observed in the extraction of Bioactive Compounds from Eggplant Peel By-Product by MAE method(Doulabi, Golmakani, & Ansari, 2020). The Ethanol concentration enhances the solubility of phenolic compounds. The increase in the concentration of ethanol in the solutions leads to a decrease in the heating of the mixture and thus the thermal decomposition of the recovered compounds is less. However, high concentrations of ethanol can lead to protein denaturation, thus preventing the dissolution of polyphenols and affecting the rate of extraction. On the other

hand, water is considered one of the best solvents because it increases the contact surface between polyphenols and the solvent, as it is a swelling agent for cells(Doulabi et al., 2020). Therefore, the higher the percentage of water in the solvent solution leads to a decrease in the concentration of the solvent and thus the rate of extraction of polyphenol is higher.

Additionally, optimum conditions to achieve the highest TPC and TFC yields are given in Table 9. The optimum conditions are 77 sec of extraction time, 9000 rpm of mixing speed and 30% (v/v) ethanol solution for the best extract yields (17.51 mg-GAE/g-DP of TPC and 271.37 mg-CE/g-DP of TFC). Confirmation experiments were also carried out under the derived conditions of the software. The difference between the actual and predicted values (< 2%) verifies the acceptability of the generated second-order models. In fact, the adeqacy of model also can be evaluated by finding the correlation between the amount of (TPC and TFC) actual and predicated. Figure 3 also explains this situation visually. Where the correlation between actual and predicted values of TPC and TFC was (R^2< 0.98) and RMSE was 0.582 for TPC and 8.30 for TFC. Where the RMSE is a good measure of how accurately the model predicts the response, and it is the most important criterion for fit. The lower value of RMSE preferred the better model performance, conversely, the higher value of R^2 (closer to 1) shows that the regression line fits the data well and the model performance is better(Vafaeipour, Rahbari, Rosen, Fazelpour, & Ansarirad, 2014).

Conclusions

Automated solvent extraction method has been used to obtain an extract rich in polyphenol and flavonoid components from *Moringa oleifera* leaves. Since the aim of this study is to develop a method using green technologies, environmentally friendly and generally known as safe (GRAS) ethanol and its aqueous solutions were chosen as the solvent system. This is significant due to the fact that target products are recommended as natural additives in areas such as food, medicine and cosmetics. According to the optimization study of the Central Composite Design with the Desing Expert software, the best process conditions required to obtain the highest total phenolic (17.51 mg-GAE/g-DP) and total flavonoid (271.3 mg-CE/g-DP) yields were obtained with 77 sec, 9000 rpm and 30% ethanol solvent system. While the most effective parameter in terms of total phenolic substance was extraction time, the most statistically significant variable in terms

of flavonoids was solvent concentration. It can be concluded that the quadratic model equations derived for both systems successfully represent the experimental data depending on the high coefficients of determination (> 0.98), statistically significant *P* values (<0.05), C.V. values (< 10%), and non-significant lack of fit value (*P* > 0.05).

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9

THE EFFECT OF DIFFERENT BIOSTIMULANTS APPLICATIONS ON CORM CHARACTERS OF SAFFRON (*CROCUS SATIVUS* L.)

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ABSTRACT

Saffron is considered “red gold” and is generally among the most expensive and valuable spices in the world. The main purpose of this study is to represent the effects of some biostimulants (activators, hormones, biostimulants, bacterial isolates, and plant nutrients) applied to the corm and leaf of saffron on the amount of corm production for seed production. One of the biggest factors restricting saffron production is that seed corm production should be limited. In this study, it has been revealed that the annual production can be done economically. The highest yield of 308.3 g parcel of corm for the production of corm from the protection biostimulants applications and the highest yield of 271.0 g of corm for the leaf biostimulants application were obtained in the BİO6 biostimulants application. It is of great importance for the production of corm used as seed material in valuable plants such as saffron and for studies on the production of corm.

Keywords:bacterial isolates; biostimulant; Crocus sativus L; hormones; plant nutrients

INTRODUCTION

Saffron is considered “red gold” and is generally among the most expensive and valuable spices in the world. Saffron contains more than 150 volatile ingredients known for their aroma, color, taste, and scent effects. The chemical composition of saffron contains protein, moisture, mineral, fat, crude fiber, and sugar and small amounts of thiamine and riboflavin. The four basic components of saffron are crocin, crocetin, picrocrocin and safranal (Asil, 2018; Rikabad et al., 2019; Caid et al., 2020; Asil, 2021).

There is a growing interest in plant biostimulantss (hormone and foliar fertilizers) and microbial fertilizers to protect the nutrition and health of plants (Rubio-Moraga et al., 2014). The effects of hormones and plant growth regulators on the production of bulbous plants are not known. Hormones and plant growth regulators have been reported to be effective for corm growth and its proliferation (Asil and Ayanoğlu, 2018). Plant growth regulators can be applied from germination to the harvest stage and even after harvest. It is important to determine appropriate concentration and application time for applying plant growth regulators to obtain the desired impact. It is generally known that hormones applied to the corm affect the existence of primary metabolites (sugars, amino acids, organic acids and fatty acids) in the corm (Göktürk and Asil, 2018; Rubio-Moraga et al., 2014).

In a recent research, they reported that biostimulants play very important roles in modulating plant stress responses. Inoculating plants with plant growth-promoting rhizobacteria (PGPR) improves plant tolerance to abiotic stresses (e.g. drought, temperature, and salinity). In recent years, the use of biostimulants has been increasing and the use of synthetic fertilizers and agricultural chemicals has been gradually decreasing (Backer et al., 2018; Sungur et al., 2014; Sungur et al., 2018).).

Saffron is grown for saffron spice production. However, corm production and leaves are an important side income source for seed material. (Shajari et al. 2021). The main purpose of this study is to represent the effects of some biostimulants (activator, hormones, biostimulant, bacterial isolates and plant nutrients) applied to the corm and leaf of saffron on the amount of corm production for seed production.

METHODS

Material

This study was carried out in Kırıkhan district of Hatay province in September 2018- May 2019. In the study, saffron corms were weighed as the production material and corm with an average weight of 8 g was used. Also, the study was planned as a different removal period, and the corm data removed in the first year are given in this study.

Obtaining the samples

Samples were collected from the corm and leaves of the saffron applied biostimulants (activator, hormones, biostimulant, bacterial isolates, and plant nutrients). Some of the biostimulants were applied only to the corm or the leaf, and others were applied to both of them. Samples were collected from the corm and leaves of the saffron applied activators (hormones, bacterial isolates and plant nutrients). Some of the biostimulants were applied only to the corm or the leaf, and others were applied both of them. Control, BİO1 (microbial biopolymer PHB (Poly--hydroxybutyrate)), BİO2 (Pseudo-monasfluorescens bacteria isolate), BİO3 (as a microbial fertilizer containing natural Bacillus subtilis, Bacillus megaterium and Lactococcus spp.), BİO4 (as a biologic fertilizer containing Mycorrhiza, Bacillus and Trichoderma), BİO5 (as a plant nutrition product containing 6% Metallic iron (EDDHA) and Na), BİO6 (GA3 hormone), BİO7 (liquid organomineral fertilizer with NK) were used as activators applied to the corm. Control, BİO2, BİO5, BİO6, BİO7, BİO8 (liquid organic fertilizer containing amino acids), BİO9 (liquid micro plant nutrient) and BİO10 (NPK liquid foliar fertilizer) activators were used to apply to the leaf of Saffron. Samples were collected in the first and second year of the applications of biostimulants.

Methods

Two different trials were set up as the separate application of biostimulants to corm and leaves. In the application of corm, corms were applied in 500 ml water at the doses indicated for ornamental plants on the labels of Control, BİO1, BİO2, BİO3, BİO4, BİO5, BİO6, and BİO7 products, by keeping them for half an hour. In leaves application, Control, BİO1, BİO5, BİO6, BİO7, BİO8, BİO9, and BİO10 products were added to 500 ml of water at the doses indicated for or-

namental plants on the labels, and after the plants reached a leaf length of 10 cm, the leaves were sprayed.

The experiment was established according to the randomized parcel trial pattern with 3 repetitions in two different environments with the same characteristics and application of corm and leaf. 10 corms were planted in each parcel to a depth of 10 cm and the first irrigation was applied immediately after planting. No other irrigation was done in field conditions. Weed control was done manually. No drugs or fertilizers were used (Asil and Ayanoğlu, 2017).

Statistical analysis was performed in the MSTAT-C computer program and the differences between the mean values were determined by the Duncan test.

RESULTS

Effects of corm biostimulants applications on the important characters of saffron in terms of agriculture

The effects of the applications of biostimulants to the corm and leaf of saffron were different for both of them. According to the mean values and variance analysis obtained from the plants for the number of harvested corm (over 10 g) and harvested corm weight (over 10 g), the effect was found to be statistically significant at the 0.05 level after biostimulants applications to the corm of saffron. In addition to that, the effect was statistically significant at 0.01 level for total harvested corm weight, units of increasing corm coefficient, and ratios of daughter corm formation (Table 2).

After applications of biostimulants to the corms, the highest number of leaves in the plant was found to be 36.6 pieces/plant for BİO1 application. The longest leaves with an average of 40.7 cm were obtained for BİO6 application. The number of harvested corms (5 g and lower) with an average of 39.7 was obtained for BİO1 application. The highest number of harvested corms (5.1-10 g) with an average of 10.0 was observed for BİO1 application. The highest number of harvested corms (over 10 g) was obtained for the BİO1 application. The highest number of total harvested corm with an average of 60.4 was obtained for the BİO1 application. Also, the harvested corm weight (5 g and lower) was found to be 89.0 g for the BİO6 application. The harvested corm weight (5.1-10 g) was 66.3 g for the BİO1 application. The highest harvested corm weight (over 10 g) was obtained

for the BİO1 application. In harvested corm weight (Over 10 g), the highest BİO1 application yielded 157.7 g of the corm. In the total harvested corm weight, the new highest corm weight was obtained in BİO1 application with 308.0 g. Also, the highest units of increased corm coefficient with 367.0% and daughter corm formation ratios with 642.0% were observed in the BİO1 application (Table 1).

Table 1. Mean values and Duncan groups of corm biostimulants applications for the characters measured

Biostimulants	Plant Leaf Number (pieces)	Leaf lenght (cm)	Number of Harvested Corm (5 g and lower) (pieces	Number of Harvested Corm (5.1-10 g) (pieces)	Number of Harvested Corm (Over 10 g) (pieces)	Total Number of Harvested Corm (pieces)
Control	30.1	38.3	27.0	6.7	10.3ab**	44.0
BİO1	36.6	35.0	39.7	10.0	10.7a**	60.4
BİO2	29.0	34.0	27.7	5.3	7.7a-c**	40.7
BİO3	34.1	38.0	31.7	6.0	9.3a-c**	47.0
BİO4	21.3	38.3	29.0	6.0	4.0c**	39.0
BİO5	26.9	38.7	24.0	8.0	5.3a-c**	37.3
BİO6	29.2	40.7	29.7	8.7	8.7a-c**	47.0
BİO7	27.9	34.3	35.0	5.0	5.0bc**	45.0

** The differences between the mean values were statistically significant at the 0.05 level.

* The differences between the mean values were statistically significant at the 0.01 level.

After biostimulants applications to the leaf of saffron, the effect was statistically found to be significant at 0.05 according to the average values obtained from the number of harvested corms (over 10) and harvested corm weight (over 10 g) and variance analysis. Also, the units of increased corm coefficient and daughter corm formation ratios were found to be statistically significant at 0.01 level (Table 2).

Table 2. Mean values and Duncan groups of corm biostimulants applications for the characters measured

Biostimulants	Harvested Corm weight (5 g and lower)	Harvested Corm weight (5.1-10 g) (g)	Harvested Corm weight (Over 10 g) (g)	Total Harvested Corm weight (g)	Unit Corm İncrease Coefficient (%)	Daughter Corm Formation Ratios (%)
Control	71.7	46.7	152.0a**	270.3a-c*	321.7a-c*	465.0bc*
BİO1	84.3	66.3	157.7a**	308.3a*	367.0a*	642.0a*
BİO2	63.3	39.7	114.0ab**	216.7a-c*	258.0a-c*	424.7bc*
BİO3	86.7	39.7	137.0ab**	263.0a-c*	313.7a-c*	506.3a-c*
BİO4	68.0	37.3	62.7b**	168.3c*	200.3c*	403.7c*
BİO5	60.3	56.0	78.3ab**	195.0bc*	232.3bc*	482.3bc*
BİO6	89.0	60.0	143.7ab**	292.7ab*	348.3ab*	566.0ab*
BİO7	73.0	26.0	71.0ab**	169.7c*	202.3c*	483.7bc*

** The differences between the mean values were statistically significant at the 0.05 level.

* The differences between the mean values were statistically significant at the 0.01 level.

Effects of leaf biostimulants applications on the important characters of saffron in terms of agriculture

According to the average values of the applications leaf the highest number of leaves with an average of 36.3 units/plant was observed in the BİO1 application. The highest leaf length with 40.7 cm was observed in BİO5 application. The highest number of harvested corms (5 g and lower) was observed in BİO6 application. The highest number of harvested corms (5.1-10 g) with 9.7 units was observed in the BİO7 application. The highest number of harvested corms (over 10 g) was observed in the Control application. The highest number of harvested corms with 57.3 units was observed in the BİO6 application. In addition to that, the harvested corm weight (5 and lower) was observed in BİO1 application. The harvested corm weight (5.1-10 g) with 70.0 g was observed in BİO7 application. The highest harvested corm weight (over 10 g) was achieved in the BİO5 application with 154.0 g. The highest harvested corm weight was achieved in the Control

application with 270.0 g. The highest ratio for the units of increased corm coefficient with 322.7% and the highest daughter corm formation ratios with 610.0% were observed in BİO6 and BİO8 applications, respectively (Table 3-4).

Table 3. Mean values and Duncan groups of leaf biostimulants applications for the characters measured

BİOSTİMULANTS	Plant Leaf Number (pieces)	Leaf lenght (cm)	Number of Harvested Corm (5 g and lower) (pieces)	Number of Harvested Corm (5.1-10 g) (pieces)	Number of Harvested Corm (Over 10 g) (pieces)	Total Number of Harvested Corm (pieces)
Control	30.0	38.3	27.0	6.7	10.3a**	44.0
BİO1	29.0	39.3	33.7	7.3	5.7bc**	46.7
BİO5	35.3	40.7	25.3	7.0	9.7ab**	42.0
BİO6	35.7	34.4	44.0	4.7	8.7ab**	57.3
BİO7	34.0	34.7	35.3	9.7	3.7c**	48.7
BİO8	21.0	35.0	30.7	6.3	5.3bc**	42.3
BİO9	33.0	40.3	41.3	7.3	7.0a-c**	55.7
BİO10	36.3	33.3	40.7	3.7	7.3a-c**	51.7

** The differences between the mean values were statistically significant at the 0.05 level.

* The differences between the mean values were statistically significant at the 0.01 level.

In the literature, the leaf length of saffron was found to be between 21-24 cm (Unal and Çavuşoğlu, 2005). A study carried out using different soil and corm weights (0.1-4 g, 4-8 g, and 8-12 g) revealed that the number of leaves per plant was 04.05, 06.58, and 11.04, respectively, and the height of the plant was measured as 16.88, 19.55 and 20.39 cm, respectively (Fallahia et al.2017). In this study, excellent results were obtained regarding the length of leaves and

the number of leaves per plant. In the biostimulants application to the corm, the average length of leaves per plant was 36.5 cm for the BİO1 biostimulants. BİO6 biostimulants application to the leaves of saffron resulted in an average of 40 cm leaf length.

Table 4. Mean values and Duncan groups of leaf biostimulants applications for the characters measured

BİOSTİMULANTS	Harvested Corm weight (5 g and lower)	Harvested Corm weight (5.1-10 g) (g)	Harvested Corm weight (Over 10 g) (g)	Total Harvested Corm weight (g)	Unit Corm İncrease Coefficient (%)	Daughter Corm Formation Ratios (%)
Control	71.7	46.7	152.0a**	270.3	321.7	465.0
BİO1	82.7	49.0	81.3bc**	213.0	253.7	486.0
BİO5	53.3	50.3	154.0a**	257.7	307.0	508.7
BİO6	108.0	31.3	131.0ab**	271.0	322.7	590.0
BİO7	79.0	70.0	47.3c**	197.3	235.0	502.0
BİO8	77.0	45.0	76.0bc**	198.0	235.0	610.0
BİO9	104.7	47.0	111.7ab**	263.3	313.3	573.7
BİO10	118.0	27.3	97.0a-c**	242.0	288.0	554.3

The effects of different dismantling times were also studied and results have shown that 50.67 pieces/parcel of corms planted 5 cm deep were removed from the parcels in the second year. They reported that 14.44 pieces/parcels of corms planted in 5 cm deep were obtained every year (Yıldırım et al., 2017a). In a study conducted on different core lengths and planting depths of saffron, an average of 6.67-21.67 pieces/plot of corm was obtained (Yıldırım et al., 2017b). In the study of corm weight from 1 to 16 g were planted and 40 corms were obtained by planting 16 g corm. An average of 3.7 g of corms with a similar weight was used in this study (Khan et al., 2011). Regarding the number of corms, quite good yields were obtained in this study. The total number of harvested corms was obtained in the BİO1 biostimulants application in the application of the corm biostimulants, and 57.3 in the BİO6 biostimulants application on the leaf (Yıldırım et al., 2017a).

In our study, the fact that the number of corms which is normally reached in the second year of planting was reached in the first year is of great importance economically regarding seed corm production.

In a study conducted by Yıldırım et al., 2017a, the highest corm weight was obtained as 527.33 g / parcel from large size corms, which were removed in two years and sewn 15 cm deep. The lowest was obtained as 84.33 g / parcel from small size corms that were removed every year and sewn 5 cm deep (Hajyzadeh et al., 2017). Obtained a corm yield between 493 and 1910 kg/da in their study conducted Hatay conditions in 2017. According to Yıldırım et al., 2017b, corm yield was obtained in 19-219 g / 150 m2 in the study conducted under Hatay conditions.

When the harvested onion weights were evaluated according to the literature, very promising results were obtained. One of the biggest factors limiting saffron production is the limited production of corm seed. The effects on corm yield were investigated by using biostimulants. In the next process, the application doses of biostimulants with important results should be studied.

CONCLUSIONS

In conclusion, the main purpose of this study is to represent the effects of some biostimulants (hormones, biostimulant, bacterial isolates and plant nutrients) applied to the corm and leaf of saffron on the amount of corm production for seed production.

It has been revealed that annual production can be made economically with the use of suitable biostimulants for seed material. It is of great importance for the production of corm used as seed material in valuable plants such as saffron and for studies on the production of corm.

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