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Research Article

Antioxidant Capacity of *Micromeria fruticosa* L. Druce Methanol Extract

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ABSTRACT:
Micromeria species have various biological activities including antimicrobial, antibacterial, antifungal and antioxidant activities, and therefore it has been reported that they are used in traditional treatment in many areas. This study was carried out to investigate the antioxidant effects of *Micromeria fruticosa* L. Druce methanol extract obtained from the leaves of *Micromeria fruticosa* L. Druce plant. Extracts were prepared at the concentrations of 125, 250 and 500 µg/mL. Antioxidant activity of the samples were analyzed by FRAP (Iron ion reducing antioxidant power) and DPPH (1,1-diphenyl-2-picrylhydrazil) methods. Equivalent antioxidant capacity was determined by using different concentrations of 1-100 µg/mL of the reference samples. It was determined that the methanol extract of *Micromeria fruticosa* L. Druce plant exhibited antioxidant capacity according to FRAP and DPPH methods, thus further studies are needed to investigate therapeutic effects of this plant.

Keywords *:* Antioxidant, DPPH, FRAP, methanol extract, *Micromeria fruticosa* L. Druce.

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1. INTRODUCTION

Micromeria fruticosa L. Druce is a member of the Lamiaceae family. *Micromeria fruticosa* L. Druce is a Mediterranean herb with medicinal properties. *Micromeria fruticosa*, also called 'Taş nanesi' in Turkey, is a perennial herb that grows up to 20-60 cm high in the rocky regions of the southern and eastern Anatolian region of Turkey. *Micromeria* species are commonly used as herbal teas. Traditionally it is used in heart diseases, headaches, wounds and skin infections. *Micromeria* species have biological activities such as antimicrobial, antibacterial, antifungal and antioxidant, and are used as a sedative, anesthetic, antiseptic, abortifacient, antirheumatic, and in the treatment of colds [1-3]. The existence of protective mechanisms that are effective in reducing and treating oxidative stress may be crucial. Antioxidants are molecules that prevent the formation of radical groups in living metabolism and neutralize the formed radicals.

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Antioxidants are among the defense systems that resist the harmful effects of free radicals [4]. The antioxidant activity of methanol extract of *Micromeria fruticosa* L. Druce was determined using two antioxidant capacity assessment techniques (FRAP and DPPH).

2. MATERIALS & METHODS

2.1. Materials

Micromeria fruticosa (L.) Druce was collected from Uzundere district of Erzurum province in August 2022. Trolox was purchased from Fluka Chemica (Switzerland) and NH4Ac from Riedel De Haen (Germany). TPTZ (2,4,6-Tri(2-pyridyl)-s-triazine) and DPPH (1,1-diphenyl-2-picrylhydrazil) were obtained from Sigma Chemicals Co. (St. Louis, USA). $FeCl_3.6H_2O$ was purchased from Merck (Germany). Millipore (Direct-Q® 3UV, USA) was used to obtain ultrapure water. All of the other solvents and reagents were of the analytical grade.

2.2. Extraction

For the preparation of methanol extract, dried *Micromeria fruticosa* (L.) Druce leaf samples taken as representative of the leaves were ground into powder with the help of a laboratory blender. 250 mL of solvent was added to 50 g of dried herb and mixed in a horizontal shaker water bath for 72 hours (filtered every 24 hours) at 50 ^oC. The solvents were evaporated at 50°C with the aid of an evaporator and the extract was made dry. The extract was kept in dark and at 2-8 ^oC in an airtight bottle for further studies.

2.3. Determination of Antioxidant Capacity

2.3.1. FRAP method

When FRAP interacts with a potential antioxidant, it transforms a colorless Fe(III)-TPTZ complex into a bright blue Fe(II)-TPTZ complex. In this method proposed by Benzei and Strain, the total amount of antioxidants is evaluated by the reducing capacity of Fe(III) in acidic medium. The reduced Fe(II)-TPTZ complex has blue color and a maximum absorbance at 593 nm. Since the color change in the FRAP method indicates the presence of antioxidant substances, it is a method in which the antioxidant capacity is directly determined [5,6]. To obtain the FRAP solution, 10 mM solution of TPTZ (2,3,5-Triphenyltetrazolium chloride) in 40 mM HCl, 20 mM $FeCl_3$ solution and 300 mmol/L acetate buffer solution (pH 3.6) were prepared separately. A total of 30 mL of FRAP solution was obtained by taking 2.5 mL of TPTZ, 2.5 mL of $FeCl_3$ and 25 mL of acetate buffer from these prepared solutions. For the 96-well plate, 200 µL of FRAP solution and 10 µL of extract sample were added to each well. Absorbances were measured at 593 nm after 30 minutes of incubation.

2.3.2. DPPH method

The spectrophotometric method developed by Brand-Williams was used to determine the antioxidant capacity based on measuring the inhibition response of the samples against the DPPH radical [7] 39 mg of DPPH was dissolved in ethanol, and then the volume was made up to 100 mL to obtain DPPH solution. To the 96-well plate, 70 µL of DPPH solution

and 210 µL of the extract sample were added to each well. After shaking for about 1 minute, it was incubated in the dark for 30 minutes and then the absorbance at 517 nm was measured. Trolox was used as the standard antioxidant for the control sample. The sign of the presence of an antioxidant in the DPPH solution is the decrease in its color, the decrease in the color intensity facilitates the measurement in the spectrophotometer. This test has advantages such as ease of laboratory work, low cost, repeatability, automation options and applicability at room temperature [8].

3. RESULTS & DISCUSSION

3.1. Antioxidant Capacity Findings

3.1.1. Findings of iron ion reducing antioxidant power (FRAP)

The absorbance values corresponding to the iron (III) reducing/antioxidant power at 595 nm of the methanol extract prepared from the leaves of *Micromeria fruticosa* (L.) Druce plant and standard antioxidant compounds were measured spectrophotometrically. The analyzed concentration range (1-100 µg/mL) was determined as a result of studies on standard antioxidant compounds. Trolox was used as the reference compound and results are shown as Trolox equivalent. Trolox solutions were prepared in various concentrations (range 1-100 µg/mL), and by measuring their absorbance, the calibration line and the line equation were obtained.

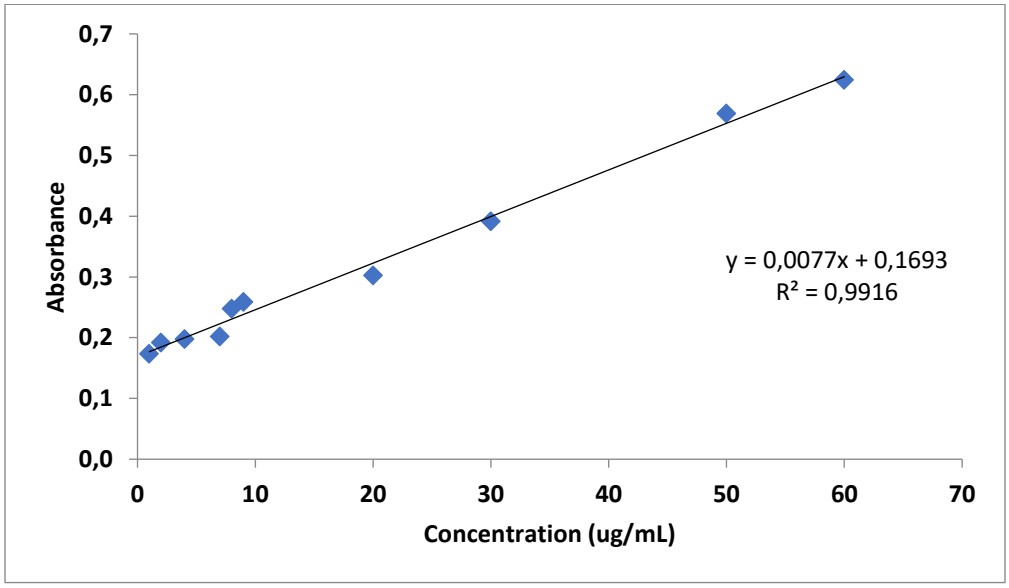

Figure 1. The calibration curve for the FRAP method.

The comparison of iron (III) reducing/antioxidant powers at 593 nm with spectrophotometric method at 125, 250 and 500 µg/mL concentrations in terms of µg Trolox equivalent Antioxidant Capacity (TEAC) is shown in Table 1.

Table 1. Antioxidant capacities of *Micromeria fruticosa* (L.) Druce methanol extract as measured by FRAP method.

Concentration (ug/mL)	Trolox (Eq µg/mL)
125	3,038
250	11,264
500	17,519

3.2. DPPH radical scavenging activity

DPPH radical scavenging activity determinations of methanol extract prepared from leaves of *Micromeria fruticosa* (L.) Druce plant and standard antioxidant compounds were performed. Trolox was used as the reference compound and results are shown as Trolox equivalent. Trolox solutions were prepared in the concentration range of 1-100 µg/mL and by measuring their absorbance, the calibration line and the line equation were obtained.

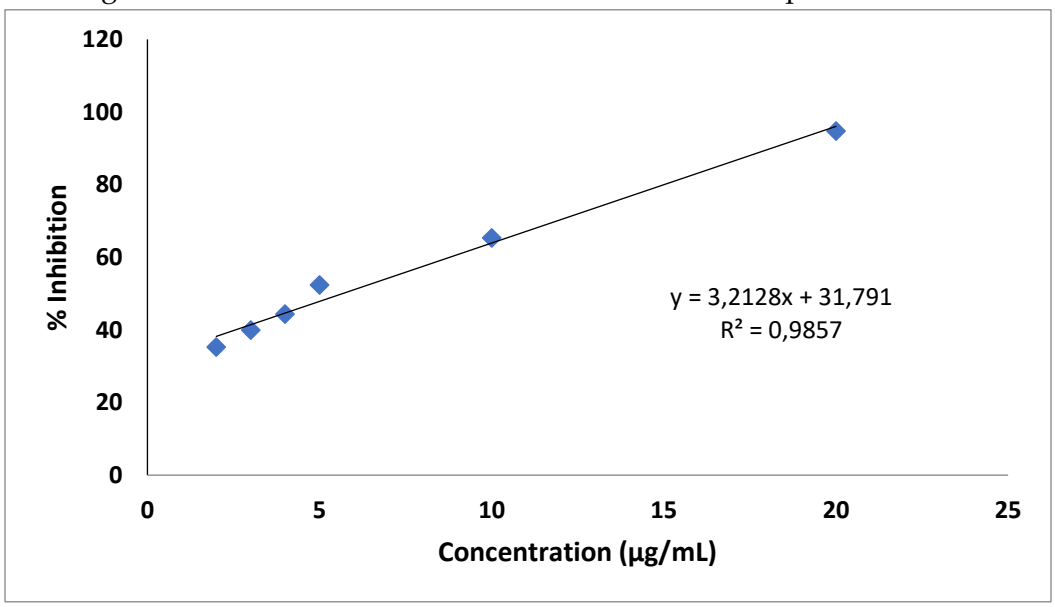

Figure 2. The obtained calibration curve for the DPPH method.

The DPPH radical scavenging capacities of the methanol extract prepared from the leaves of *Micromeria fruticosa* (L.) Druce plant at 125, 250 and 500 µg/mL concentrations are shown as % inhibition (Table 2). It was determined that the concentration with the highest DPPH free radical scavenging capacity from the methanol extract prepared from the leaves of *Micromeria fruticosa* (L.) Druce plant was 500 µg/mL.

Table 2. DPPH % Inhibition Values

Concentration (ug/mL)	% Inhibition
125	3,977
250	9,670
500	12,834

4. CONCLUSIONS

Micromeria fruticosa (L.) Druce extract has high antioxidant capacity, thus, the extracts can used as antioxidant in pharmaceutical preparations. It is thought that this study will contribute to the studies on *Micromeria fruticosa* (L.) Druce.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

The authors declare that they have contributed equally to the article.

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Research Article

Investigation of The *In Vitro* Antioxidant Properties of Methanol Extract of *Olea europaea* L. (Olive) Leaf

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ABSTRACT:

Olea europaea L. (Olive) plant has been used by humans for many years for the treatment of diseases. The aim of this study was to investigate the total phenolic compound and antioxidant activities of methanol extract of the leaves of *Olea europaea* L. (Olive) plant. Total phenolic content of *Olea europaea* L. (Olive) plant leaves was determined by a method using Folin Ciocalteu reagent. Antioxidant activities were determined by DPPH (1,1-diphenyl-2-picrylhydrazil) and FRAP (Iron ion reducing antioxidant power) methods. Different concentrations of reference samples between 1-100 µg/mL were prepared to determine the equivalent antioxidant capacity of the extract. The phenolic compound of methanol extract of the leaves of the *Olea europaea* L. (Olive) plant was the highest at a concentration of 1000 µl/ml. DPPH radical scavenging capacity (inhibition %) values were found to be significantly higher in the extract at 1000 µl/ml than the extracts at other concentrations. This study supports the potential use of *Olea europaea* L. (Olive) in folk medicine.

Keywords *:* Antioxidant, DPPH, FRAP, methanol extract, *Olea europaea* L.

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1. INTRODUCTION

Plants have been used by humans to treat diseases since ancient times in the world. Considering the plant existence in our country, approximately 8500 plant species are grown and it is known that only 650 of them are used for therapeutic purposes. Some natural chemicals found in these plants, which have been the subject of research, draw attention as antioxidants that are very beneficial for human health, and these plants have been consumed by humans and animals since the first civilizations, and the demand for consumption is increasing day by day [1]. Turkey has a rich flora for medicinal plants and there are many plants used in traditional medicine [2]. Antioxidants are molecules that prevent the formation of radical groups in living metabolism and neutralize the resulting radicals. Preventing the spoilage of food and preserving the value of the nutrients in it is

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another area of use. Fruits and vegetables are used in the treatment of some diseases due to these effects. Toxic substances that occur due to the applications in the production process of the foods we consume in our body increase the intake of free radicals to the organism and cause them to accumulate in our body. Therefore, it is extremely important to consume foods containing antioxidants in order to remove toxic substances from our body and protect them from their damaging effects. One of these plants is Olive (*Olea europaea* L.) and it is a plant belonging to the Oleaceae (Zeytingiller) family. Olive leaves (*Olea europaea* L. folium) are a natural source of bioactive components. It has been reported that many of the phenolic compounds contained in olive leaves have antioxidant effects [4]. In this study, it was aimed to determine the phenolic compound and antioxidant activity of olive (*Olea europaea* L. folium) leaf methanol extract. Although scientists have made various studies on many plant species, there are still many plant species that have not been researched yet. Our study differs from other studies in terms of the fact that the plant material used was collected from Antalya Finike district and the width of the tested concentration range.

2. METHODS

2.1. Plant Material

Olive leaves collected from Finike district of Antalya province in the Mediterranean Region of our country in September 2021 were cleaned and dried in the shade at room temperature.

2.2. Preparation of Plant Extracts

After drying the leaves of *Olea europaea* L. (Olive) plant, it was ground into powder in a porcelain mortar with liquid nitrogen. Olive leaves treated with methanol were extracted by filtering every 24 hours at 50˚C in a shaker water bath for three days. The resulting filtrate was evaporated by an evaporator. After the methanol was removed, the extract remaining in the glass flask was taken into a glass petri dish, left to dry and stored in a refrigerator at +4˚C for determinations.

2.3. Determination of Total phenolic content

Total phenolic compound content of olive (*Olea europaea* L.) leaf methanol extract was determined by Folin-Ciocalteu method (FC) method [4]. The amount of total phenolic compounds in the plant methanol extract was determined by using the modified version of the method developed by Slinkard and Singleton [5]. First, 50 ml of 7.5% Na_2CO_3 was prepared. Then, after weighing 25 mg of Gallic acid for the standard, it was completed with methanol to 25 ml in a test tube. Finally, Folin Ciocalteu reagent was taken into beaker for phenolic compound determination. Stock solutions were prepared and necessary dilutions were made. First, 40 µL of the sample was pipetted onto the plates. Then, 200 µL of Folin & Ciocalteu reagent was pipetted and incubated for 5 minutes. Finally, 160 µL of Na_2CO_3 was pipetted and incubated for 30 minutes. After the incubation period was over, absorbance was measured at 765 nm with a spectrophotometer device.

Using the standard graph prepared using gallic acid, the results were given as mg Gallic Acid equivalent (GAE)/g.

2.4. Antioxidant Capacity Tests

Total antioxidant activity was determined by DPPH (1,1-diphenyl-2-picrylhydrazil) and FRAP Iron(III) Ion Reduction methods.

2.5. Determination of DPPH (2,2-diphenyl-1-picrylhydrazil) radical scavenging activity

The DPPH radical scavenging capacities of the methanol extract obtained from the leaves of *Olea europaea* L. plant were determined according to the Brand Williams method [6]. After the DPPH solution was prepared according to this method, stock solutions of plant extracts in the range of 2-20 µL/mL were prepared. First, 210 µL of extract sample was pipetted into the plate wells, and then 70 µL of DPPH solution was pipetted into each well. The plate was mixed for 1 minute with the help of a stirrer and incubated for 30 minutes. Trolox was used as the standard antioxidant for the control sample. All samples were measured absorbance at 515 nm against a blank consisting of methanol, and the results were calculated as percent inhibition.

2.6. Fe^{+3} TPTZ reduction capacity according to FRAP method

The antioxidant capacity determination method of the extracts obtained from the leaves of *Olea europaea* L. plant, based on electron transfer, was applied by Huang et al. [7]. First, 300 mmol/L acetate buffer (pH=3.6) was prepared. 10 mM TPTZ was taken into a 100 mL flask, 40 mM HCl was added and the final volume was made up to 100 mL. Finally, 20 mmol/L $FeCl_3$ solution was prepared. A total of 30 mL of FRAP solution was obtained by taking 2.5 mL of TPTZ, 2.5 mL of $FeCl_3$ and 25 mL of acetate buffer from these prepared solutions. 10 µL of the extract sample and 200 µL of FRAP solution were pipetted into the plate wells and allowed to incubate for 30 minutes, and then the absorbance was measured at 593 nm.

3. RESULTS

3.1. Total Phenolic Compound Quantification

The amount of total phenolic compounds of the methanol extract prepared from the leaves of *Olea europaea* L. (Olive) plant was determined by Folin-Ciocalteu Reagent (FCR). Gallic acid was plotted with calculations using a standard phenolic compound (Figure 1). The total phenolic compound amounts of the methanol extract prepared from the leaves of *Olea europaea* L. (Olive) plant were calculated as gallic acid equivalents (GAE) (Table 1).

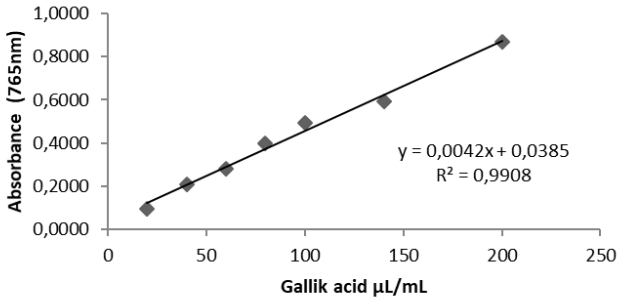

Figure 1. Standard graph of gallic acid

Table 1. Total phenolic compound amounts of methanol extract of *Olea europaea* L. (Olive) leaves

Concentration µg/mL	**Total Phenolic Compound (µg GAE/mg extract)**
250	14.976
500	26.555
1000	63.103

GAE: Gallic acid equivalent

Total phenolic contents of methanol extract of *Olea europaea* L. (Olive) leaves were determined at different concentrations. Accordingly, the highest total phenolic content was determined at the concentration of 1000µg/mL.

3.2. Antioxidant Capacity Findings

3.2.1. DPPH radical scavenging studies

The analyzed concentration range (1-100 µg/mL) was determined as a result of studies in the literature and preliminary trials on standard antioxidant compounds. As a standard antioxidant, the radical scavenging effect of trolox DPPH reached its highest level at a concentration of 20 µg/mL (Figure 2).

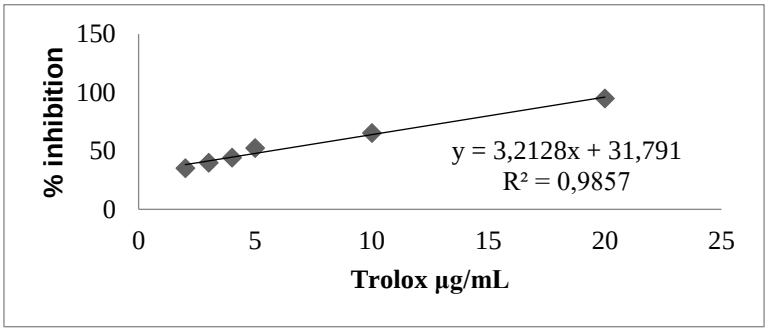

Figure 2. Concentration-% inhibition plot of Trolox

DPPH radical scavenging capacities in the range of methanol extract (250-1000 µg/mL) of *Olea europaea* L. (Olive) leaves are shown in Table 2 as % inhibition.

Table 2. DPPH % Inhibition Values

Concentration (µg/mL)	Trolox Eq (µL/mL)
250	4.774
500	9.682
1000	17.620

Olea europaea L. (Olive) leaves showed the highest DPPH free radical scavenging effect of methanol extract at 1000 µg/mL concentration of the extract.

3.2.2. Iron ion reducing antioxidant power (FRAP)

Spectrophotometric measurement of iron (III) reduction/antioxidant equivalent absorbance at 595 nm of methanol extract of *Olea europaea* L. (Olive) leaves and standard antioxidant compounds was performed. As a result of the studies, the analyzed concentration value range (1-100 µg/mL) was made over standard antioxidant compounds. Since the antioxidant power capacity of trolox, which is one of the standard antioxidant compounds, reached the maximum level at a concentration value of 40 µg/mL, the study was performed in the range of 1-100 µg/mL (Figure 3).

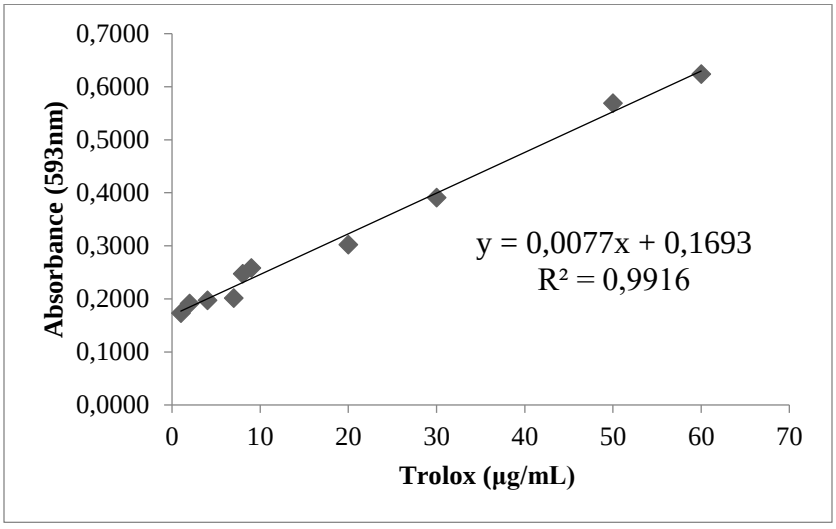

Figure 3. Standard graph of Trolox

The comparison of the iron (III) reducing/antioxidant powers of *Olea europaea* L. (Olive) leaves at 593 nm by spectrophotometric method in terms of µg/mL Trolox equivalent Antioxidant Capacity (TEAC) is shown in Table 3.

Table 3. FRAP method Equivalent Trolox Eq Values

Concentration (µg/mL)	Trolox Eq µg/mL
250	3.316
500	6.259
1000	11.324

4. DISCUSSION

In this study, the total phenolic compound amount and antioxidant capacity (DPPH, FRAP) of the methanol extract of the leaves of *Olea europaea* L. (Olive) plant were determined. Accordingly, the phenolic content was found to be 63.103 µg GAE/mg at 1000 µg/mL concentration. DPPH, FRAP tests carried out to determine the antioxidant capacities of the methanol extract obtained from the leaves of *Olea europaea* L. (Olive) plant showed that, antioxidant activity was found to be higher at 1000 µg/mL concentration. The antioxidant effects were found to be 1000 µg/mL 17.620 Trolox Eq (µL/mL) in the DPPD method and 1000 µg/mL 11.324 Trolox Eq (µL/mL) in the FRAP method.

Olea europaea L. (Olive) is a rich source of active compounds and has been used in folk medicine in Turkey as well as worldwide. Antiarrhythmic and hypotensive effects of olive leaf have been demonstrated, as demonstrated in animal studies. In the literature, it has been stated that the extract made by drying the olive leaf can regulate blood coagulation and circulation and therefore has preventive effects on heart diseases. In another aspect, it has been determined that it has an effect on regulating blood pressure and preventing cardiovascular diseases by preventing low-density lipoprotein (LDL) oxidation. It has been determined that it has an inhibitory effect on free radicals that occur as a result of inflammation in the lung epithelial cells, and it has been suggested to be used in the treatment of the disease. As a result of experiments in mice, genetic structures were also examined in mice in the reproductive and gestational period, and no acute or chronic toxic effect was detected in the study. Oleuropein, one of the main compounds of olive leaf, has been shown to have anti-inflammatory, anti-bacterial and antitumor properties, and in addition, it has strong antioxidant activity due to its binding to endogenous peptides [8]. In this study, it was determined that the methanol extract of the leaves of *Olea Europaea* L. (Olive) plant was rich in high antioxidant activity and total phenolic compounds. We think that this feature is due to the compounds contained in the leaves of the *Olea Europaea* L. (Olive) plant. The reason why methanol extract has been studied is because it has been studied in detail at different concentrations from a single substance (methanol). In addition, in the extract studies in the literature, it is given with the comparison of the methanol extract made with ethanol, water and other different substances. This study was conducted in order to find studies based on these comparisons, to show that the antioxidant activity capacity and the amount of phenolic compounds in

the methanol extract are intense, and to draw attention to the biological effects of olive leaf, which is produced in many countries around the world and has a place in the field of health. Lee and Lee studied the antioxidant and antibacterial effects of phenolic compounds in olive leaf extract and determined that olive leaf extract has radical quenching effects such as superoxide dismutase (SOD) [9]. Since methods for determining antioxidant activity depend on various parameters, there is no single standard method for determining the antioxidant activity of a compound. For this reason, many methods are used to measure antioxidant activity. Different results in the literature may be due to different methods, growing and drying conditions of plants.

5. CONCLUSIONS

The methanol extract obtained from the leaves of the *Olea europaea* L. (Olive) plant analyzed in this study was rich in antioxidant activity and amount of phenolic compounds. It has been determined that olive tree leaves can prevent the formation of damaged cells caused by free radicals due to their high antioxidant effect, and can also be used as an alternative to the standard antioxidants BHA and BHT for various purposes because they are cheap, safe and easily accessible. It is thought that this study will contribute to the studies on *Olea europaea* L. (Olive) plant.

Conflict of Interest

The authors of the article declare that there is no conflict of interest.

Author Contributions

The authors declare that they have contributed equally to the article. This study was presented as a graduation Project of first author (Cenk Güven) in Ataturk University Faculty of Pharmacy in December 2022.

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Review

Cisplatin Nefrotoxicity and Treatment Approaches

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ABSTRACT:
Cancer is one of the most important health problem in the world. Cisplatin is an effective chemotherapeutic drug that is widely used in many cancer types such as lung, cervical, head and neck cancer, stomach cancer, testicular, ovarian, breast cancer. However, the clinical use of cisplatin is limited due to serious side effects and drug-induced resistance. Acute kidney injury (AKI) develops in 20-35% of patients after cisplatin administration. Long-term use of cisplatin results in tubular kidney damage, acute kidney failure, and chronic kidney disease in patients. Mechanisms of kidney injury induced by cisplatin use include proximal tubular damage, oxidative stress, ER stress, apoptosis, and inflammation in the kidneys. There is no completely effective drug or method for kidney damage due to cisplatin use. In vitro and in vivo studies have proven that many natural products and chemicals are effective against cisplatin-induced kidney damage in recent years. In this review, the molecular mechanisms of nephrotoxicity due to the use of cisplatin are described and the findings on current treatment approaches against cisplatin-induced kidney injury are summarized.

Keywords*:* Cisplatin, nephrotoxicity, apoptosis, oxidative stress, inflammation.

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1. INTRODUCTION

Cancer is one of the important problem that threaten human health and result in high rates of death. Today, it is reported that one out of every four people has a lifetime risk of cancer and cancer cases are over 19 million worldwide [1-3]. Cisplatin is an effective chemotherapeutic drug that has been used for a long time in the treatment of cancer [4,5]. Cisplatin is a platinum compound that is in the form of a white or dark yellow crystalline powder at room temperature, also called cis-diamminedichloroplatinum (II). It was first synthesized in 1844 by M. Peyrone and its chemical structure was elucidated by Alfred Werner, and Rosenberg drew attention to the possible use of platinum-based compounds in cancer chemotherapy. Cisplatin shows anticancer activity in a variety of tumors, including ovarian cancer, testicular, head and neck solid tumors. In 1978 it became the first FDA-approved platinum compound for the treatment of testicular and bladder cancer [6].

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Since the discovery of cisplatin, five platinum drugs such as carboplatin, oxaliplatin, nedaplatin, lobaplatin and heptaplatin have been approved in more diverse countries [7].
The mechanism of the anticancer activity of cisplatin is not fully understood. It is generally thought to exert its anticancer effect by binding to DNA. When cisplatin enters cells, hydrolyzed products with a strongly electrophilic structure are formed, which can react with DNA bases. Interaction of cisplatin with DNA; It has many effects such as inhibition of DNA replication and transcription, disruption of protein production, necrosis and apoptosis [4,8]. Although cisplatin has been used in many cancer types for many years, there are two important factors limiting its clinical use. The first is the serious side effects caused by the use of cisplatin, the second is the resistance to cisplatin [6,9]. Cisplatin has many side effects that limit its use in cancer patients. These are ototoxicity, nephrotoxicity, hepatotoxicity, neurotoxicity [10]. The most common side effect of cisplatin in clinical use is nephrotoxicity [4]. Nephrotoxicity may vary depending on the dose used, and the rate of development of nephrotoxicity in patients is 30-40%. Cisplatin nephrotoxicity initially shows symptoms such as hypocalcemia, hypomagnesemia, and acute kidney injury. It then manifests in various forms such as proximal tubular damage and chronic renal failure. Patients who develop kidney damage have a decrease in renal plasma flow and glomerular filtration rate (GFR). At the same time, serum creatinine (Cr) and blood urea nitrogen (BUN) concentrations increase [8,11].
Many methods have been developed to combat the side effects that limit the use of cisplatin, such as developing cisplatin analogues with lower toxicity and hydrating patients during cisplatin treatment. Despite these precautions, especially nephrotoxicity remains a serious side effect that limits the use of cisplatin.

1.1. Nephrotoxicity Mechanisms of Cisplatin

Cisplatin is taken into the cell by the copper transporter 1 (CTR1) and organic cation transporter 2 (OCT2) located in the plasma membrane. These transporter proteins are localized in the kidney, so cisplatin tends to accumulate in the kidneys at higher concentrations, particularly in the proximal tubule [12,13]. The toxicity of cisplatin in the kidney is due to its accumulation in high concentrations in the proximal tubules. Even doses that would not normally cause toxicity can reach toxic levels in the kidney tubules and cause damage [8]. Clinically, the development of nephrotoxicity occurs within 10 days after cisplatin administration [4] and is manifested by disturbances in electrolyte balance and abnormalities in renal function tests [14].
Cisplatin, which causes tubular cell damage, achieves this effect by activating many signaling pathways [4]. Cisplatin tends to bind to mitochondrial DNA in the cell. Cisplatin tends to bind to mitochondrial DNA in the cell. The renal proximal tubule is the region with the highest mitochondria density in the kidney, so it is more susceptible to cisplatin toxicity [15].

The mechanisms of nephrotoxicity caused by cisplatin are quite complex. Many pathways are activated simultaneously. Activated pathways mainly include oxidative stress, inflammation, and apoptosis. It is stated that cisplatin doses that cause kidney damage cause an increase in necrosis and apoptosis in the tubules [16,17]. In the nephrotoxicity of cisplatin, apoptotic pathways activated by tumor necrosis factor receptors 1 and 2 (TNFR1 and TNFR2) or death receptors such as Fas and intrinsic pathways such as mitochondrial and endoplasmic reticulum (ER) stress pathway have been identified [15]. In caspase-independent pathways, a nuclear factor responsible for DNA regulation activates a factor on the mitochondrial membrane and apoptosis is induced. The apoptotic response can also be activated by Bcl/Bax and caspases. Bax travels to the mitochondria and induces the release of cytochrome c, then activation of caspase-3 and 9 occurs for activation of apoptosis [14].

Cisplatin-induced apoptosis can also be triggered by ER stress (ERS). As a result of physiological and pathological events, unfolded and/or misfolded proteins accumulate in the ER lumen. ERS activates the unfolded protein response (UPR) to prevent protein aggregation and maintain homeostasis. If ER stress continues for a long time, the UPR leads to apoptosis [18]. In vitro study showed activation of GRP78/BiP and PERK pathways after cisplatin treatment [19]. Another study found that CHOP, GRP94 and GRP78 were upregulated in cisplatin-treated rats [20]. Oxidative stress caused by reactive oxygen species (ROS) is one of the critical mechanisms underlying cisplatin nephrotoxicity. Oxidative stress occurs due to the deteriorated balance of oxidant and antioxidant production in the body [21]. ROS can attack and alter multiple target molecules such as lipids, proteins, and DNA, creating cellular stress. In cisplatin-induced nephrotoxicity, ROS molecules activate apoptotic pathways and important signaling pathways that lead to cell death. NADPH oxidase, cytochrome P450 system and electron transport system can cause oxidative stress by generating ROS in the cell [22].

Inflammation is an important mechanism in the pathogenesis of many diseases [23] and plays an active role in the nephrotoxicity mechanisms of cisplatin. Proinflammatory cytokines play an important role in many inflammatory diseases. It has been determined that cytokines accompanying inflammation such as tumor necrosis factor-α (TNF-α), IL-1β, transforming growth factor-beta 1 (TGF-β), RANTES, MIP2 and MCP1 are increased in cisplatin-induced kidney damage [22]. Studies show that TNF-α plays an important role in the pathogenesis of cisplatin nephrotoxicity. TNF-α triggers the inflammatory response in cisplatin-induced nephrotoxicity [24,25]. After cisplatin administration, oxidative stress pathways are activated and ROS is induced. In addition, the transcription factor NF-kB is activated and TNF-α is induced. TNF-α and ROS are two inducers that affect each other's activation [22]. In addition, p53, a tumor suppressor protein, has been reported to be a contributing factor to cisplatin's renal damage. In the experiment performed on rabbits, it

was determined that p53 played a role in apoptosis due to tubular damage caused by cisplatin, and pifithrin-α, a pharmacological inhibitor of p53, suppressed apoptosis [26].

1.2. Renoprotective Treatment Approaches for the Cisplatin Nephrotoxicity

Low glomerular filtration rate, decreased serum magnesium and potassium levels, and elevated serum Cr occur after 10 days of use in cancer patients using cisplatin. In addition, loss of sodium, potassium, and magnesium causes tubular damage and tubular dysfunction [27]. Patients treated with high-dose cisplatin are treated with hydration, magnesium supplementation, or mannitol-induced forced diuresis to alleviate or prevent nephrotoxicity [16]. However, a sufficient level of effect cannot be seen with these treatment approaches. In clinical practice, hydration and diuresis increase cisplatin excretion and decrease renal exposure. However, this method has a disadvantage. Intravenous administration of large volumes (3–6 L per day) of isotonic saline is required before and after cisplatin use to reduce nephrotoxicity [28]. Many pathways are activated when cisplatin produces toxicity. Therefore, blocking only one of these pathways does not provide a full protective effect for the target tissue. Many potential therapeutic approaches have been developed against cisplatin nephrotoxicity. These approaches areaim to reduce cisplatin uptake, inhibit its bioactivation, regulate the mitochondrial system, inhibit oxidative stress, reduce inflammation, regulate ER stress and UPR, inhibit apoptosis and/or regulated necrosis [18]. In a study examining the protective effects of cimetidine against cisplatin nephrotoxicity, it was shown that high-dose and continuous infusion of cimetidine reduced nephrotoxicity. It has been reported that cimetidine competitively inhibits OCT2-mediated cisplatin transport and affects cell toxicity [29]. In another study, cilastatin, a renal dehydropeptidase-I inhibitor, was found to have protective effects in vitro and in vivo against cisplatin-induced kidney injury by inhibiting apoptosis and oxidation. [30] In addition, studies have shown that β-adrenoceptor blockers (carvedilol, propranolol) reduce cisplatin toxicity by preventing oxidative stress [31,32].

Nigella sativa (N. sativa) is a plant known as black cumin and used in the treatment of a wide variety of diseases. In the study, the protective effects of N. sativa and Vitamin E on cisplatin-induced nephrotoxicity in rats were evaluated. It has been observed that N. sativa extract and Vitamin E weaken nephrotoxicity and reduce oxidative stress [33]. Hesperetin is a flavonoid found in citrus fruits. Kidney damage was induced in rats using cisplatin, and the protective effect of Hesperetin was investigated. It has been observed that Hesperetin treatment normalizes kidney function by reducing oxidative stress, lipid peroxidation and inflammatory cytokines formed in renal tubules [34]. In another study, it was shown that administration of gallic acid (GA), an antioxidant substance, decreased the expression of Bcl-2, Bax and caspase-3 in cisplatin-induced nephrotoxicity in rats. Likewise, TAC level increased and kidney malondialdehyde (MDA) content decreased with GA administration. GA also reduced levels of inflammatory factors, including IL-1β and TNF-α. In addition, GA reduced plasma BUN and Cr, leading to amelioration of renal

dysfunction [35]. Studies have evaluated the effects of Curcumin on cisplatin-induced nephrotoxicity in rats. The polyphenol Curcumin has pharmacological effects with antioxidant, anti-inflammatory and anti-cancer properties. Curcumin treatment prevented the elevation of serum BUN, Cr and renal MDA levels [36]. Curcumin nanoparticles caused significant increases in bilirubin, urea, uric acid and Cr levels with aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities. In addition, MDA, nitric oxide and serum TNF-α levels were also increased [37]. The protective activity of linalool, an essential oil, against cisplatin-induced nephrotoxicity was investigated. According to biochemical results and molecular studies, linalool has been found to reduce oxidative stress and have an antioxidant effect. In addition, linalool reduced inflammatory cytokine levels induced in the kidney and attenuated cisplatin-induced apoptotic markers. These data showed that linalool may protect against the nephrotoxic effects of cisplatin and against tissue damage [38]. In another study, the protective effects of Phloretin (PH) and its glycosylated form Phloridzin (PZ), which are natural compounds found in apple fruit, for nephrotoxicity caused by cisplatin were evaluated. They are compounds with high antioxidant properties thanks to the hydroxyl group in their PH and PZ structures. PH and PZ ameliorated cisplatin-induced renal dysfunction. It was also found that it reduced oxidative stress and protected the kidneys from the toxic effects of cisplatin by suppressing the inflammatory response [39].

One of the important side effects of the use of chemotherapy drugs in cancer patients is vomiting. Aprepitant, which is used as an antiemetic drug in patients treated with cisplatin in the clinic, is a selective neurokinin 1 receptor antagonist (NK1RA) drug. A study was conducted to investigate the protective effects of Aprepitant on nephrotoxicity and hepatotoxicity in patients using cisplatin. According to this study, it was determined that Aprepitant reduced cisplatin-induced kidney and liver damage by reducing oxidative stress, inflammatory cytokines such as TNF-α and NF-kB, and serum levels of ALT, AST, ALP, BUN and Cr [40]. Only natural compounds have not been tested on cisplatin nephrotoxicity. Platelet-rich plasma (PRP) has curative properties that have been used for many diseases. PRP is obtained from blood and has a rich content of active growth factors. PRP is obtained by centrifuging the blood sample and isolating the supernatant. In this study, PRP was administered to rats with cisplatin-induced nephrotoxicity by subcapsular renal injection. According to the results, Cr, BUN and N-acetyl glucosaminidase levels were decreased. In addition, while PRP increases epidermal growth factor, intercellular adhesion molecule-1 (ICAM-1), kidney injury molecule-1 (KIM-1), caspase-3 and TGF-β1 levels suppressed. According to these data, PRP showed protective effects in cisplatin-induced kidney damage in rats. It has been shown that PRP application can also be used to reduce the side effects of cisplatin [41]. These treatment approaches under development should not harm the anticancer activity of cisplatin. Therefore, further studies are needed

to evaluate the effects of the kidney-protecting agents cisplatin on anticancer therapy in animals with cancer.

2. CONCLUSIONS

Cisplatin is widely used in the treatment of various types of cancer, but its clinical use is limited due to its side effects. The most important side effect of cisplatin is nephrotoxicity. Many studies on this subject have tried to elucidate the cellular and molecular mechanisms of cisplatin nephrotoxicity. Cisplatin generally acts through many mechanisms such as DNA damage, mitochondrial dysfunction, oxidative stress, ERS, inflammation and apoptosis. More research is needed to determine the activation pathways of these mechanisms that cause severe renal damage and to determine the roles of critical molecules involved in cisplatin nephrotoxicity. Many potential therapeutic approaches for cisplatin nephrotoxicity have been tried to be described. The effects of these developed renoprotective approaches on the anticancer activity of cisplatin need to be comprehensively evaluated. Further studies on cisplatin nephrotoxicity will protect the kidney without reducing its chemotherapeutic efficacy.

Conflict of Interest

The authors of the article declare that there is no conflict of interest.

Author Contributions

The authors declare that they have contributed equally to the article.

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Review Article

HIV Integrase Inhibitors

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ABSTRACT:

HIV infection is incredibly detrimental and fatal to people. Unfortunately, despite recent advancements and medications, it has not yet been completely eradicated. Opportunistic infections are added to the list of disorders in AIDS (Acquired Immune Deficiency Syndrome), an infectious disease that develops as a result of an impaired immune system. In 2021, there were 38.4 million [33.9-43.8 million] persons living with HIV worldwide, up from 26.0 million [22.9-29.7 million] in 2000. The benefits of vastly expanded access to antiretrovirals, which have contributed to decreasing the number of individuals dying from HIV-related causes, can be observed in the persistence of HIV transmission despite declines in incidence. HIV-1 integration (IN), a critical stage in the integration of viral DNA into the host genome, is vital for retroviral replication. Numerous HIV integrase inhibitors have been created since the identification of this pathway, including Raltegravir, Elvitegravir, Dolutegravir, Bictegravir, and Cabotegravir. HIV integrase inhibitors and their synthesis are covered in this review.

Keywords *:* AIDS, HIV, integrase inhibitors.

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1. INTRODUCTION

In AIDS (Acquired Immune Deficiency Syndrome), an infectious disease brought on by the immune system's dysfunction, opportunistic infections are added to the list of illnesses. AIDS is caused by HIV (Human Immunodeficiency Virus) [1]. Circumstances and trends In 2021, there were 38.4 million (33.9–43.8 million) persons infected with HIV worldwide, up from 26.0 million (22.9–29.7 million) in 2000. The fact that HIV transmission persists despite declines in incidence is a reflection of the advantages of considerably expanded reach to antiretrovirals, which have assisted to minimize the number of people dying owing to HIV-related causes, notably since 2004 when mortality peaked. In Sub-Saharan Africa, where roughly one in every twenty-five individuals (3.4%) and two-thirds of all HIV-positive persons globally live, the situation is still the worst. That amount (23.4-28.6 million)

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increased to 25.6 million in 2021 [2]. The HIV replication cycle offers numerous crucial targets for pharmacological intervention. The N/NtRTIs, for exampleZidovudine and Zalcitabine, and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs), for instance Delavirdine andEfavirenz, the Entry- Fusion Inhibitors, such as the CCR5 antagonist Maraviroc, the Integrase Inhibitors, such as Dolutegravir, and lastly the Protease Inhibitors, like Ritonavir [3].

2.HIV INTEGRASE

Retroviral replication requires HIV-1 integrase (IN), which is a crucial step in the integration of viral DNA into the host genome. Although it can happen anywhere in the host genome, particular DNA regionsare favored [4]. In long-lived cells, integrated viral DNA is duplicated alongside host DNA during a period of cell divisions and serves as a latent source for the return of elevated viral loads as soon as the treatment is stopped or the environment is conducive. Pre-integration complex (PIC) is a nucleoprotein complex that transports reverse-transcribed viral DNA into the nucleus of the host cell, where it is integrated with the DNA of the host cell. PIC consists of a few viral core proteins as well as host proteins additionally an IN tetramer plus viral DNA.As part of the PIC in the cytoplasm, which is the first stage of the integration process, IN removes the 2 terminal nucleosides (G, T) from the three ends of the long terminal repeat (LTR) domain of reverse transcribed viral DNA. This process, known as 3-processing (3-P), involves the hydrolysis of the phosphodiester bond.When PIC translocates to the infected cell nucleus, the terminal 3'-OH of the viral DNA assaults the host DNA through a process named as strand transfer. Nucleic acid repair enzymes are then activated after the strand transfer process, completely closing the viral and host strands. Integration is viewed as a fascinating therapeutic aim for the development of anti-HIV lead compounds since it is anessential and distinctive phase in the HIV-1 replication cycle and because IN has nohuman counter part [5,6].

3.HIV INTEGRASE INHIBITORS

Raltegravir (MK-0518), the first anti-integrase inhibitor developed by Merck, was approved by the US Food and Drug Administration after a successful clinical trial (U.S. FDA). It was discovered that it was metabolized through glucuronidation and is a member of the diketo acidclass of inhibitors [7]. A successful clinical trial was conducted for Raltegravir, Merck's initial anti-integrase inhibitor. Elvitegravir was created as a result of Sato and colleagues' 2006 demonstration that 4-quinolone-3-carboxylic acids can replace diketoacids (GS-9137) [8]. The U.S. FDA granted approval to the Shionogi-ViiV Healthcare-GlaxoSmithKline joint venture's dolutegravir (S/GSK1349572 or GSK572) drug in August 2013. Treatment with the sodium salt of dolutegravir, an organofluorine, monocarboxylic, heterocyclic substance [9]. Another integrase inhibitor by the name of Bictegravir (BIC; GS-9883) was introduced by Gilead Sciences in 2016 and received FDA approval in 2018. A bridging bicyclic ring and a distinctive benzyl moiety make up the distinctivestructure of BIC [10].

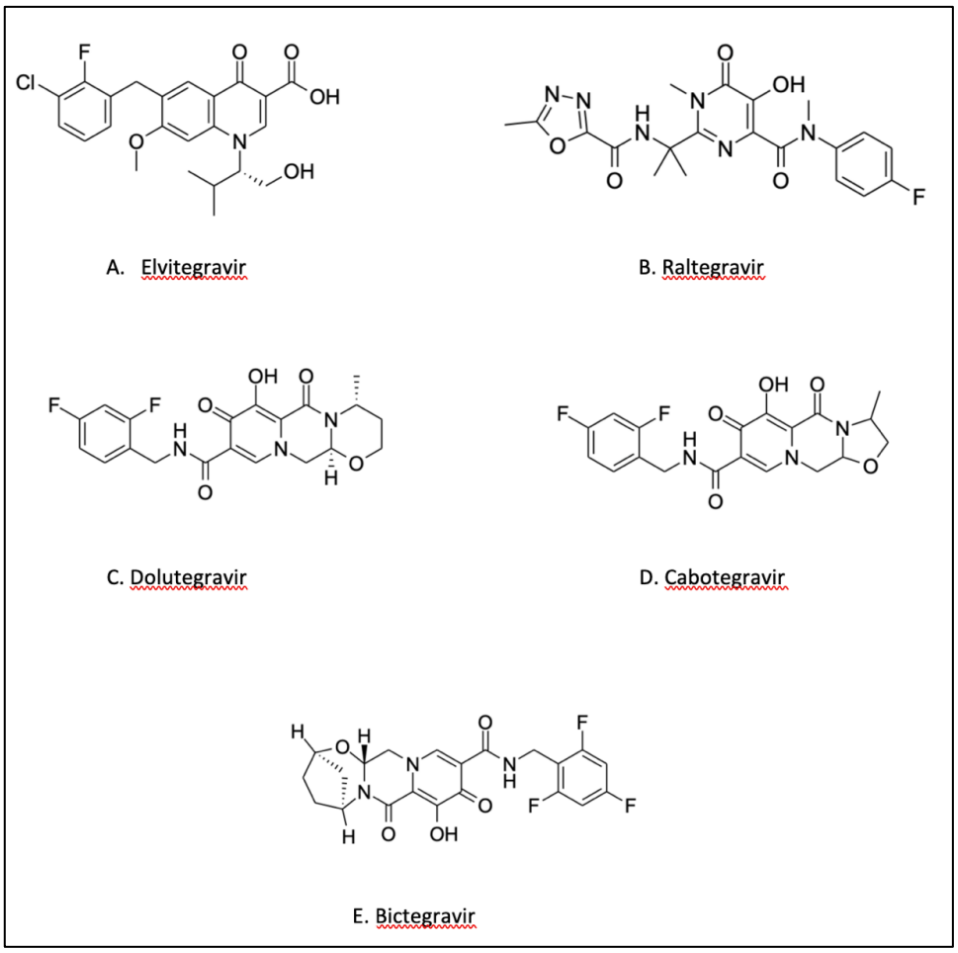

Fig. 1: HIV Integrase Inhibitors.

4.SYNTHESIS OF HIV INTEGRASE INHIBITORS

4.1.Synthesis of Raltegravir

From the commercially accessible 2-amino-2-methylpropanenitrile hydrochloride (2), raltegravir potassium (1) was created over the course of seven synthesis steps. When compound 2's amino group was protected by methylchloroformate in the diisopropylethylamine, (cyano-dimethyl-methyl) carbamic acid methylester was created (3). A crystalline substance known as [1-(N-Hydroxycarbamimidoyl)-1-methyl-ethyl] carbamic acid methylester (4) was created when hydroxyl amine was added to 3. Dimethyl acetylenedicarboxylate (DMAD) was added by Michael to yield 2-(1-methyloxycarbonylamino-1-methyl-ethyl)-5-hydroxy-6-oxo-1,6-dihydropyrimidine-4-carboxylic acid methyl ester through a subsequent thermal rearrangement (6). N-[(4-fluorophenyl)methyl] was created by reacting 6 with 4-fluorobenzylamine in a triethylamine solution.

1,6-dihydro-5-hydroxy-2[(1-methyl-1-[(methoxy)carbonyl]amino]ethyl carboxamide of -6-oxo-4-pyrimidine (7). In the existence of either magnesium hydroxide, compound 7 was N-methylated toproduce N-[(4-fluorophenyl)methyl]. 1,6-dihydro-5-hydroxy-1-methyl-2-[(1-methyl-1[(methoxy)carbonyl]amino]ethyl] oxo-4-pyrimidine carboxamide, 6 (8). NaOH was used to deprotectthe MOC group in 8 to produce 2-(1-amino-1-methyl-ethyl)-N-[(4-fluorophenyl)methyl]. 1,6-dihydro -1,6-methyl-6-oxo-4-pyrimidine carbo xamide (9). Raltegravir was created by amidating 9 with oxadiazole carbonyl chloride (10) when N-methylmorpholine was present (11). Raltegravir (11) wasconverted into Raltegravir Potassium (1), a crystalline, white substance, by treatment with aqueous KOH in ethanol [11].

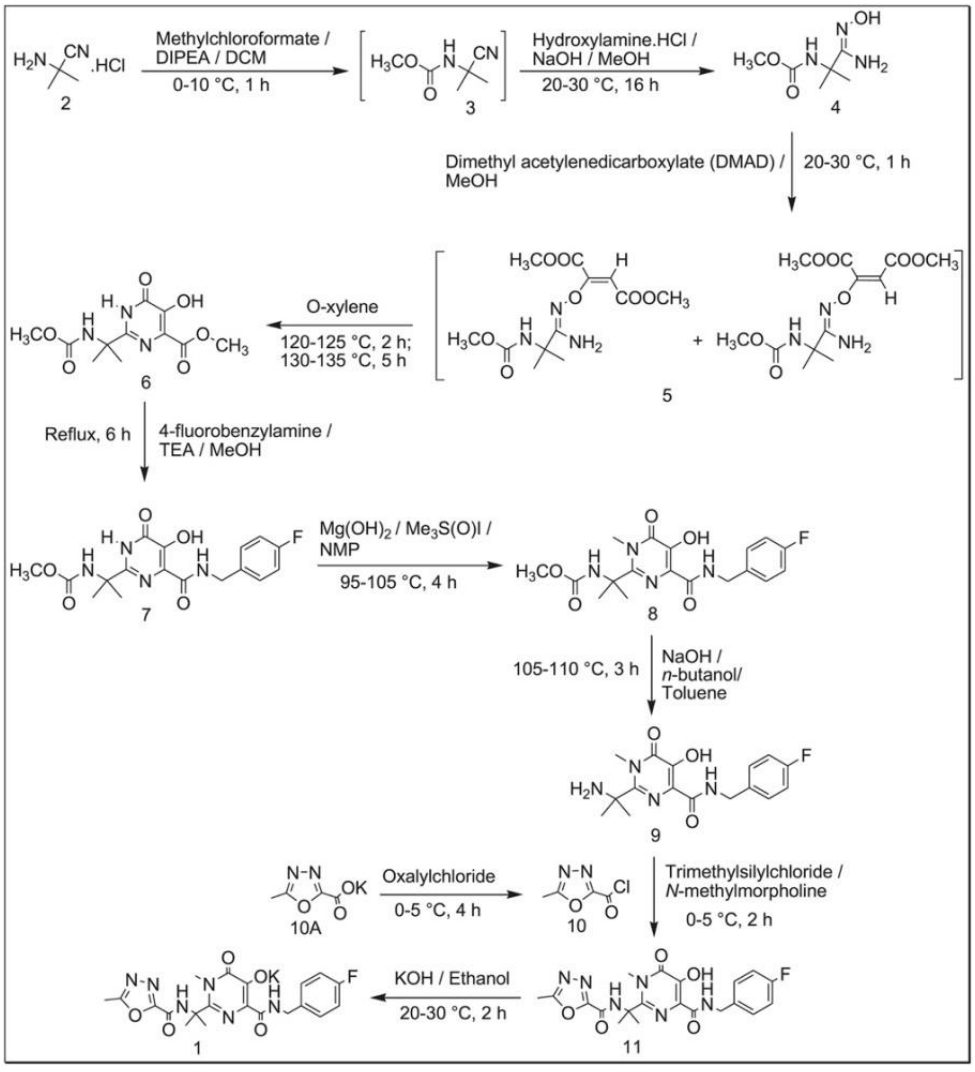

Fig. 2: Synthesis of Raltegravir [14]

4.2.Synthesis of Cabotegravir

In order to create vinylogous dimethyl amide 13, ketoester 12 was treated to neat DMF-DMA treatment. Following the addition of MeOH, aminoacetaldehyde dimethyl acetal, and concentration to eliminate extra DMF-DMA, vinylogous amide 14 was created.After the mixture was concentrated, pyridone 15 was produced by adding dimethyl oxalate and LiOMe in MeOH. Pyridone-acid 16 was produced as a white solid in an overall 61% yield by selective hydrolysis with LiOH. According to the authors, LiOH had a selectivity of 10:1 for hydrolyzing the target ester, compared to only 3:1 for NaOH and KOH.The way the unfavorable hydrolysis product is eliminated or if the unpleasant monoacid, diacid, or both are the primary by products are not mentioned, though. The original process entailed simultaneously hydrolyzing the methyl ester and the acetal in water, rendering the resulting acid-acetal inert for use in the next step. Hydrolysis utilizing $MeSO_3H$ and HOAc in CH_3CN was developed as a result of research into anhydrous conditions to create acid-aldehyde 17.Without conducting any work-up, (S)-alaninol (10) was incorporated into the mixture and heated to 64 °C for 18.5 hours, resulting in ring closure and the formation of tricycle 18 with 34:1 dr. 18 was crystallized from MeOH with a yield of 74% and a dr of 41:1. After activating the carboxylic acid in DME with CDI, 95% of the reaction's yield was amino 20. at 80 °C and then treated with 2,4-difluorobenzylamine (19). The susceptible aminal moiety, which was difficult to demethylate conventional circumstances utilizing silica/ boron reagents, presented a problem. Clean demethylation was completed using Mg salts, such as $MgCl_2$, $MgBr_2$, and MgI_2. This was done under the direction of the cabotegravir mechanism of action, which states that chelation to Mg is essential for integrase inhibition. Cabotegravir was produced with an isolated yield of 93%, but the authors emphasize in the accompanying information that demethylation with LiBr is a morescalablemethod [12].

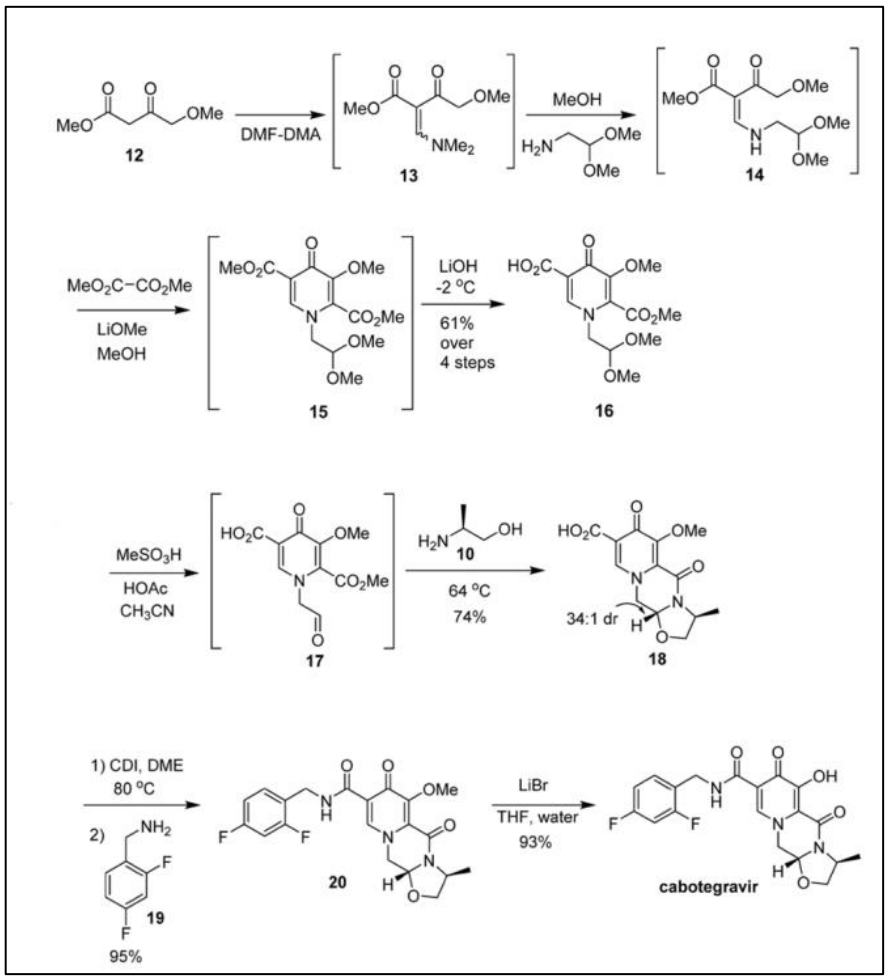

Fig. 3: Synthesis of Cabotegravir [14]

4.3.Synthesis of Bictegravir

Meldrum's acid is first converted to methoxyacetic acid in MeCN, where it is activated with pivaloyl chloride to produce intermediate 22. 2,4,6-Trifluorobenzyl amine (23) and TFA were added to this solution of 22 in MeCN. The reaction has been worked up and thenextracted by flash chromatography to produce 24 after an 18-hour reaction period at 45–50 °C. After installing the enamine-protected aldehyde 25, the technique then follows chemistry similar to that used to prepare other integrase inhibitors, cyclizing it with diethyl oxalate to produce 26, and so forth. Utilizing either the oxalate/ benzoate salt of (1R,3S)-3-aminocyclopentanol (27) to install the bicyclic ring system, it was possible to produce bicegravir by deprotecting the methyl group together with $MgBr_2$. For any of the phases, noyieldsare listed [13].

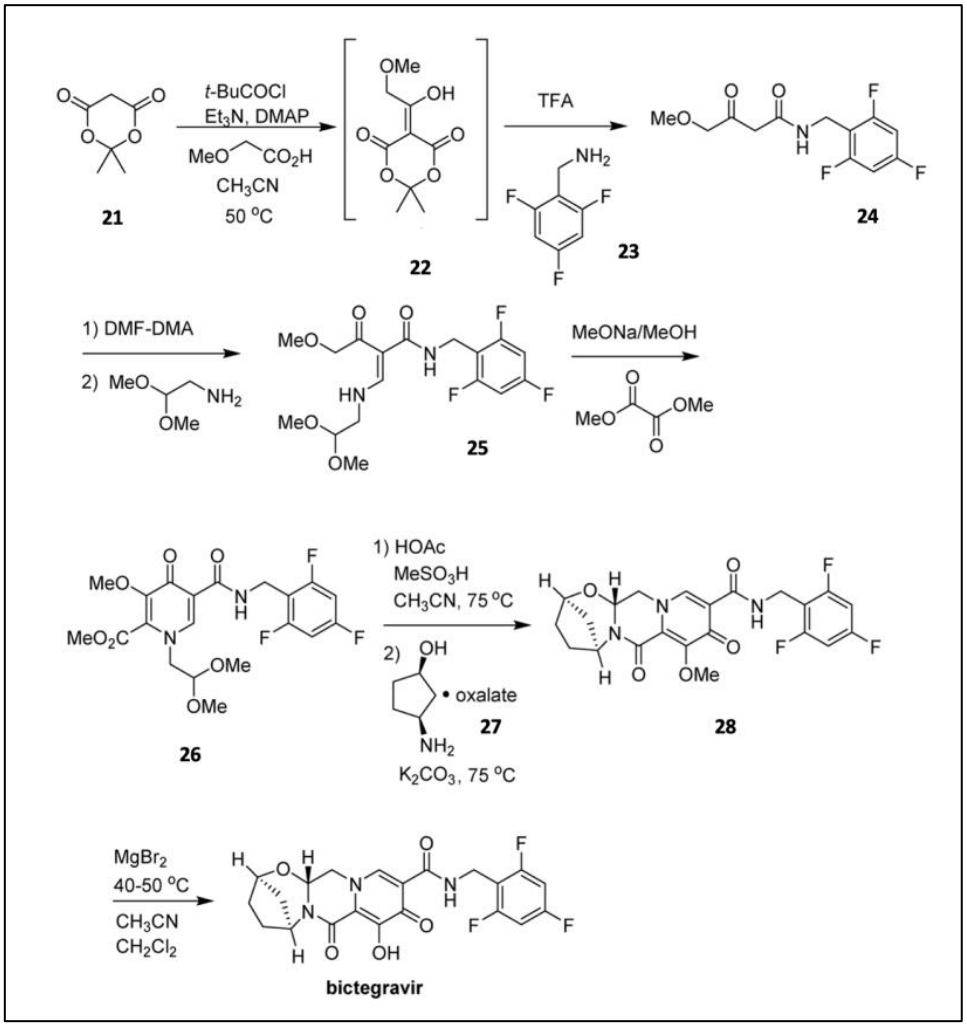

Fig. 4: Synthesis of Bictegravir[14]

5. CONCLUSIONS

As a result, AIDS has a significant place in drug discovery research due to the disease cannot be completely eradicated. Research on HIV Integrase inhibitors has advanced significantly in recent years as a result of innovations. The current effective use of developed drug molecules in therapy highlights the importance of the possibility of finding undiscovered therapeutic compounds.

Conflict of Interest

The authors of the article declare that there is no conflict of interest.

Author Contributions

The authors declare that they have contributed equally to the article.

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