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Review Article

Assessment of Antioxidant Potential of Herbal Extracts and their Therapeutic Applications

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Abstract

Antioxidants are necessary to prevent adverse effects arising from free radicals and oxidants. Synthetic antioxidants have been proved to have several side effects, hereby increasing the demand to identify and extract natural antioxidants. Medicinal plants are rich sources of phenolic compounds as the most important natural antioxidants. In recent years, significant portions of medical-related researches have attempted to detect and extract the phenolic compounds found in various plant. The plant extracts due to the presence of antioxidants have high potential in prevention and treatment of various diseases and the production of safe antioxidant-based drugs. In the present review article, the features of free radicals and their production procedures were assessed initially. Then, different types of antioxidant found in the plant extracts and their extraction methods were studied. Subsequently, some of studies conducted on the antioxidant properties of plants were reviewed; finally, the applications of antioxidants obtained from the plant extracts were investigated in the prevention and treatment of various diseases.

Keywords: *Antioxidants; Plants; Free Radicals; Oxidative Stress*

Introduction

The human cells are constantly exposed to enzymatic and non-enzymatic oxidizing agents [1,2]. Fortunately, the presence of valuable substances, such as antioxidants, prevents the destructive effects of oxidizing agents including free radicals and oxidants. There is a balance between the production of oxidants and antioxidants in the human body. The antioxidant defense system makes balance between the antioxidant activity and the production of oxidants [3,4]. Any disruption in this balance refers to "oxidative stress". Excessive production of oxidants or weakened antioxidant defense system can lead to destructive effects of oxidants on macromolecules of the body such as DNA, protein, lipids, etc [5,6]. Free radicals are of the most important oxidants in the body, and have a great impact on preventing various diseases such as cancers, cardiovascular diseases, atherosclerosis, Alzheimer's disease, aging, Parkinson's disease and ischemia [7-12]. Therefore, the use of antioxidants has a remarkable importance to prevent the harmful effects of oxidants. Various studies demonstrated that many plants contain a wide range of antioxidants [13,14]. The antioxidants obtained from plant extracts can impede the complications of oxidants, expand life expectancy and improve life quality by preventing the development of various diseases.

Free radicals and their formation

Oxidative reactions occur regularly in the body. Reactive oxygen species (ROS) create owing to the presence of unpaired free electrons in oxygen molecule. The ROS make in result of intra cellular interactions. They can corporate in adjusting many important body responses

such as gene expression, cell division, cell migration and so on [15,16]. The ROS are divided into two groups of free radicals and non-radicals. The non-radical ROS can be formed by sharing unpaired electrons of two radicals [17]. The free radicals are highly active due to their free unpaired electrons and can exert irreversible harmful impact on the body by oxidizing macromolecules such as proteins, lipids, lipoproteins, carbohydrates, and nucleic acids [18-21]. Some of the endogenous ROS include hydroxyl radical (·OH), superoxide anion (O_2^{-}·), hydrogen peroxide (H_2O_2), peroxyl radicals (ROO·), hypochlorous acid (HOCL), and hydroperoxyl radical (HOO·).

The ROS are formed in the mitochondrial electron transfer chain by releasing small number of electrons and the reduction of oxygen molecule into superoxide. The superoxide dismutase (SOD) changes superoxide into hydrogen peroxide. As the consequence of collision between hydrogen peroxide and heavy metals such as iron, the hydroxyl radicals are generated. Among different types of ROS, hydroxyl radicals are more active rather than the others [16,22] (Figure 1). In addition to the mentioned endogenous ROS, there are also several exogenous oxidants applying the same destructive effects in the body. Ionizing radiation, viral infections, cigarettes and heavy metals are of the production resources of exogenous oxidants [23-26].

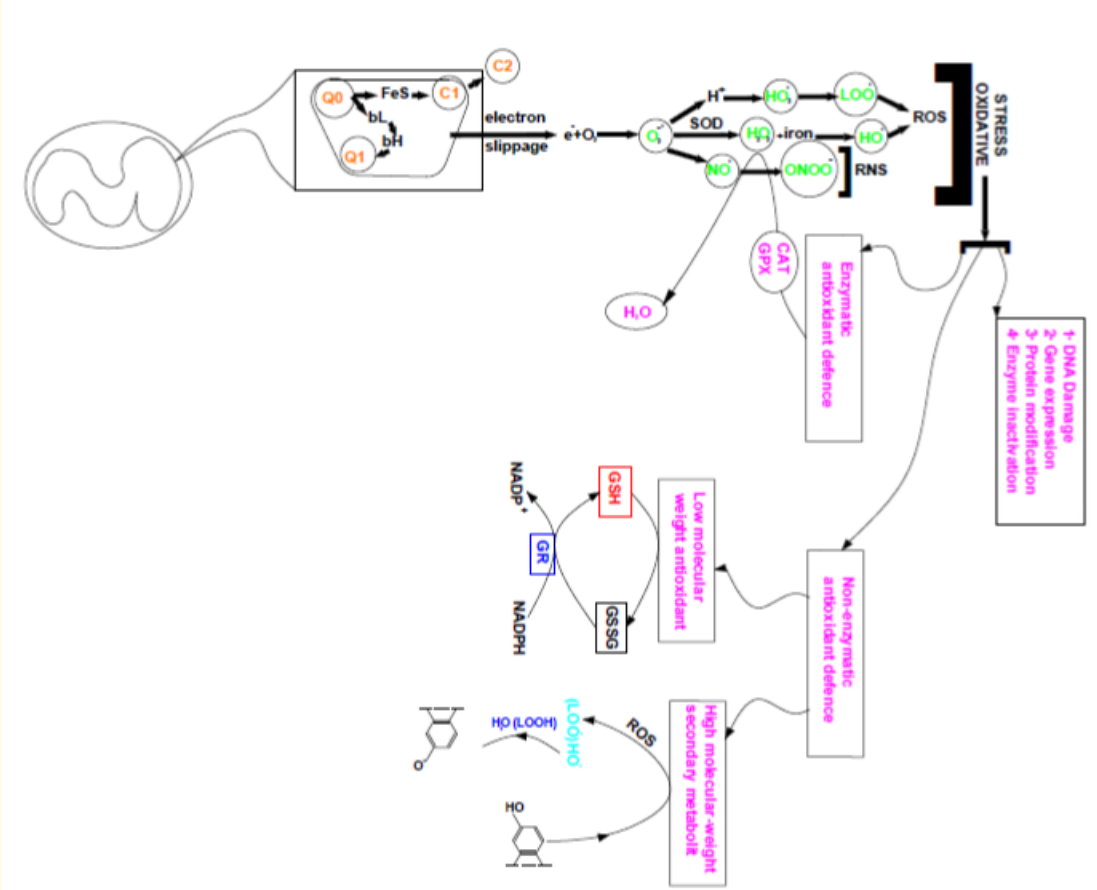

Figure 1: *Diagram of free radicals production and defense systems: Due to electron slippage of electron transfer chain of mitochondria resulted to reaction with O_2, free radicals make such as superoxide anion (O_2^{-}.), hydroxyl radical (HO.), peroxynitrite ($ONOO^{-}$) and etc. By different defense mechanisms, free radicals are neutralized.*

Antioxidants

Antioxidants are the compounds inhibiting side effects of free radicals through different ways and reducing cell damage and apoptosis [27,28]. The history of antioxidants usage goes back to early nineteenth century. One of the first reported applications for antioxidants is delay in lipid oxidation. The industrial use of antioxidants was gradually found to be more pervasive in the late nineteenth century [29,30]. The antioxidants were exploited in the food packing industry in 1940.

Classification of antioxidants

Natural or primary antioxidants: these antioxidants present in the nature include vitamins, phenolic compounds and minerals. Vitamins C, E, and B have antioxidant properties. Phenolic compounds are found in different portions of the plants like seeds, bark, flowers. Copper, iron, zinc, and magnesium as minerals, can act as a cofactor in antioxidants activity [31].

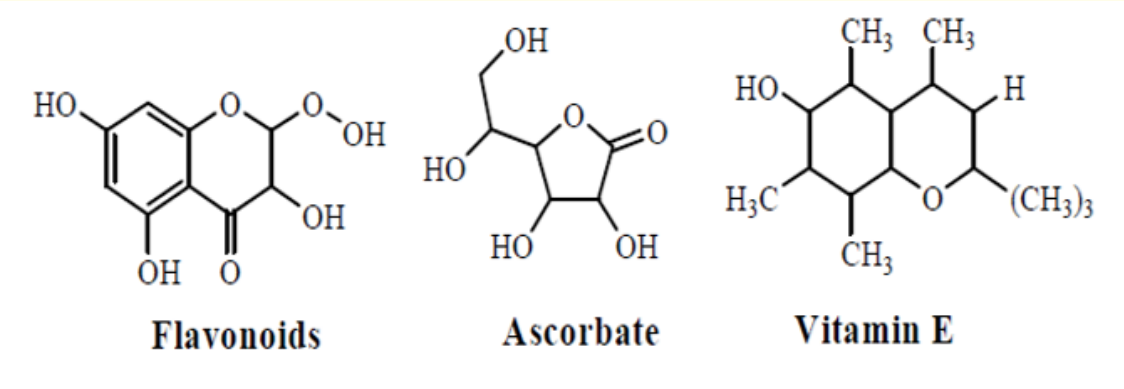

Figure 2: *Chemical structure of natural antioxidants.*

Synthetic or secondary antioxidants: these antioxidants are generally synthetic phenolic compounds like butylated hydroxyl anisole (BHA), butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ), nordihydroguaretic acid (NDGA) and so on. Different studies have shown carcinogenicity and toxicity properties of these antioxidants [32,33] (Table 1). Hence, their application in treatment is associated with several serious problems. Accordingly, the antioxidant properties of various plant extracts have attracted the attentions in treatments. The phenolic compounds are of the widely distributed herbal antioxidant compounds presence in many plants [34].

Types of Antioxidants	
Natural	**Synthetic**
β-carotene- Provitamin A	Carotenoids (Lycopene, Xanthophyll)
Ascorbic acid- Vitamin C	Lutein, α-and γ-carotenes
Tocopherols	Anthocyanins
Sulphur amino acids: Cysteine	Propyl Gallate (E-310)
Selenium	TBHQ (E-319)

Table 1: *Antioxidants of natural and synthetic.*

The antioxidant properties of phenolic compounds are mainly associated with their high reductive effect. Phenolic compounds can inhibit lipid oxidation through electron donation to free radicals. Different plants with many natural antioxidants have a remarkable contribution in preventing and treating of various diseases. Studies indicate that 20,000 to 35,000 different species of the plants are employed in the pharmaceutical, cosmetic and medical industries [35]. Several antioxidant-based drugs have been developed for prevention as well as treatment various diseases such as Alzheimer's disease, cancer, Parkinson's disease, diabetes and so on [36].

Extraction methods of plants

There are different methods to extract the effective compounds from medicinal plants. The extraction of these compounds can be divided into two main steps, including pre-extraction and extraction. The pre-extraction step involves sampling, drying, and grinding. These steps have great influence on the efficiency of phytochemicals extraction from medicinal plants [37,38]. Drying plant samples is performed through a variety of methods; some of them used in different researches are presented below.

Pre-extraction

- **Air-drying method:** Different parts of the plant are exposed to the air in order to eliminate moisture content. The drying duration may last three days to a month, depending on the used part of the plant [37].
- **Microwave-drying method:** Electromagnetic waves are used to heat and dry the various parts of the plant. This method is much faster than air-drying, but it may change the structure of phytochemicals in some cases [39].
- **Freeze-drying method:** The plant is frozen, and then the **moisture content** is converted to steam through the sublimation process to complete the drying process. This method is expensive and used in cases where the plant ingredients are sensitive to heat [37].
- **Oven-drying method:** The moisture content of the plant is removed by heat to be dried, which is a fast and easy method to dry the plants [40].

Extraction

After sampling and appropriate drying of the plant, a suitable method should be selected to extract the effective substances the plant. The method involves the use of suitable solvent for extraction of the therapeutic parts of the plant through the standard and repeatable process. Some of these common methods are as follows:

Maceration method: The dried plant samples are soaked in a container with a suitable solvent for at least three days at room temperature. After extracting the phytochemicals, the resulting mixture is filtered. This method is easy and widely used for extraction of medicinal plants [41].

Soxhlet method: the sample is placed inside a porous container. The proper solvent is heated at the bottom of the container and the resulting steams pass through the sample container and then fall down in droplet after condensation. This is a widely used but slow method for extraction of the plants [42]. In addition to the two conventional and common methods of Maceration and Soxhlet, newer methods have been introduced that significantly reduce the duration of plant extraction. Some of these methods are listed below.

Microwave-assisted extraction (MAE): generally speaking, in this method polar molecules of chemical compounds can absorb microwave energy. The absorbed energy is proportional to the dielectric constant of the object, which causes a dipole rotation in the electric field. The extraction is performed at a high temperature. A solvent with high dielectric constant, which absorbs the highest microwave waves, is used in this method so that the solvent reaches the boiling point faster. Therefore, increasing the temperature and pressure make faster the extraction of compounds from plants. Low solvent consumption and extraction duration are of the advantages of this method compared to the conventional methods. However, this method has limitations in the extraction of small phenolic compounds, such as phenolic acids [43].

Ultrasound-assisted extraction (UAE): this approach utilizes ultrasound waves ranged from 20KH up to 2000KH. The basic principles of this method are to destroy the plant cell wall by colliding with high-energy ultrasound waves, increasing the permeability of the cell to its contents so that phytochemicals are easily extracted from the plant and release into the solvent [44].

Supercritical fluid extraction (SFE): in this method, an extracting fluid for example, CO_2 is used. Some factors such as temperature, sample volume and pressure are determinants to deliver the substance to critical point. Properties such as low cost, suitable solvent for nonpolar samples and accessibility make CO_2 as first choice [45].

Accelerated solvent extraction (ASE): a major advantage of this method in compared to conventional techniques is much less quantity of required solvent. Also, the time of extraction is less than other methods [46,47] (Figure 3).

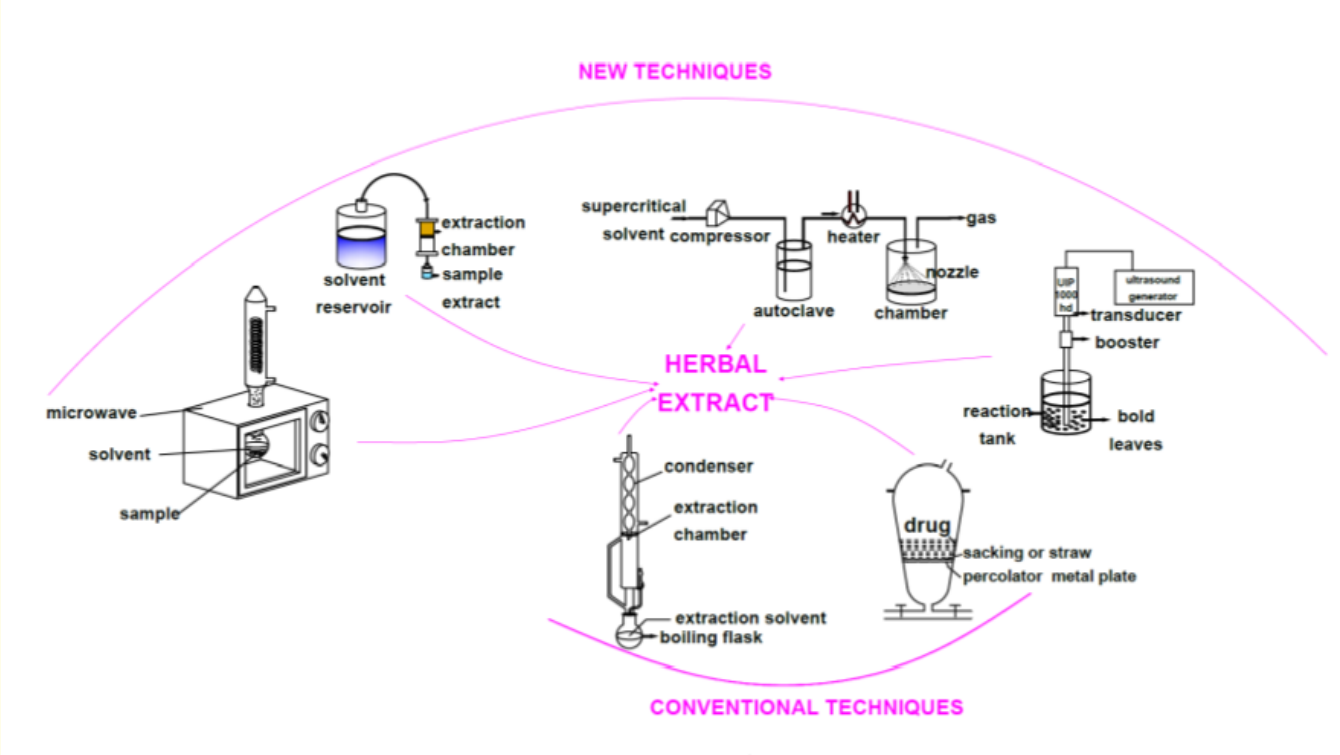

Figure 3: *Outline of various techniques of herbal extraction.*

Assessment of antioxidant property of plant extracts

The assessment of antioxidant capacity of plant extraction is very important. There are different techniques for studying the antioxidant activity and capacity, categorized into three general groups of chromatography, spectrometry and electrochemical techniques [48].

Spectrometry technique: This method records the color change of certain oxidant in exposure to the studied antioxidant using the spectrometers, and determines the activity and capacity level of antioxidant [49]. Various methods are used in this technique; some of them are described below.

DPPH method: Although the absorption maximum of DPPH• free radical or 2,2-diphenyl-1 picrylhydrazyl occurs at a wavelength 517 nm, the absorption rate can be reduced by reacting with antioxidants, the which the solution color is disappeared. The absorption rate at the respective wavelength represents the amount of antioxidant present in the sample [50].

ABTS method: The reduction of ABTS radical or (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)) in reaction with hydrogen donating antioxidant causes the appearance of blue-green color in solution. The color change rates indicate the presence of antioxidant concentrations [51].

FRAP (ferric reducing antioxidant power) method: The TPTZ-Fe^{3+} complex is converted to (TPTZ-Fe^{2+}) because of reacting with the desired antioxidants, appearing a color solution that shows maximum absorption at a wavelength of 593 nm [52].

Chromatography technique: This method is applicable for detection and separation of the antioxidants. The chromatography is performed in two common ways, involving gas chromatography (GC) as well as high performance liquid chromatography (HPLC) [53,54].

Electrochemical technique: This approach is recruited to evaluate the antioxidant content and antioxidant capacity. The two methods of cyclic voltammetry and biamperometry are mostly used in this technique [55].

Various studies have used different methods to evaluate the activity and capacity of antioxidants for the extract of plants. For example, a study (1999) evaluated the total antioxidant power of the tea extract using the FRAP method [56]. In a similar study, Alexander Yashin., *et al.* reported total antioxidant content of green tea extract by electrochemical technique [57]. In another study, Effat Souri., *et al.* (2008) used the DPPH radical inhibition method to assess the antioxidant capacity of thirteen herbal extracts [58]. Vahid Afshari., *et al.* (2016) tested the antioxidant properties and antigenotoxic effects of *Thymus kotschyanus* plant, who applied gas chromatography to investigate existing antioxidants and also DPPH to assess the antioxidant activity [59] (Table 2).

	Antioxidant capacity assay	Principle of the method	Determination method
Spectrometry	DPPH	Antioxidant reaction with organic radical	Colorimetry
	ABTS	Antioxidant reaction with organic cation radical	Colorimetry
	FRAP	Antioxidant reaction with a Fe(III) complex	Colorimetry
	PFRAP	Antioxidant reduction by potassium ferricyanide	Colorimetry
	CUPRAC	Cu (II) reduction to Cu (I) by antioxidants	Colorimetry
	ORAC	Antioxidant reaction with peroxyl radicals	Fluorescence detection
Chromatography	Gas chromatography	Separation of compounds in a mixture is based on the repartition between a liquid stationary phase and a gas mobile phase	Thermal conductivity detection
	HPLC	Separation of the compounds in a mixture is based on the repartition between a solid stationary phase and a liquid mobile phase	Fluorescence Mass spectrometry
Electrochemical methods	Cyclic voltammetry	The working electrode potential is ramped linearly versus time then is ramped in the opposite direction	Measurement cathodic/ anodic peak
	Biamperometry	The potential of the working electrode is set at a fixed value with respect to a reference electrode	Measurement of oxidation/ reduction of electroactive analyte

Table 2: *Categories of antioxidant capacity assays.*

A review of studies to identify the antioxidants of various plants

Chang., *et al.* investigated the antioxidant properties and the capacity to eliminate the radicals released by several herbs native to Korea. This study proved the presence of abundant antioxidants in the extraction of these plants [60].

Lie-Fen Shyur (2005) examined the DPPH scavenging property of 26 different herbal extracts being common in Taiwan's folk medicine. Based on the findings, *Ludwigia octovalvis*, *Linderina anagallis*, *Vitis thunbergii*, *Zanthoxylum nitidum*, and also *Rubus parvifolius* had the most antioxidant capacity [61].

In another study, Stephanie Dudonn., *et al.* examined total phenolic content and antioxidant properties in the extracts of 30 medicinal herbs. As per of findings, the antioxidant capacity of aqueous extracts from oak (*Quercus robur*), cinnamon (*Cinnamomum zeylanicum*) and pine (*Pinus maritima*) was higher than other plants. In contrast, total phenolic content in extracts of Mate (*Ilex paraguariensis*) as well as clove (*Eugenia caryophyllus clovis*) was more than the rest [62].

A study was conducted in 2012 on lipid oxidation inhibitory effects of several Amazon native plants. Except for a plant, all herbs had the potential to prevent lipid oxidation. These plants also contained a large amount of flavanols [63].

Maja Kazazic., *et al.* examined the antioxidant activity of several Herzegovina medicinal plants. Among them, the plant *Papaverrhoeas aqueous* showed the highest amount of phenolic compounds. In addition, the mentioned plant accounted for the most antioxidant activity. Significant relationship between the phenolic compounds content and the level of antioxidant activity in these plants showed that phenolic compounds play key role in the antioxidant activity of plants in this region [64].

A study (2016) has been conducted on the antioxidant properties of the Seri Lanka medicinal herbs extract. Comparing the results of this study revealed a substantial relationship between phenolic amount and antioxidant capacity of the extract of these plants, indicating the main role of these polyphenolic compounds present in the extract of these plants in antioxidant activity [65].

The results of assessing antioxidant properties of ten Iranian herbs by Soheila Moein., *et al.* showed that antioxidant activity in the extract of *Verbascum sinuatum L. Var* and *Rosa damascena Mill* plants is higher than others [66].

Feras Q Alali., *et al.* investigated the antioxidant activity and phenol content in 95 species extracts of Jordanian herbs. The results of this study found a significant linear correlation between these two characteristics [67].

Therapeutic application of plant antioxidants

The plants involve the enzymatic and non-enzymatic antioxidant defense system to prevent damages caused by free radicals. The main enzymatic inhibitors consists of catalase (CAT), SOD, glutathione peroxidase (GPx), and ascorbate peroxidase (APX) enzymes. The latter one includes antioxidants with low molecular mass such as vitamin C, glutathione (GSH), vitamin E, proline, phenolic acids, and so on. The effective antioxidant defense system in plants is able to control the adverse effects of free radicals and plant survival. The enzymatic system of antioxidant defense in human cells has also been developed and is effective, but the non-enzyme system is not well developed. Therefore, the human body appears to have a constant need for taking antioxidants through daily food to prevent the destructive effects of the free radicals [68,69].

Medicinal plants are gradually known as complementary treatments and even alternative to conventional methods. The medicinal plants have attracted the attention of many researchers because of two basic features of availability and low-harm [70].

A study on hemodialysis patients applied anthocyanin/polyphenolic-rich fruit juice to reduce oxidative stress. The amount of DNA damage was evaluated in a blood sample collected weekly from patients. In the patients receiving antioxidants, the levels of DNA damage and protein and lipid peroxidation were decreased significantly compared to the control group [71].

Saverio Bettuzzi., *et al.* conducted a clinical trial on the effect of using green tea catechins in patients suffered from high-grade prostate intraepithelial neoplasia (HG-PIN). The results of this study showed that the patients with HG-PIN who used green tea antioxidants experienced prostate cancer less than in other people [72].

Navneet Dhillon., *et al.* in a clinical trial investigated the effect of oral consumption of curcumin on inhibiting the growth of human pancreatic tumor, which was attributed to inhibit the expression of genes regulating nuclear factor-kB (NF-kB) [73].

A study examined the effect of *Withania somnifera* extract on improving chemotherapy-induced fatigue in 100 patients with breast cancer. Disability and chemotherapy-induced fatigue in cancer patients are among the major problems in the treatment of the disease, which is a main contributor to the life of these patients. The results of this study indicate that a dose of 2 grams of root extract of this plant every eight hours during chemotherapy has a significant effect on the quality of life and the reduction of fatigue in these patients [74].

Clinical trials indicate that *Melissa officinalis* extraction plays a significant role in improving the Alzheimer's disease. The results of studies by Akhondzadeh., *et al.* showed that patients with Alzheimer's disease who used this extraction showed better symptoms after four months, and the Alzheimer's disease assessment scale was improved. Meanwhile, this extraction had no destructive effect and decreased agitation in the patients [75].

Tang., *et al.* [76] in an *in vitro* study assessed the effect of antioxidants in the *Petroselinum crispum* extraction on the duplication as well as migration of MCF-7 cells. They found that addition of this plant extraction to the medium of MCF-7 breast cancer cell line led to inhibition of cell proliferation and migration. Moreover, the extract antioxidant protects DNA damages caused by H_2O_2.

A research has shown that oxidative stress in human alveolar macrophages exposed to cigarette extract causes increased production of interleukin-8 (IL-8) and expression of MMP-1, both of which are involved in the pathophysiology of COPD. Gema Flores., *et al.* (2013) examined the potential therapeutic effects of flavonoid-rich extraction from the Costa Rican guava (*Psidium friedrichsthalianum*) in the treatment of chronic obstructive pulmonary disease (COPD). Their results showed that the presence of antioxidants in the extract would significantly reduce IL-8 production and inhibit the expression of MMP-1 in small airway epithelial cells and could be effective in the treatment of COPD [77].

The use of antioxidant potential of medicinal herb extracts in the treatment of diabetes has been studied in many studies. For example, in an experimental study, the anti-diabetic potential of *Terminalia paniculata* bark was investigated in diabetic model rats. The levels of blood sugar, creatinine, HbA1c, SGBT and SGOT were significantly reduced in diabetic rats receiving oral extracts compared to controls [78].

Arika WM., *et al.* (2016) studied the anti-diabetic properties of *Azardirachta indica* extract and showed that oral and intraperitoneally injected extraction of this plant reduced blood glucose and improved the symptoms of diabetic rats [79].

Somogyi A., *et al.* within clinical trial on 14 patients with type II hyperlipidemia reported that silymarin extract at a dose of 420 mg reduced cholesterol and increased HDL cholesterol [80].

In a study by Koppula., *et al.* by assessing the role of *Foeniculum vulgare* Mill (Umbelliferae) extract in reducing stress, it was found that the antioxidant activity of this extract, in addition to reducing stress, improved memory in rats [81].

In addition to the aforementioned items, the antioxidants present in medicinal plants have huge capability in prevention and therapy of many diseases, for instance skin disorder, male reproductive system damage, malaria, colon cancer, hepatotoxicity, renal injury, cardiovascular disorders, etc [82-87].

Discussion and Conclusion

The timely performance of antioxidants in impeding free radical oxidation prevents the occurrence of various diseases. Due to the lower production of some low molecular weight antioxidants in the human body, the supply of different kind of antioxidants by nutrition is necessary to avoid the adverse effects of antioxidants. The medicinal plants are nowadays considered as one of the key sources of antioxidant compounds and various studies have been conducting on the effect of these plants extracts on the prevention and treatment of diseases. Exploring different studies on extraction methods of plant antioxidants suggest that the most influential parameter is solvent types and strength and the ethanol extract method is more widely employed than other techniques [88,89]. The DPPH and ABTS are the most widely used methods for investigating the antioxidant properties of the plant extracts [50].

The research results mentioned in this study indicate that the antioxidants available in the plant extracts have high ability to control various diseases. One of the most important antioxidant potentials is antitumor feature. Various considerations show that antioxidants have potential to inhibit the proliferation of cancer cells via various pathways, driving them to death through the induction of apoptosis [90,91]. Cancer disease is treated using varied approaches that have many side effects, in addition to high costs. Availability, inexpensiveness, lack of side effects and anti-cancer potency are features that make antioxidants more effective compared to other methods. At present, some of anticancer drugs are composed of natural plant compounds. The researches on other diseases also indicate the potent antioxidant ability to treat these diseases.

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