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A REVIEW ON ANTIOXIDANT POTENTIAL OF VARIOUS HERBS AND IT'S THERAPEUTIC EFFECTS

A. Saravanakumar* and N. Pranuthi

Department of Pharmacology, Sri Venkateswara College of Pharmacy, R.V.S. Nagar, Tirupathi Road, Chittoor-517127, Andhra Pradesh, India.

ABSTRACT

The present review focused on *In vitro* methods of antioxidant activity and many medicinal Indian herbs that have great antioxidant potential. This review covers medicinal species from a variety of countries (Africa, Algeria, The United States of America, Australia, Brazil, Bulgaria, China, India, Iran, Italy, Japan, Malaysia, Poland, Portugal, Thailand and Turkey, The purpose of this review is to survey the antioxidant capacity and to evaluate potential sources of natural antioxidants for food and medicinal purposes. According to a phytochemical database USDA 2003 the number of different antioxidants in some plants can reach up to 40 (soybean 42, tea 36, fennel 35, oregano 34, onion 32, thyme 32, etc). In this database, plants with the highest contents of antioxidants are walnut, betel nut, guava, coconut, and other less known plants. A list of some known substances with antioxidant activity in some very common spices is reported.

Keywords: Antioxidant, Therapeutic Effects, Medicinal plants.

INTRODUCTION

The adverse effects of oxidative stress on human health have become a serious issue. The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts and their active components [1]. Under stress, our bodies produce more reactive oxygen species (ROS) (e.g., superoxide anion radicals, hydroxyl radicals and hydrogen peroxide) than enzymatic antioxidants (e.g., superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid (vitamin C), tocopherol (vitamin E), glutathione, carotenoids, and flavonoids). This imbalance leads to cell damage [2-6] and health problems [7, 8]. A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases [9], including cardiovascular diseases, cancers, neurodegenerative diseases, Alzheimer's disease [10] and inflammatory diseases.

One solution to this problem is to supplement the diet with antioxidant compounds that are contained in natural plant sources [11]. These natural plant antioxidants can therefore serve as a type of preventive medicine. Recent reports indicate that there is an inverse relationship between the dietary intake of antioxidant-rich foods and the incidence of human disease [12]. However, synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), have been widely used as antioxidants in the food industry and may be responsible for liver damage and carcinogenesis. For this reason, interest in the use of natural antioxidants has increased. Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to humankind; a great deal of effort has therefore focused on using available experimental techniques to identify natural antioxidants from plants. Several authors have reviewed the beneficial uses of these plant species [13].

Recently, reviewed twenty-four medicinal Indian herbs that have great antioxidant potential. This review covers medicinal species from a variety of countries (Africa, Algeria, The United States of America, Australia, Brazil, Bulgaria, China, India, Iran, Italy, Japan, Malaysia, Poland, Portugal,

Thailand and Turkey, The purpose of this review is to survey the antioxidant capacity and to evaluate potential sources of natural antioxidants for food and medicinal purposes.

The number of contributions to isolation methods, techniques and activity testing of plant origin antioxidants

Corresponding Author :- **A.Saravanakumar** Email:- saravanacology@gmail.com

has significantly increased in recent years. Oxidation is one of the major causes of chemical spoilage, resulting in rancidity and/or deterioration of the nutritional quality, colour, flavour, texture and safety of foods. There is, at present increasing interest both in the industry and in scientific research for spices and aromatic herbs because of their strong antioxidant and antimicrobial properties, which exceed many currently used natural and synthetic antioxidants. These properties are due to many substances, including some vitamins, flavonoids, terpenoids, carotenoids, phytoestrogens, minerals, etc. and render spices and some herbs or their antioxidant components as preservative agents in food [14]. Except basic plant antioxidants some specific ones are characteristic for some important aromatic herbs and spices. Some examples of specific antioxidants are pimento from all spice; gallates, biflorin, its isomer eugenol and eugenyl acetate in clove [15]; carnosol, carnosic acid, rosmanol, rosmaridiphenol, rosmadial and rosmariquinone, and various methyl and ethyl esters of these substances in rosemary and sage [16]; diarylheptanoids, gingerol and zingerone in ginger, curcumin and tetradehydrocurcumin in turmeric; flavonides, ferulic acid, piperine, phenolic amide feruperine in black pepper; thymol and carvacrol in essential oils from Algerian origanum; derivatives of phenolic acids, flavonoids, tocopherols, rosmarinic acid and carvacrol in oregano [17], etc.

According to a phytochemical database USDA 2003 the number of different antioxidants in some plants can reach up to 40 (soybean 42, tea 36, fennel 35, oregano 34, onion 32, thyme 32, etc). In this database, plants with the highest contents of antioxidants are walnut, betel nut, guava, coconut, and other less known plants. A list of some known substances with antioxidant activity in some very common spices is reported in Table 1. More information is available for examples on USDA food antioxidant database USDA, 2003.

METHODS OF ANTIOXIDANT ACTIVITY

Spices can be added to foods in several forms: as whole spices, as ground spices, or as isolates from their extracts. Spices are aromatic and pungent food ingredients, therefore, their direct use as antioxidants is limited. The extraction procedure is determined by the types of antioxidant compounds to be extracted. Selection of a suitable extraction procedure can increase the antioxidant concentration relative to the plant material. For polyphenols and other antioxidants in plant materials three principal extraction techniques may be used: extraction using solvents, solid-phase extraction and supercritical extraction. It is advisable to complete the extraction using dry, frozen or lyophilized samples since some antioxidants are unstable or can be degraded by enzyme action in undried plant material. Ultrasound-assisted extraction is often used for the extraction of plant material using liquid solvents. The extraction can be also performed in a Soxhlet apparatus, thus combining percolation and immersion techniques. Several extraction techniques have been patented using solvents with different polarities, such as petrol ether, toluene, acetone, ethanol, methanol, ethyl acetate, and water. In addition, supercritical CO2-extraction and medium-chain triglycerides as carrier in a mechanical extraction process have been applied [18]. Extraction using edible oil or fat is a very simple method. Natural material containing antioxidants, such as herbs and spices, is mixed with fats and/or oils, and the mixture is left at a room or moderately increased temperature for a defined time. The mixture is then filtered and used [19]. Methanol is the solvent most commonly employed. Extraction of anthocyanins is commonly carried out under cold conditions with methanol containing a small amount of acid. It has been indicated that methanol is the best solvent for catechin extraction, whereas a better yield for procyanidins is obtained with 70% acetone. It has also been indicated that aqueous methanol, due to its polarity, extracts polyphenols linked to polar fibrous matrices more effectively, while acetone/water mixtures are more useful for extracting polyphenols from proteic matrices, since they appear to degrade the polyphenol protein complexes. Microwave-assisted extraction is a new extraction technique that combines microwave and traditional solvent extraction with advantages that include shorter time, less solvent used, or higher extraction rate. Pressurized liquid extraction has been recently introduced for phenolic compound extraction. In this technique high temperature and high pressure are used to accelerate the extraction.

Very interesting results were obtained in the study of sage antioxidant activity, where pressurized hot water extraction was found to be the most effective extraction procedure, followed by maceration with 70% ethanol, hydrodistillation, and ultrasonication assisted methanol extraction [20]. For industrial purposes ethanol would probably be better than methanol as eventual solvent residues would be less toxic. The extracts obtained using organic solvents may be further concentrated, for instance, by molecular distillation. Essential oils present in spice extracts, are responsible for the characteristic aroma of the spices and can be removed by steam distillation at normal atmospheric pressure or in a vacuum, but antioxidant activity may be partially lost. Commercial antioxidant extracts from spices are available in powder form or as oily oleoresins.

AN OVERVIEW OF THE ASSAY METHODS USED TO ESTIMATE ANTIOXIDANT CONTENT

Antioxidants, including phenolic compounds (e.g., flavonoids, phenolic acids and tannins) have diverse biological effects, such as anti-inflammatory, anti-carcinogenic and antiatherosclerotic effects, as a result of their antioxidant activity [21]. The antioxidant extracts were evaluated in terms of their total phenols (TP), total flavonoids (TFA), total flavonols (TFO), phenolic acids, catechins, lignans and tannins [22]. The antioxidant properties were evaluated using the following methods: 1,1-

diphenyl-2-picrylhydrazine (DPPH) radical scavenging assay food and bio products processing 8 9 (2 0 1 1) 217–233 219 , carotene linoleic acid bleaching assay, inhibition of linoleic acid peroxidation, ferric reducing antioxidant power (FRAP) , total radical trapping antioxidant potential (TRAP) assay, oxygen radical absorbance capacity (ORAC) assay, 15-lipoxygenase inhibition, lipid peroxidation (LPO) method, nitro blue tetrazolium (NBT) reduction assay or superoxide anion scavenging activity, hydroxyl radical scavenging activity or non-site- and site-specific deoxyribose degradation assay, hydrogen peroxide scavenging activity [23], and non-enzymatic *in vitro* antioxidant assay, 2,2azinobis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical scavenging method[24], reducing power assay, 50% inhibition of a particular assay (IC50), Briggs Rauscher (BR) method [25], Trolox equivalent antioxidant capacity (TEAC) method, phenazine methosulfate nicotinamide adenine dinucleotidereduced (PMS-NADH) system superoxide radical scavenging [26], linoleic acid peroxidation, ammonium thiocyanate (ATC) method, ferric thiocyanate (FTC) method, thiobarbituric acid (TBA) method and luminol-photochemiluminescence (PCL) assay.

Similarly, the phenolic concentration was determined using the Folin-Ciocalteau (FTC) method, while the total phenol content , the total flavonoid content , the tannin content and the total flavanol content were also determined by known methods. Although many methods are available to determine antioxidant activity, it is important to employ a consistent and rapid method. While each method has its own merits and drawbacks, it has been found that the most common and reliable methods are the ABTS and DPPH methods; these have been modified and improved in recent years.

DPPH method

The 1, 1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging assay was first described by Blois in 1958 and was later modified slightly by numerous researchers. It is one of the most extensively used antioxidant assays for plant samples. DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom. This method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolourizes the DPPH solution. The antioxidant activity is then measured by the decrease in absorption at 515 nm. In this method, a 0.1mMsolution of DPPH in methanol is prepared, and 4ml of this solution are added to 1ml of the sample solution in methanol at varying concentrations. Thirty minutes later, the absorbance was measured at 517 nm. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound.

ABTS method

The ABTS radical scavenging method was developed by Rice- Evans and Miller in 1994 and was then modified by Re et al. in 1999. The modification is based on the activation of met myoglobin with hydrogen peroxide in the presence of ABTS•+ to produce a radical cation. This improved method generates a blue/green ABTS•+ chromophore via the reaction of ABTS and potassium persulfate and is now widely used. Along with the DPPH method, the ABTS radical scavenging method is one of the most extensively used antioxidant assays for plant samples. The ABTS radical cation is generated by the oxidation of ABTS with potassium persulfate, and its reduction in the presence of hydrogen-donating antioxidants is measured spectrophotometrically at 734 nm. This decolourisation assay measures the total antioxidant capacity in both lipophilic and hydrophilic substances. The effect of the antioxidant concentration and the duration of the inhibition of the radical cation's absorption are taken into account when the antioxidant activity is determined. Trolox, a water-soluble analog of Vitamin E, is used as a positive control. The activity is expressed in terms of the Trolox-equivalent antioxidant capacity of the extract (TEAC/mg).

ORAC Assay

The ORAC assay uses beta-phycoerythrin (PE) as an oxidizable protein substrate and 2, 2- azobis (2-amidinopropane) dihydrochloride (AAPH) as a peroxyl radical generator or a Cu2+-H2O2 system as a hydroxyl radical generator. To date, it is the onlymethod that takes the free radical reaction to completion
and uses an area-under-the-curve (AUC) technique for quantification, thereby combining both the inhibition percentage and the length of the inhibition time of the free radical's action into a single quantity. The assay has been widely used in many recent studies of plants.

PCL Assay

The PCL assay measures the antioxidant capacity of a compound against the superoxide radical in lipid (ACL) and aqueous (ACW) phases. This method allows the quantification of the antioxidant capacity of both hydrophilic and lipophilic substances either as pure compounds or as a component in a complex matrix from various origins, including synthetic, vegetable, animal, or human sources. The PCL method is based on an approximately 1000-fold acceleration of the oxidative reactions *in vitro* when compared to normal conditions because of the presence of an appropriate photosensitiser. The PCL method is a very quick and sensitive method of measurement. Using the PCL assay, researchers have determined the antioxidant properties of marigold flowers.

Carotene linoleic acid bleaching assay

The carotene linoleic acid bleaching assay was first described by and is one of the antioxidant assays suitable for plant samples. In this assay, the antioxidant capacity is determined by measuring the inhibition of the production of volatile organic compounds and the formation

of **220** food and bioproducts processing 8 9 (2 0 1 1) 217–233 conjugated diene hydroperoxides arising from linoleic acid oxidation, which results in the discolouration of carotene. Carotene (0.5 mg) in 1 ml of chloroform is added to 25µl of linoleic acid and 200mg of the Tween 40 emulsifier mixture. After evaporation of the chloroform under vacuum, 100 ml of oxygen-saturated distilled water is added with vigorous shaking. Next, 4ml of this mixture is transferred into test tubes containing different concentrations of the sample. As soon as the emulsion is added to each tube, the zero time point absorbance is measured at 470nm using a spectrophotometer. The emulsion is incubated for 2h at 50 ◦C. A blank, devoid of carotene, is prepared for background subtraction. Quercetin, BHT and tocopherol are used as standards.

Reducing power Assay

The reducing power of the samples is determined according to the method described by [27]. The sample in 1ml of methanol is mixed with a phosphate buffer (5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5 ml, 1%), and the mixture is incubated at 50 ◦C for 20 min. Next, 5ml of trichloroacetic acid (10%) are added to the reaction mixture, which is then centrifuged at 3000 RPM for 10 min. The upper layer of the solution (5 ml) is mixed with distilled water (5 ml) and ferric chloride (1 ml, 1%), and the absorbance is measured at 700 nm. A stronger absorbance indicates increased reducing power.

NBT Assay or the superoxide anion scavenging activity assay

The superoxide anion scavenging activity assay was first described by [28]. The scavenging potential for superoxide radicals is analysed with a hypoxanthine/ xanthine oxidase-generating system coupled with a nitroblue tetrazolium (NBT) reduction (measured spectrophotometrically). The reaction mixture contains 125µl of buffer (50mM KH2PO4/KOH, pH 7.4), 20µl of a 15mMNa2EDTA solution in buffer, 30µl of a 3mM hypoxanthine solution in buffer, 50µl of a 0.6mM NBT solution in buffer, 50µl of xanthine oxidase in buffer (1 unit per 10 ml buffer) and 25µl of the plant extract in buffer (a diluted, sonicated solution of 10µg per 250µl buffer). Microplates (96wells) are read at 540nm 2.5 min after the addition of the xanthine oxidase using the series 7500 Microplate Reader. The superoxide scavenging activity is expressed as percent inhibition compared to the blank, in which buffer is used in place of the extract. When using this system, any inhibition by tannins in the plant extracts would have to be due to their antioxidant activity and not to their action upon the enzyme.

Total flavonoid content:

Total flavonoid content has been discussed by several authors [29]. The measurement of an extract's flavonoid concentration is based on the method described by **[30]** with a slight modification, and the results are expressed as quercetin equivalents. An aliquot of 1ml of amethanol solution containing 1mg of extract is added to test tubes containing 0.1 ml of 10% aluminium nitrate, 0.1 ml of a 1Mpotassium acetate solution and 3.8 ml of methanol. After 40 min at roomtemperature, the absorbance is measured at 415 nm. Quercetin is used as a standard.

Folin-Ciocalteu method

The Folin-Ciocalteu reagent assay is used to determine the total phenolics content (Singlenton and Rossi, 1965). The sample (0.2 ml) is mixed with 0.5 ml of the Folin-Ciocalteu reagent previously diluted with 7ml of deionised water. The solution is allowed to stand for 3min at 25 ◦C before 0.2 ml of a saturated sodium carbonate solution is added. The mixed solution is allowed to stand for another 120 min before the absorbance at 725nmis measured. Gallic acid is used as a standard for the calibration curve. The total phenolics content is expressed as mM gallic acid equivalents (GAE) per l of sample (mM/l).

ELECTRO CHEMICAL BIOSENSORS

Free radicals, such as reactive oxygen species (ROS), are highly unstable molecules with available electrons, generated *in vivo* during metabolic processes. These molecules are neutralised by antioxidants, naturally produced by the body. However, environmental or behavioural stressors (pollution, sunlight exposure, cigarette smoking, excessive alcohol consumption, etc.) or simply a malfunction of the antioxidant production may lead to a free radical excess, resulting in oxidative stress. Oxidative stress produces damage to lipids, proteins or DNA, impeding normal cell functioning. These biochemical alterations are implicated in a growing list of human diseases, such as cancer and Alzheimer's disease, as well as in the aging process. Since antioxidants are naturally present in vegetables, a balanced diet helps the body to prevent these diseases. The determination of free radicals and antioxidants has been widely investigated in the food technology and human health fields.

Traditional techniques such as spectrophotometry, fluorescence, and gas or liquid chromatography [31], are being replaced by other innovating technologies. In this direction, electrochemical biosensors are promising tools, suitable for fast analyses, based on inexpensive instrumentation and simple operation protocols. Whereas in the medical field the main objective is the evaluation of the ability of some compounds to scavenge free radicals, in food science research aims to detect and quantify them. In this sense, two different kinds of biosensors are reported in the antioxidant domain. On one hand, several amperometric biosensors for the detection of mono and polyphenols (the main antioxidant compounds in food) have been developed on the basis of enzymes such as tyrosinase, lactase or peroxidase [32]. These configurations allow the evaluation of the usually named "Total phenol content". On the other hand, biosensors for the assessment of the antioxidant capacity are based on the free radical scavenging activity.

This reviewis focused on the biosensors for measuring the antioxidant capacity.

All biosensors developed for this purpose are electrochemical and use ROS in their configurations. ROS are not commercially available because of their highly reactive nature and their very short lifetime. Consequently, the first step in the development of such biosensors is their generation *in vitro*. In the following section, the biochemical, chemical and physico-chemical processes to produce these radicals are summarised. Afterwards, cytochrome *c* (cyt *c*) sensors, superoxide dismutase (SOD) sensors and DNA sensors for the assessment of the antioxidant capacity are described.

Generation of ROS for electrochemical antioxidant biosensors

One of the key factors when developing a biosensor for evaluating the antioxidant capacity is the generation of the radicals that will be subsequently scavenged by the antioxidant compounds. There are several processes to produce ROS. Below, the reactions that generate these radicals for use in electrochemical biosensors are summarised.

• In the Fenton reaction, reduced transition metal ions, such as

Fe(II), Cu(I) or Cr(II), react withH2O2 in a one-electron redox reaction producing hydroxyl radical (OH•) and hydroxide anion:

Me*n* +H2O2→ Me*n*+1 +OH− +OH• (1)

The addition of a reducing agent increases the radical generation rate. Alternatively, the transition metal can be reduced by the application of an appropriate electrode potential [33].

• The radical generation by photocatalysis starts with the absorption of light of a wavelength higher than the band gap of TiO2, which results in the transition of an electron from the valence band (VB) to the conduction band (CB), leaving a hole behind (Eq. (2)). Then, adsorbed water or hydroxide ions are trapped by holes to produce OH• (Eqs. (3) and (4)). Subsequently, electrons are trapped by the reaction with adsorbed O2 to produce superoxide radical (O_2 •−) (Eq. (5)), which then forms more OH• (Eq. (6):

TiO_2 +h*v*→ h+ VB +e− CB (2)

h+ VB +H_2O (ads)→ OH• + H+ (3)

h+ VB +2OH− (ads) → OH− +OH• (4)

e− CB +O2→ O2 •− (5)

O_2 •− +2H_2O → 2OH• + 2OH− +O_2 (6)

• Xanthine oxidase (XOD) catalyses the oxidation of xanthine or hypoxanthine with concomitant reduction of O2 to H_2O_2;

O2 •− is formed as an intermediate of this reaction [6]:

xanthine + O_2 + H_2O → uric acid + 2H+ + O_2 •− (7)

• The addition of NaOH to dimethylsulphoxide (DMSO) generates O_2 •− [7].

ThisO2 production is inversely proportional to the water concentration in DMSO and solutions obtained are stable up to three days.

• The simple injection of KO2 in aprotic organic solvents, specially DMSO also results in O2 •− generation by means of the following reaction [8,9]:

KO_2→ K+ +O2 •− (8)

Whereas DNA-based sensors mainly use the Fenton reaction, cyt *c*- and SOD-based sensors usually incorporate XOD as radical generator.

Biosensors for determination of the antioxidant capacity

The presence of antioxidant substances in a sample where radicals are generated involves their decomposition. Therefore, evaluation of the antioxidant capacity of different compounds can be determined based on the variation of the ROS concentration in the reaction medium. With the aim to assess antioxidant capacity based on the measurement of O_2 •− concentration two main types of biosensors have been developed, using cyt *c* or SOD enzyme. O2 •− determination using a cyt *c*-based sensor lacks in selectivity, since this heme protein is not specific for O_2 •−, moreover, its inherent property as a peroxidase, able to reduce H2O2 endogenously coexisting in biological systems, greatly limits its application for detection of O_2 •− in real samples. SOD-based biosensors, on the contrary, use to be much more specific and sensitive. Another method to determine the antioxidant capacity is by measuring the damage produced to DNA by free radicals. In this case, the presence of antioxidants involves a decrease in DNA alterations. In what follows, the three different types of biosensors are described.

Cyt c-based antioxidant biosensors

The detection principle of these biosensors is based on the redox reaction of cyt *c* (Fig. 1). The immobilised cyt *c* is reduced by O_2 •− and immediately regenerated at the surface of the electrode polarised at the oxidation potential. The current generated due to the electron transfer from the radical, via cyt *c*, to the electrode is proportional to the radical concentration [34].

In order to avoid interference from H2O2, generated by spontaneous dismutation of O_2 •− (Eq. (9)), sometimes catalase enzyme is added to the reaction media. The addition of antioxidants reduces the radical concentration and thus the oxidation current, allowing the quantification of the antioxidant capacity.

2O_2 •− +2H+→ O_2 + H_2O_2 (9)

Usually, the electron transfer between the electrode and the redox protein is extremely slow. Consequently, traditional electrochemical methods cannot detect it [35]. An elegant way to modify gold electrodes, the most commonly used in this area, is the formation of self-assembled monolayers (SAMs). Shortchain alkanethiols show a high efficiency of communication between cyt *c* and the electrode. However, they cannot form dense films and thus, do not effectively block the electrode from interfering substances. In order to eliminate electroactive interferences, e.g. H_2O_2 and uric acid, products of the XOD-catalysed

reaction, cyt *c* has been immobilised on longchain thiol (mercaptoundecanoic acid) -modified electrodes.

These cyt *c*-based biosensors can be used for O_2 •− detection and for the analysis of antioxidant activities. Following this strategy, analysed the ability of SOD to dismutate O_2 •− radicals. Besides, [36] evaluated the antioxidant capacity of flavonoids with the same cyt *c*-modified electrode. They established the following trend: flavanols > flavonols > flavones > flavonones > isoflavonones. In order to increase the electron transfer rate of cyt *c*, employed mercaptoundecanoic acid/ mercaptoundecanol mixed SAM-modified gold electrodes. This procedure had already been described and applied by to the O2 •− sensing. However, they still used short-chain modifiers, which limited the selectivity. These mixed SAM-modified biosensors showed more sensitivity to O_2 •− and were applied to the study of the antioxidant capacity of hydrophobic antioxidants [37] tested the antioxidant capacity of ascorbic acid and Biochanin A in a mixture of 40% DMSO and 60% phosphate buffer. Results concluded that Biochanin A was less effective than ascorbic acid as an antioxidant. They also studied the antioxidative properties of some cosmetic creams in the same medium.

Some authors tried to simultaneously detect both O_2 •− and H_2O_2 produced in the course of its spontaneous dis mutation. Following the concept of "lab-on-a-chip", [38] developed a fluidic chip that combined the generation of O_2 •− and H_2O_2 with a two-electrode biosensor chip for the detection. In this way, the antioxidant capacity of different potential scavengers of the respective reactive species was quantified in a flow-injection mode. The antioxidant capacity of ascorbic acid found using this strategy was very close to previous works. The same group has recently applied this fluidic chip to the determination of the antioxidative capacity of complex lipophilic mixtures, such as cosmetic creams. The antioxidative properties of a model cosmetic cream doped with green tea extract were also characterised. The study of such SAM-modified electrodes showed that the sensitivity of the sensor was directly proportional to the amount of immobilised protein.

With the aim of increasing the amount of immobilised biomolecules, constructed multilayer structures of cyt *c* and poly (anilinesulfonic acid) (PASA) on long-chain mixed SAM-modified electrodes. These multilayer electrodes were successfully applied for the quantitative detection of O_2 •−. Besides, they were much more sensitive than monolayer electrodes. Apart from *in vitro* analysis, cyt *c*-based biosensors allow the measurement of the O_2 •− produced *in vivo*, e.g. during ischemia and reperfusion injury. Beissenhirtz et al. compared the *in vitro* O_2 •− scavenging activity and the *in vivo* antioxidant potential of methanolic extracts prepared from 10 Chinese tonifying herbs. Results did not show quantitative correlation. However, for 8 out of 10 samples a similar tendency was found.

Superoxide dismutase-based biosensors

SOD biosensors are shown as a promising alternative to cyt *c* biosensors for the evaluation of the antioxidant capacity. SOD enzyme is involved in cell protection mechanisms against oxidative damage from ROS. It specifically catalyses the dismutation of the O2 •− producing O2 and H2O2 via a cyclic

MEDICINAL SPECIES WITH HIGH ANTIOXIDANT POTENTIAL

Several authors have also reviewed medicinal species with great antioxidant potential. In this work, I have reviewed the antioxidant potential of a number of additional plants.

Diospyros abyssinica

Diospyros species have been used in many traditional medical systems around the world, including traditional Ayurvedic, African and Chinese medicine. Nearly every part of these plants has been used as a medicine in some way, for example as an astringent remedy and to cure biliousness [39]. In India, a juice made from the bark and leaves of *Diospyros peregrine* combined with the root juice of *Albizia lebbeck* is used as a remedy for snakebites. In Japan, the leaves of *Diospyros kaki* are used in combination with jasmine to make anti-smoking candies. The most frequently-isolated compounds from *Diospyros abyssinica* are the triterpenoids betulin, betulinic acid and lupeol. All of these compounds are well-known anti-inflammatory compounds. This species has a significant medicinal value demonstrated by its use in traditional medicine. The root bark from *D. abyssinica* has been tested regarding its antioxidant activity. It was extracted with a series of solvents, including petroleum ether, dichloromethane, chloroform, 80% aqueous ethanol, andwater (at 50 ◦C and 100 ◦C). It was determined that the root bark from *D. abyssinica* is the richest source of extracted compounds; 36.7% of the weight of the plant material is composed of antioxidants. *D. abyssinica* exhibited the greatest radical scavenging activity and the greatest 15-lipoxygenase inhibition in the 80% ethanol and methanol extracts. Thus, this plant appears to be an excellent source of antioxidants.

Pistacia lentiscus

Pistacia lentiscus is extensively used in folk medicine by rural populations in Algeria. Algeria is home to at least 3164 species of vascular plant, of which 7.9% are endemic. *P. lentiscus* is important because of its medicinal value. The reducing power and radical scavenging activity of the extracts from the leaves of *P. lentiscus* in solvents, such as ethanol, ethyl acetate, aqueous/ ethyl acetate, hexane, aqueous/hexane, chloroform, andaqueous chloroform has been studied *in vitro*. Using the DPPH scavenging activity assay, itwas found that all of the *P. lentiscus* extracts, except for the chloroform extract, have a high radical scavenging activity (90%) equivalent to that of the standard, BHA (89%). Overall, *P. lentiscus* exhibited

outstanding reducing power, good radical scavenging activity against DPPH and H_2O_2, slow inhibition of lipid peroxidation and richness in tannins; however, it also showed a lack of flavonoids. A strong correlation was found between reducing power and the total amount of phenols present in *P. lentiscus*, indicating that the phenol compounds play an important role in the beneficial effects of these medicinal plants. This finding is in agreement with the work of Chryssavgi et al. (2008), which demonstrated that the greatest phenolic content in *P. lentiscus* is 588mg gallic acid/g of plant material and consists mainly of monoterpenes (81.6%).

***Geranium sanguineum L*.**

Geranium sanguineum L., commonly found in Bulgaria, has significant antioxidant activity and antiviral activity. Its root extracts are used in traditional medicine to treat gastrointestinal disorders, infections and inflammatory conditions. It is also frequently used in folk medicine for the treatment of eruptive skin diseases and as a disinfectant bath and poultice for the affected area. The polyphenolic compounds of this plant species include tannins (11.02%), flavonoids (0.14%), catechins and proanthocyanidines (2.1 mg/kg) . Using three separate, complementary methods (the DPPH assay, the carotene-linoleic acid assay and the NBT-reduction assay), it was established that a polyphenol-rich extract from *G. sanguineum* L., which had a strong anti-influenza activity, possessed antioxidant and radical scavenging capacities. In this study, caffeic acid and the synthetic antioxidant BHT were used as positive controls.

The root extract of this plant exhibited a strong antioxidant capacity in the DPPH assay (IC_{50} = 13.86±0.84µg/ml) when compared to BHT (IC_{50} = 19.81±0.05µg/ml). In the carotenelinoleic acid test system, the root extract achieved 88–89% inhibition, which is as strong as BHT's inhibition. Furthermore, the total extract and ethyl acetate fraction exhibited a strong superoxide dismutase (SOD) activity, comparable to that of caffeic acid. In addition, the total methanol-soluble phenolic constituentswere measured with the Folin-Ciocalteu reagent andwere found to be 34.6% (w/w). In another study, it was found that *G. sanguineum* L. reduced the accumulation of TBA-reactive products in rat liver microsomes *in vivo* in the induced LPO method, but the non-induced LPO method was not affected.

***Sargentodoxa cuneata* Rehd. Et Wils**

In the classification of Chinese medicinal plants, *Sargentodoxa cuneata* Rehd. Et Wils falls into the "heat-clearing" category. The plants in this category have significant anti-inflammatory, anti-tumour, anti-allergic, anti-viral and anti-bacterial activities. China is the only country on Earth in which there are unbroken connections among tropical, subtropical, temperate and boreal forests. This unbroken connection has fostered the formation of rich plant associations rarely seen elsewhere in the world. China's plant life is enormously rich. Some 31,000 plant species are native to China, representing nearly one-eighth of the world's total plant species, including thousands found nowhere else on Earth. Chinese medicinal plants contain a wide variety of natural antioxidants, such as phenolic acids, flavonoids and tannins and possess more potent antioxidant activity than common dietary plants. Sargentol, tyrosol, salidroside, methyl protocatechuate, vanillic acid, syringic acid, *p*-hydroxy benzoic acid, and ferulic acid have been identified in this plant. Using the FRAP and TEAC assays, *S. cuneata* Rehd. Et Wils was found to have the greatest antioxidant capacity with 453.53µmol Fe (II)/g and 265.43µmol Fe(II)/g, respectively. In addition, *S. cuneata* Rehd. Et Wils had the highest phenolic content (52.35mg GAE/g). A strong correlation was also found between the TEAC and FRAP values, which implies that the extracts from this plant are capable of scavenging free radicals and reducing antioxidants. This study concludes that this medicinally-important species is a valuable source of natural antioxidants, both for the preparation of crude extracts and for the further isolation and purification of antioxidant components.

***Polyalthia cerasoides* (Roxb.) Bedd**

Polyalthia cerasoides (Roxb.) Bedd. (Annonaceae) is a mediumsized tree distributed in almost all of the forests of Deccan India at elevations of up to 3000 ft. India is one of the richest countries in the world with respect to medicinal and aromatic plants. The plant life of India constitutes 11% of the world's total known flora that have medicinal properties. The number of plant species in India is estimated to be over 45,000. The tribal people of Tamil Nadu and Andhra Pradesh (states of India) use the fruits of this plant, while tribes in Africa use the fruits, roots and leaves to treat rheumatism and toothaches, as an aphrodisiac, as a deparasitant and as an anti-inflammatory. Pharmacological studies confirmed that the stem bark of P. cerasoides reduces brain stress. The antioxidative potential of the alcohol extract of P. cerasoides was evaluated using the DPPH, hydroxyl radical, superoxide anion scavenging, and reducing power assays. The methanol extract of P. cerasoides exhibited a significant dosedependent inhibition of DPPH scavenging activity with 50% inhibition occurring at a concentration equivalent to 25µg/ml of tannic acid. The total phenolic content of the alcohol extract of P. cerasoides was equivalent to 0.589µg of tannic acid per mg of extract. The phenolic compounds present in the extract may contribute directly to the antioxidative action of the plant, suggesting that the polyphenols present in the extract could be responsible for its beneficial effects.

***Crataeva nurvala* Buch-Ham**

Crataeva nurvala Buch-Ham. is used extensively in traditional medicine as a blood purifier. The bark of *C. nurvala* is used in herbal powders to treat urinary stones, thyroid disorders, obesity and cancer. *C. nurvala* has a higher total antioxidant capacity than catechin. In this study, *C. nurvala* showed the highest SOD mimetic activity

(122.53 unit/min/mg), which was determined spectrophotometrically by measuring inhibition in the nicotinamide adenine dinucleotide (reduced)-phenazine methosufatenitroblue tetrazolium reaction system. *C. nurvala* was found to have the highest LPO inhibitory potential. *C. nurvala* was more efficient at scavenging peroxide radicals than catechin. Using the ABTS assay, the total antioxidant potential of *C. nurvala* was found to be 0.39 mmol/l TEAC/mg of extract. In addition, a study of this plant's phytochemicals revealed that the stem bark of *C. nurvala* contained triterpenoids such as phragmalin triacetate and lupeol.

***Acacia auriculiformis* A. Cunn**

Acacia auriculiformis A. Cunn is a vigorously-growing deciduous or evergreen tree. It can reach heights of up to 30m and belongs to the family *Mimosaceae*. It is rich inmethylglucuronic acid, glucuronic acid, galactose, arabinose, and rhamnose. Tannins and triterpenoid saponins are present in the species . In addition, extracts from the *Acacia* species are rich in phenols and polyphenols and have strong antimutagenic and antioxidant activities. The hydroxyl radical scavenging potency of the extracts of *A. auriculiformis* increased with solvent polarity andwas greatest in thewater fraction, followed by the ethyl acetate fraction, and the crude extract. The water fraction had a higher phenolic content (720 mg) than the ethyl acetate fraction (600 mg) or the crude ethyl acetate extract (390 mg) when expressed as GAE/g of extract/fraction.

***Teucrium polium* L**

Teucrium polium L. is a wild flower species belonging to the *Lamiaceae* family, which is composed of numerous species with exploitable antioxidant activity. An infusion of the leaves and flowers of the plant is consumed as a refreshing beverage. This infusion is also used for liver ailments, gastrointestinal diseases, fevers, colds, diarrhoea, stomach pains and fevers. In this study, the aerial part of the plant was extracted with petroleum ether, chloroform, methanol and water. The antioxidant flavonoids were separated from the methanol extract and were identified as rutin, apigenin, 3,6-dimethoxy apigenin and 4µ,7-dimethoxy apigenin; their IC50 values in the DPPH assay were found to be 23.7±1.9µg/ml, 30.3±2.1µg/ml, 31.5±3.4µg/ml and 37.4±3.4µg/ml, respectively. The DPPH assay IC50 value for the methanol extract was found to be 20.1±1.7µg/ml; this value is similar to the IC50 value of the synthetic antioxidant, butylated hydroxytoluene (18.3±1.9µg/ml). The potential antioxidant activity and the rich flavonoid content of *T. polium* suggests that its extracts may be added to various food products in place of synthetic antioxidants. The aqueous extract of *T. polium* can effectively inhibit oxidative processes and has substantial antioxidant activity *in vitro*. The ethanol extract prepared from *T. Polium* exhibited the same antioxidant activity as tocopherol. The antioxidant activity of *T. polium* was also demonstrated in a recent *in vivo* study of rats. Ratswere treated with a *T. polium* extract that showed significant antioxidant activity in the DPPH test compared to the positive control (tocopherol). The *T. polium* extract given to rats at doses of 50 and 100 mg/kg significantly increased the total antioxidant power (TAP) and decreased the thiobarbuteric acid reactive substances (TBARS) relative to the control.

***Dracocephalum moldavica* L**

The Moldavian balm (*Dracocephalum moldavica* L., *Lamiaceae*) is a perennial herb that is native to central Asia and is naturalised in eastern and central Europe. It is used as a food ingredient, a tea, and as an herbal drug used to treat stomach and liver disorders, headaches and congestion. The antioxidant activity of this species has been studied by several researchers suggested that the components responsible for its activity were hydroxycinnamic acids and flavonoids, including caffeic acid, ferulic acid, rosmarinic acid, luteolin, luteolin-7-*O*-glucoside and apigenin. The extract yields ranged from 3.7 mg/g in the ethyl acetate and n-butanol extracts to 109.2 mg/g in the methanol extract; they increased in the following order: ethyl acetate and n-butanol, acetonitrile (ACN), dichloromethane, petrol, water and methanol. The total phenolic content of the extracts ranged from 0.0±0.0mg GA/g in the petrol extract to 488.4±1.8mg GA/g in the methanol extract and increased in the following order: petrol, dichloromethane, ACN, ethyl acetate, water, n-butanol and methanol. The HPLC-determined total phenolic content of the raw material was found to be 476.59±25.22 mg/g (sum of the individual extracts). Rosmarinic acid was the most abundant component identified (247.95±24.78 mg/g), followed by chlorogenic acid (41.46±2.76 mg/g) and apigenin-7-*O*glucoside (26.55±2.20 mg/g). The greatest quantities of phenolic substances were found in the n-butanol (39%), methanol (31.1%) and water (11.5%) fractions.

***Urtica dioica* L**

Urtica dioica L. (*Urticaceae*) leaves have been used in Sardinia, Italy as a medicinal tea or decoction as diuretic and antidiabetic therapies and to treat stomach disorders. The flora of Italy is the richest in Europe. As of 2004, 6759 species had been recorded in the data bank of Italian vascular flora, of which 700 are endemic. *U. dioica* L. leaves are also used to treat stomachaches in Turkish folk medicine. The antioxidant capacity of this plant was evaluated using several *in vitro* methods (BR, TEAC, DPPH, and FC). The BR method determined that the antioxidant activity of *U. dioica* at an acidic pH was 0.013±0.001µg/ml resorcinol equivalents (Re eq.); the DPPH method in methanolic solutions determined that the antioxidant activitywas 419±10µg/ml. The total phenolic content was found to be 0.35±0.02 mg/l GAE. Concentrations of *U. dioica* L. extract of 50, 100 and 250µg/ml showed 39%, 66% and 98% inhibition, respectively, of the peroxidation of a linoleic acid emulsion. However, tocopherol, positive control, at 60µg/ml, exhibited

only 30% inhibition. It can be concluded that *U. dioica* L. has powerful antioxidant activities.

***Ficus microcarpa* L. fil**

Ficus microcarpa L. fil. (Chinese banyan tree, *Moraceae*) is a popular ornamental tree grown widely in many tropical regions. It is native to areas including Ceylon, India, southern China, the Ryukyu Islands, Australia and New Caledonia. It is also a popular ornamental plant in Taiwan. Its dried leaves, aerial roots and bark have been used as folk remedies to decrease perspiration, alleviate fever and relieve pain in the Okinawa Islands. There are about 7000 species of vascular plants in Japan, and about 40% of these, approximately 2900 species, are recognized as endemic. In the study, two isoflavones comprised of 28 components were identified in the bark of the *F. microcarpa* tree. In addition, the methanol extracts of this tree's bark, fruits and leaves exhibited strong antioxidant activity when assayed by the DPPH method, the ABTS free radical scavenging method, the PMS-NADH system superoxide radical scavenging assay and the carotene-linoleic acid system. The methanol extract of the bark showed stronger antioxidant activity than the extracts of the leaves or fruits in the ABTS method, the PMS-NADH method and the carotene-linoleic acid system.

However, no significant difference was found between the bark and the fruits in the DPPH assay. Furthermore, the bark contained a significantly higher amount of total phenolics (237mg GAE/g extract) than the fruits (179mg GAE/g extract) or the leaves (127mg GAE/g extract). Furthermore, the total phenolics in the bark were present in greater amounts in the ethyl acetate fraction than in the aqueous fraction and the hexane fraction; the values were 436, 194 and 41.7mg GAE/g extract, respectively. The ethyl acetate fraction contained twelve phenolic compounds, of which seven were quantified by HPLC: protocatechuic acid (6.60±0.20 mg/g extract), catechol (11.1±0.00 mg/g extract), *p*-vinylguaiacol (4.40±0.07 mg/g extract), syringol (173±1.12 mg/g extract), *p*-propylphenol (10.5±0.78 mg/g extract), vanillin (4.27±0.02 mg/g extract) and syringaldehyde (8.96±0.29 mg/g extract).

***Bidens pilosa* Linn. Radiata**

Bidens pilosa Linn. *Radiata* (family *Asteraceae*) is widely distributed in subtropical and tropical regions. It is 30–100cm in height with yellow flowers and is commonly known as "hairy beggar ticks," "sticks tights," and "Spanish needles." The plant is used in various folk medicines for its anti-inflammatory, antiseptic, liver-protective, blood-pressure lowering, and antihypoglycaemic effects. The plant has been widely used in Taiwan as a traditional medicine and as a major ingredient of an herbal tea that is believed to prevent inflammation and cancer. Phenylpropanoid glucosides, polyacetylenes, diterpenes, flavonoids and flavone glycosides have been identified as the bioactive components of this plant and are thought to be involved in its antioxidant activity. The methanol extract of *B. pilosa* was shown to prevent the onset of hypertension and to reduce blood pressure in rats. In addition, the fresh leaves and flowers of *B. pilosa* were subjected to steam-distillation, and colourless and yellowish essential oils were obtained in amounts of 0.08% and 0.06% (w/w), respectively. GC-MS analysis of these essential oils resulted in the identification of forty-four compounds including the major essential oils, caryophyllene (10.9% and 5.1% in the leaves and flowers, respectively and cadinene (7.82% and 6.13% in the leaves and flowers, respectively). Both of these essential oils are terpenes. The other chemical components were pinene, limonene, trans-ocimene, cis-ocimene, muurolene, bourbonene, elemene, cubebene, caryophyllene, caryophyllene oxide and megastigmatrienone. The essential oils in the leaves and flowers were able to reduce the stable free radical DPPH to the yellow coloured diphenylpicrylhydrazine with IC50s of 57 and50µg/ml, respectively, whereas the synthetic and natural antioxidant activities were 21 and 36µg/ml, respectively.

This study revealed that the flowers of *B. pilosa* have an antioxidant activity that is similar to that of synthetic antioxidants. In addition, the aqueous extracts of the flowers and leaves were found to be less efficient in radical scavenging and had IC_{50} values of 172µg/ml and 61µg/ml, respectively. Furthermore, the essential oils of the leaves and the aqueous extracts of the leaves and flowers exhibited higher antioxidant activities than did the flower oils. The lower activity of the essential oils of *B. pilosa's* flowers may be due to their volatility at higher temperatures. The study showed that the antioxidant effects of essential oils depend not only on the temperature but also on other factors such as their structural features, the characteristics of the lipid system, and the binding of the fatty acids.

Leea indica

Leea indica, a member of the *Leeaceae* family, was studied for its antioxidant and nitric oxide inhibitory properties because of its traditional use for various medicinal purposes. It is commonly found in Malaysia. Malaysian tropical rainforests contain many species that are important sources of traditional medicines. About 10,000 species of higher plants and 2000 species of lower plants are available in Peninsular Malaysia; 16% of these are used for traditional medicinal purposes. The leaves of *Leea indica* contain 23 relevant chemical compounds, including eleven hydrocarbons, phthalic acid, palmitic acid, 1-eicosanol, solanesol, farnesol, three phthalic acid esters, gallic acid, lupeol, beta-sitosterol and ursolic acid . This study used the FTC and TBA methods to demonstrate that methanol extracts of *Leea indica* had strong antioxidant activity that is comparable to, or higher than, that of tocopherol, BHT and quercetin. confirmed that extracts from *Leea indica* had strong activity compared with the standards (i.e., vitamin C, quercetin and BHT). The high antioxidant activity of *Leea indica* extracts may be due to the presence of gallic acid.

***Lamiaceae* species**

Six Lamiaceae species (i.e., Leonurus cardiaca, Lamium album, Marrubium vulgare, Stachys officinalis, Lamium purpureum and Galeopsis speciosa) are rich in antioxidant activity. *Leonurus cardiac* L. is a mild cardiac drug containing flavonoid and phenolic glycosides. The chemical composition, therapeutic uses and pharmacological properties of these species have been reported. *Lamium album* L. (dead nettle) has antispasmodic, diuretic and haemostatic properties and is used to alleviate bladder, kidney and menstrual problems. *Lamium purpureum* L. is used for similar medicinal purposes. *Marrubium vulgare* L., which contains diterpenoids, iridoids, flavonoids and terpenoid, is used to treat coughs and digestive disorders. *Stachys officinalis* Franch. is used for antiseptic, astringent, tonic, anthelmintic and digestive purposes. *Galeopsis speciosa*, which contains tannins, flavonoids, soluble silica and saponins, is used as an astringent, diuretic and expectorant. The antioxidant activities of these six species have been studied by several authors , but it is difficult to compare their results because of the methodological differences between the studies. In addition, the antioxidative effects of the methanolic extracts from six wild European *Lamiaceae* species have been studied using three *in vitro* assays. In the DPPH scavenging assay, the order of these species from strongest to weakest antioxidant activity was: *Leonurus cardiaca*, *Lamium album*, *Marrubium vulgare*, *Stachys officinalis*, *Lamium purpureum* and *Galeopsis speciosa*. In the LPO assay, *S. officinalis* and *M. vulgare* reached a maximum inhibition of 78%, *Lamium sp*. and *L. cardiac* slightly exceeded 70% while *G. speciosa* reached 65%. All of the extracts contained a considerable quantity of phenolic metabolites, ranging from13.2% GAE in *S. betonica* to 20% in *L. cardiaca*. *L. cardiaca* has significant antioxidant potential, as demonstrated by several authors.

***Uncaria tomentosa* (Willd.) DC**

Uncaria tomentosa (Willd.) DC., commonly known as cat's claw, belongs to the family *Rubiaceae* and is found in South and Central America. It is used for the treatment of asthma, cancer, cirrhosis, fevers, gastritis, diabetes, dysentery and inflammation of the urinary tract. In addition, it is used as an anticancer remedy and has anti-inflammatory properties. Due to the chemical structure of its components, this plant is expected to have strong antioxidant activity. The active chemical constituents of this species are alkaloids, quinoic acid, glycosides, polyhydroxylated triterpenes and several steroidal components. The antioxidant properties of the aqueous and ethanolic extracts of *U. tomentosa* bark have been evaluated. A higher antioxidant activity and greater number of total phenolic compounds were detected in the alcoholic preparations (TEAC= 0.57mmol of Trolox/g and SOD= 0.39 U/mg) than in the aqueous preparations (TEAC= 0.34mmol of Trolox/g and SOD= 0.1 U/mg). This study revealed that five pentacyclic oxindole alkaloids, including uncarine F, speciophylline, mitraphylline, isomitraphylline and/or pteropodine and isopteropodine, were present in the bark. The content of TPC in the ethanol extract from *U. tomentosa* bark (292 mg/g D-catechin units) was two times higher than in the aqueous extract (111 mg/g). These values are very high compared to other TPC-containing cereals (from 0.481 to 0.896 mg/g), vegetables (e.g., 11.7 mg/g for broccoli, 9.9 mg/g for garlic and 7.6 mg/g for pepper) and fruits (e.g., 23.1 mg/g for blackberries). The ethanol extract showed higher superoxide radical scavenging activity (0.39 U/mg) than did the aqueous extract (0.10 U/mg).

***Salvia officinalis* L**

Common sage (*Salvia officinalis* L., *Lamiaceae*) is an aromatic and medicinal plant of Mediterranean origin commonly found in Portugal and Lithuania and well known for its antioxidant properties that are mainly due to its phenolic-rich composition. Methanolic and aqueous extracts were prepared from the aerial parts of *S. offcinalis* and analysed for phenolic compounds by HPLC/DAD. Eight phenolic compounds were identified, including five phenolic acids (i.e., rosmarinic acid, caffeic acid, ferulic acid, 3-caffeoylquinic acid and 5-caffeoylquinic acid) and three flavonoids (i.e., luteolin-7-glucoside; 4,5,7,8-tetrahydroxyflavone; apigenin-7- glucoside). The methanolic extract had a higher content of these compounds than did the aqueous extract. The activity of both extracts was lower than the positive control, quercetin. In the superoxide radical scavenging assay, the aqueous extract had a greater antiradical activity (14.4±1.4µg/ml) than the methanolic extract (162±39µg/ml). A separate study showed that replacing the drinking water of rats and mice with *S. officinalis* infusions for 14 days led to improved liver antioxidant status.

***Momordica Charantia* L**

The bitter gourd (*Momordica Charantia* L.) or *Mara* (in Thai) belongs to the family *Cucurbitaceae* and has long been used in foods and medicines [40]. The bitter gourd is known by different names, such as balsam pear and karela, and it grows in tropical and sub-tropical regions of India, Malaysia, China, Africa, the Middle East, USA and Thailand. Thailand is home to a wide range of herbal plant species. Medicinal plants and herbs have long been a part of everyday life in Thailand; many are used as spices in various Thai dishes. The therapeutic efficacy of Thai medicinal plants and traditional herbal medications has been scientifically proven and described in the literature by both Thai and non-Thai scientists. The bitter gourd can be used to treat diabetes mellitus and appears to be a safe alternative to reduce blood glucose. In the DPPH radical scavenging assay, the activity of the positive control, ascorbic acid, was the highest (200 mg/ml), followed by BHT, the leaf, the green fruit, the stem and the ripe fruit fractions of the bitter gourd. The IC50 values were lowest in the leaf fraction (9.72±0.25 mg/ml), followed by the green fruit fraction

(11.00±0.76 mg/ml), the stem fraction (17.8±0.66 mg/ml) and the ripe fruit fraction (27.6±0.23 mg/ml).

In the hydroxyl radical scavenging assay, the activity of the leaf fraction was greater than that of the other fractions but lower than that of ascorbic acid and BHT. The antioxidant activity was greatest in the leaf, followed, in decreasing order, by the green fruit, the stem and the ripe fruit. In the four analysed fractions, seven phenolic compounds were identified: *p*-coumeric acid, tannic acid, benzoic acid, ferulic acid, gallic acid, caffeic acid and (+)-catechin. Gallic acid was the most predominant of the phenolic compounds in all parts of the bitter gourd, contributing from 72.8 mg/l in the extracts of the stem to 202 mg/l in the extracts of the ripe fruit. Caffeic acid was most concentrated in the leaf extract (7.77±1.02 mg/l), while *p*-coumeric acid was most abundant in the stem extract (6.73±0.21 mg/l). Ferulic acid was only found in the stem and green fruit extracts, while benzoic acid was not present in either the leaf or the stem extracts. The bitter gourd fractions are rich in phenolics and have strong antioxidant activity and radical scavenging action by all of the testing methods. Semiz and Sen have studied the fruit extract of *M. Charantia* in rats (200 mg/kg of weight) and found that there is a significant increase in the activity of the hepatic antioxidant enzymes, including SOD, catalase and glutathione peroxidase.

***Rheum ribes* L**

Rhubarb (*Rheum ribes* L.) belongs to the family *Polygonaceae.* It is used for medicinal purposes, and its fresh stems and petioles are also consumed as a vegetable. It is commonly found in eastern Turkey, Lebanon and Iran. In Turkey, 11,700 types of plants are available, of which nearly a thousand have aromatic and medicinal value. *R. ribes* is the only *Rheum* species growing in Turkey. Rhubarb roots have been used as a laxative and an antipsoriatic drug in Iran. The roots of the species are also used to treat diabetes, hypertension, obesity and diarrhoea. The young shoots and petioles of *R. ribes* are used against diarrhoea and as a stomachic and antiemetic treatment. The medicinal properties of this species are due to its anthroquinone content. Furthermore, the DPPH assay showed that methanol extracts of both the stems and the roots exhibited higher activity than BHT at concentrations greater than 50µg/ml. The methanol extract of the stems showed the highest DPPH radical scavenging activity among all of the extracts tested (87.07±0.54%), followed by the methanol extract of the roots (60.60±0.86%) and the chloroform extract of the roots (50.87±0.3%) at a concentration of 100µg/ml. In addition, the chloroform extract of the roots (48.66±1.23µg PEs/mg extract) had a higher phenolic content than the other extracts, and the extract containing the lowest quantity of phenolics was the chloroform extract of the stems (22.68±1.10µg PEs/mg extract). The most flavonoid-rich extract was found to be the chloroform extract of the roots (145.59±0.22µg QEs/mg extract), while the methanol extract of the stems (13.66±0.75µg QEs/mg extract) had the lowest flavonoid content.

Fig 1. Cyt c-based antioxidant biosensors

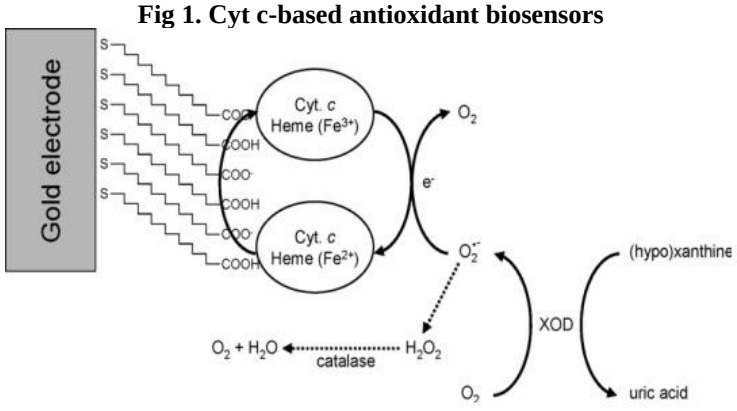

Table 1. Process of Extraction

Spices	Process of Extraction
Basil, black pepper, cinnamon, nutmeg, oregano, parsley, rosemary, sage.	(1) Trichloacetic acid extract for ascorbate determination, centrifugation 8000 g, filtration (2) Methanol extract for carotenoids and capsaicinoids determination, vacuum concentration, filtration
Allspice, clove	Methanol extract
Ginger, turmeric, cayenne pepper, rosemary, sage, thyme, oregano, green tea, spice mixture	Mechanical medium-chain triglyceride and propylene glycol extracts prepared by hydraulic laboratory press
Clove buds	Dichlormethane extract of water steam-distillation, extract reduction by a

	rotary flash evaporator.
Sage	Crude methanol extract (80% methanol, 20% water)
Sage	Pressurized hot water, maceration with 70% ethanol
Pepper (Capsicum species)	Methanol extract (50% methanol, 50% water)
Ginger, nutmeg, coriander	Ethanol extract (96% ethanol, 4% water)
Oregano, sage, thyme	Homogenization in phosphate buffer (pH7)centrifugation
Oregano, sage, thyme	SFE under specific operating conditions
Sage, rosemary, oregano	Soxhlet extraction with pure methanol
Basil	80% ethanol extract
Thyme, basil, rosemary, chamomile,lavender, cinnamon	Liquid–liquid continuous extraction following steam distillation under reduced pressure
Rosemary	CO2 supercritical fluid extraction
Chilly, black pepper, ginger	Near-critical carbon dioxide, propane, and dimethylether
Chilly, black pepper, turmeric	Aqueous extract, homogenization, centrifugation, filtration
Cinnamon	Fat-free cinnamon Soxhlet extraction with 80% methanol, then n-hexane and ethyl acetate

Table 2. Antioxidant active chemicals isolated from some of the most common and used spices (USDA, 2003)

Black pepper (*Piper nigrum*)	Ascorbic-acid fruit 0–10, beta-carotene fruit 0.114–0.128, camphene fruit, carvacrol fruit, eugenol fruit, gamma terpinene fruit, lauric-acid fruit 400–447, linalyl-acetate fruit, methyl-eugenol fruit, myrcene fruit, myristic-acid fruit 700–782, myristicin fruit, palmitic-acid fruit 12,200–13,633, piperine fruit 17,000–90,000, terpinen-4-ol fruit, ubiquinone fruit.
Caraway (*Carum carvi*)	Beta-carotene fruit 0–2, camphene fruit 1–900, camphene plant 0–30, carvacrol plant, gamma-terpinene fruit 12–14,160, gamma-terpinene plant 0–270, lauric-acid fruit 100–110, myrcene fruit 180–1560, myrcene plant 0–150, myristic-acid fruit 400–444, myristicin fruit 60–420, myristicin plant 0–120, palmitic-acid fruit 2000–6658, quercetin fruit, tannin plant, terpinen-4-ol plant 0–120.
Chilli pepper (*Capsicum frutescens*)	Alanine fruit 820–6691, ascorbic-acid fruit 350–19,992, beta-carotene fruit 1–38, caffeic-acid fruit 0–32, campesterol fruit, capsaicin fruit 100–17,900, capsanthin fruit, chlorogenic-acid stem, hesperidin fruit, histidine fruit 410–3346, kaempferol anther, lauric-acid resin, exudate, sap, lutein fruit, methionine fruit 240–1958, myrcene fruit, myristic-acid fruit 10–82, myristic-acid seed, p-coumaric-acid fruit 0–540, palmitic-acid fruit 150–1224, palmitic-acid seed, pentadecanoic-acid fruit, quercetin fruit 0–63, scopoletin fruit, stigmasterol fruit, terpinen-4-ol fruit, tocopherol fruit 0–24, tryptophan fruit 260–2122.
Coriander (*Coriandrum sativum L*.)	Apigenin fruit, ascorbic-acid leaf 780–6290, beta-carotene leaf 29–228, beta-carotene seed, beta-sitosterol fruit, caffeic-acid fruit, caffeic-acid leaf, camphene fruit 2–155, chlorogenic-acid plant 305–320, gamma-terpinene fruit 762–2626, isoquercitrin fruit, myrcene fruit 13–169, myristic-acid fruit 200–219, myristicin fruit, p-hydroxybenzoic- acid fruit 0–960, p-hydroxy-benzoic-acid plant 252–333, palmitic-acid fruit 5000–16,800, protocatechuicacid fruit 0–760, protocatechuic-acid plant 167–179, quercetin fruit, rhamnetin fruit, rutin fruit, scopoletin fruit, tannin fruit, terpinen-4-ol fruit 6–80, trans-anethole fruit 1–2, vanillic-acid fruit 0–960, vanillic-acid plant 221–347.
Dill (*Anethum graveolens*)	Alpha-tocopherol leaf 16–147, anethole plant, ascorbic-acid plant 0–1440, beta-sitosterol plant, caffeic-acid fruit, camphene plant, carvacrol plant, chlorogenic-acid fruit, eugenol plant, ferulic-acid fruit, gamma-terpinene fruit 12–77, histidine fruit 3200–3466, isoeugenol plant, isorhamnetin plant, kaempferol fruit, lauric-acid fruit 100–108, methionine fruit 1430–1549, myrcene fruit 84–924, myristic-acid fruit 100–108, myristicin fruit, myristicin root, palmitic-acid fruit 5800–6281, quercetin plant, scopoletin fruit, selenium plant 0.001–0.012, stigmasterol plant, terpinen-4-ol fruit 12–77, trans-anethole fruit 12–539, vicenin fruit.
Ginger (*Zingiber*	6-Gingerol rhizome 130–7138, 6-shogaol rhizome 40–330, alanine rhizome 310–1793, ascorbic-acid rhizome 0–317, beta-carotene rhizome 0–4, beta-sitosterol plant, caffeic-acid rhizome, camphene rhizome 28–6300, capsaicin plant, chlorogenic-acid plant, curcumin plant, delphinidin plant, ferulic-acid plant, gamma-terpinene rhizome 0.4–25, rhizome 300–1738, kaempferol plant, lauric-acid rhizome 390–3630, methionine rhizome 130–737, myrcene 2–950, myricetin plant, myristic-acid rhizome 180–1650, p-coumaric-acid rhizome 0–19, p-hydroxy-benzoic-acid plant, palmitic-acid rhizome 1200–11,220, quercetin plant, selenium rhizome 10, shikimic-acid leaf, sucrose rhizome, terpinen-4-ol rhizome, tryptophan rhizome 120–693, vanillic-acid plant, vanillin plant.
	Ascorbic-acid plant 514–555, beta-carotene plant 48–52, beta-sitosterol plant, caffeic-acid plant, carvacrol plant 1092–6261, eugenol plant 200–1152, hydroquinone plant, linalyl-acetate plant 3–17, myrcene plant

officinale)	18–103, oleanolic-acid plant 0–4700, phenol plant 1431–8204, rosmarinic-acid plant 0–33,000, tannin plant, terpinen-4-ol plant 1365–7826, trans-anethole plant 8–43, ursolic-acid plant 500–2100.
Marjoram (*Origanum majorana*)	Camphene seed 80–640, cyanidin plant, eugenol seed 40–320, gamma-terpinene seed 580–4640, isoeugenol seed 140–320, kaempferol plant, lauric-acid seed 375–1600, methyl-eugenol seed 20–900, myrcene seed 740–5920, myristic-acid seed 60–304,000, myristicin leaf 410–620, myristicin seed 800–12,800, oleanolic-acid seed, palmiticacid seed 25,000–128,000, quercetin plant, terpinen-4-ol seed 600–4800. Camphene plant 0–1, carvacrol plant 0–12, gamma-terpinene plant 0–13, linalyl-acetate plant 0–50, myrcene plant 0–5, terpinen-4-ol plant 0–220, thymol plant.
Nutmeg (*Myristica fragrans*)	Alanine fruit 350–4774, alpha-tocopherol fruit 22–284, ascorbic-acid fruit 230–20,982, beta-carotene fruit 0–462, beta-sitosterol plant, caffeic-acid fruit 0–11, campesterol fruit, camphene fruit, capsaicin fruit 100–4000, capsanthin fruit, chlorogenic-acid fruit, eugenol fruit, gamma-terpinene fruit, hesperidin fruit, histidine fruit 170–2319, lupeol seed, lutein fruit, methionine fruit 100–1364, myrcene fruit, myristic-acid fruit 10–136, pcoumaric- acid fruit 0–79, palmitic-acid fruit 500–6820, palmitic-acid seed, pentadecanoic-acid fruit, scopoletin fruit, selenium fruit 0.001–0.002, stigmasterol fruit, terpinen-4-ol fruit, tocopherol fruit 0–24, tryptophan fruit 110–1500.
Oregano (*Origanum Vulgare*)	Apigenin plant, ascorbic-acid plant 612–673, beta-carotene plant 19–21, beta-sitosterol plant, caffeic-acid plant, camphene leaf 0–23, camphene leaf 0–145, camphene plant 23–2350, camphene shoot 355–1435, camphene shoot 620–1260, camphene shoot 1035–2280, carnosic-acid plant, carnosol leaf 530–9803, carvacrol leaf 0–5, carvacrol leaf 0–6, carvacrol leaf 5–6, carvacrol plant, chlorogenic-acid plant, gamma-terpinene leaf 0–4, gamma-terpinene plant 4–400, gamma-terpinene shoot 25–50, gamma-terpinene shoot 37–225, gamma-terpinene shoot 105–300, hesperidin leaf, hispidulin plant, isorosmanol flower 0–17, labiatic-acid plant, luteolin leaf, luteolin plant.
Red (sweet) pepper (*Capsicum annuum*)	4-Terpineol plant 73–8320, alanine plant, anethole essential oil, apigenin plant, ascorbic-acid leaf, beta-carotene plant 24–25, caffeic-acid leaf 0–16,900, camphene plant 15–375, carvacrol plant 8–18,720, chlorogenic-acid plant, chrysoeriol plant, eriodictyol plant, eugenol plant, ferulic-acid plant, gallic-acid plant, gamma-terpinene plant 36–5460, isochlorogenic-acid leaf, isoeugenol plant, isothymonin plant, kaempferol plant, labiatic-acid leaf, lauric acid.
Rosemary (*Rosemarinus officinalis*)	plant 2300–2484, linalyl-acetate plant 15–4680, luteolin plant, methionine plant 1370–1980, myrcene plant 36–676, myristic-acid plant 1500–1620, naringenin plant, oleanolic-acid plant 0–6300 p-coumaric-acid leaf 0–420, p-hydroxy-benzoic-acid plant, palmitic-acid plant 17,200–18,576, rosmarinic-acid plant 0–26,000, rosmarinic-acid shoot 5000–13,500, selenium leaf 0–16, tannin plant 80,000–100,000, thymol plant 15–24,100, tryptophan plant 1860–2009, ursolic-acid plant 15,000–18,800, vanillic-acid plant.
Turmeric (*Curcuma domestica*)	Ascorbic-acid rhizome 0–293, beta-carotene rhizome, caffeic-acid rhizome 0–5, curcumin rhizome 9–38,888, eugenol essential oil 0–2100, p-coumaric-acid rhizome 0–345, protocatechuic-acid leaf, syringic-acid leaf, vanillicacid leaf.

CONCLUSION

This review discussed medicinally significant plant species from around the world and showed that many herbs have high antioxidant activity when compared to synthetic antioxidants. In addition, many of these species have a high phenolic content and a large amount of flavonoids and flavonols. However, an overall ranking of the antioxidant strength of these species cannot be determined because of the different experimental methods used in various studies.

In view of the diversity of methods used for spices and herb antioxidant isolation and their activity determination, there is a great need to standardize them for both these measurements. Modern consumers ask for natural products, free of synthetic additives. Therefore, the application of natural antioxidants will probably continue even the future, and it will be necessary to study their changes and interactions in more details. Scientists will look for new effective herbal sources with potent antioxidants. All these plants extract, their mixtures, isolates and concentrates with antioxidant effects have to meet all the requirements of human health safety.

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Nil

CONFLICT OF INTEREST

No Interest

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