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Antioxidant and *Conyza bonariensis*: A Review

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Abstract:

In the last few years, plant products and their modified derivatives have been rich sources for clinically useful drugs. According to the World Health Organization (WHO, 2008), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. Medicinal plants contain high levels of antioxidants that can delay or inhibit the oxidation of lipids or other molecules. Natural antioxidants are compounds from plant or animal sources. Phenolic phytochemicals are a large group of substances and found in significant quantities in vegetables, fruits, spices, and seeds. Since they have been regarded as possible antioxidants, their roles in food industry and in chemoprevention of diseases have become an area of active research in many fields. Phenolic compounds are one of the most diverse groups of secondary metabolites found in edible plants. The genus Conyza including: highest activity (antioxidants), alkaloids, volatile oils, terpenoids, phenolic acids, flavonoids and hydrolysable tannins. In addition to C. bonariensis has been used for three centuries as a pungent tonic, astringent to control bleeding and as a diuretic, it was approved in medicine as a hemostatic and possibly also as an

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anthelminthic and claimed to be efficient in diarrhea, diabetes, hemorrhages of the bowels, uterus.

Key words: Antioxidant, Conyza bonariensis, Phenolics Compounds, (DPPH) radical scavenging, ß-Carotene bleaching assay.

Introduction

Medicinal plants or parts of these plants (leaves, roots, seeds, flowers) can be utilized in different forms such as fresh crude form and preparations as teas, decoctions, powdered plant material, or extracted forms of medicinal agents (juices, water or alcohol extracts, tinctures, essential oils, resins, balsams). Medicinal plants are generally known and popular for a number of health benefits such as decreasing of blood pressure, prevention of cardiovascular diseases, or reducing the risk of cancer also due to their antioxidant activity. Medicinal plants contain high levels of antioxidants that can delay or inhibit the oxidation of lipids or other molecules. Many lipid-oxidation products are known to interact with biological materials to cause cellular damage, so oxidation process has been associated with chronic diseases such as cancer (Soňa et.al .2012).

Most of the beneficial properties of fruits, vegetables and whole grains have been attributed to bioactive non-nutritional chemical compounds commonly named phytochemicals. Whole foods have been estimated to have between 5,000 and 25,000 individual phytochemicals. Among these, phenolic compounds have been extensively studied due to their diverse health benefits as antioxidants, and for preventing chronic inflammation, cardiovascular diseases, cancer and diabetes.

The genus Conyza belongs to family *Asteraceae* which comprises of about fifty species, which are mainly found in tropical and subtropical regions. Some species of this genus are traditionally used for a variety of pharmacological applications including treatment of smallpox, chickenpox, sore throat, ringworm and other skin related diseases, toothache and to stop bleeding from injuries (Shinwari & Khan, 2000). *Conyza canadensis* is one of the specie belongs to genus conyza, family asteraceae. The plant is used as antirheumatism, antidiarrhoeal and as antihaemorrhoidal (Shahkirullah et al., 2011).

Numerous epidemiological studies have shown an inverse relationship between the intake of natural antioxidants from plant products and the incidence of some diseases because dietary plant antioxidants are capable of removing free radicals. Also the many constituents of plants may contribute to their antioxidant and other protective properties. The amounts of antioxidant compounds vary between different species, plant variety, environmental conditions, climatic differences, seasonal variations (Yesil-Celiktas et al., 2007), degree of ripeness, growing practices, geographical regions of growth (Yesil-Celiktas et al., 2007), and many other factors such as postharvest treatment and processing.

Conyza bonariensis

Taxonomic Tree

- Domain: Eukaryota
  - Kingdom: Plantae
    - Phylum: Spermatophyta
      - Subphylum: Angiospermae
        - Class: Dicotyledonae
          - Order: Asterales
            - Family: Asteraceae
              - Genus: Conyza
                - Species: Conyza bonariensis

Family Asteraceae (Compositeae, Sunflower or Aster family) is the largest family of flowering plants; it comprises about 23600 species distributed in 1620 genera and 12 subfamilies (Jeffrey, 2007). Compositous plants are distributed among the tropics and warm temperate regions of South, South- East and East Asia and Africa; some being cultivated as vegetable or food while others grow wild (Panero and Crozier, 2008). The genus Conyza composed of 50 species which are found on the tropical Himalaya from Nepal to Sikhim, extending to Assam, Khasia hills, Chittagong and Burma.

Conyza bonariensis (L.) Cronquist is an annual or short-lived perennial weed of the Asteraceae family and it known as sadaf in Yemen, and in Yemen found on the Hajjah, Dhamar, W. Dhahr (Al Khulaidi, 2000).

Figure 1. *Conyza bonariensis* plant

The most common Conyza species are Conyza dioscoridis (L.) Desf. and *Conyza bonariensis (L.)* Cronquist; these are mainly localized in more cities. Secondary metabolites belonging to different phytochemical groups have been reported from members of genus Conyza including: alkaloids, volatile oils, terpenoids, phenolic acids, flavonoids and hydrolysable tannins (Shahwar et al., 2012). The plant was found to exert a molluscicidal activity against Biomphalaria snails (Bakry, 2009); as well as to exhibit anti-inflammatory, antinociceptive (Atta and Abo Sooud, 2004) and antipyretic effects (Awaad et al., 2011). Also the growth inhibitory activity of this plant against a series of selected microbial strains has also been reported (Zain et al., 2012). Likewise, *C. bonariensis (L.)* is widely used as a folk medicine in treatment of rheumatism, gout, cystitis, nephritis, dysmenorrhea, tooth pain and headache; it was also reported to have an antiulcerogenic and anticoagulant activity (Favila and Antonio, 2006); in addition, the antioxidant potential of its extracts has been assessed (Shahwar et al., 2012).

The genus Conyza is found to be very rich in terpenoids such as celarodanes (Zdero et al., 1990), sesquiterpenes (Bohlmann and Wagner, 1982) and diterpenes (Ahmad et al., 1992; Mata et al., 1997). Extract of *Conyza bonariensis* has three glycosides, in addition to nine known compounds including amyrin, b-sitostero1 daucosterol, syringic acid 3-hydroxy-5-methoxybenzoic acid, eugenol 4-Oglucopyranoside, and luteolin, apigenin and takakin 8-O-glucuronide.(Konga. et al., 2001). The oil composition is dominated by the presence of sesquiterpene accounting for 51.14% of the total oil composition. Figure 2 . show the principal components of the essential oil were Aromadendrene oxide - (2) (17.38%), Cedren - 13- ol, 8 (17.30%), Caryophyllone oxide (7.23%), alpha – Bisabolol (2.32%), Isoaromadendrene epoxide (1.69%), Globulol (1.49%), Thymol (2.86%), Tetracyclo (6.3.2.0(2.5).0(1.8)tridecan-9-ol,4,4-dimethyl (8.41%), 1 - Naphthalenol , decahydro-1,4a - dimethyl-7-)1 - methylethylidene) - (1R-(1. alpha., 4abeta, 8a. Alpha) (7.20%), Butanoic acid, 3,7- dimethyl - 2,6-octadianyl ester (5.87%), n- hexadecanoic acid (4.27%), 4-Penten-2-0l (3.76%), phenol , 4 - methoxy - 2,3,6 – trimethyl (3.02%). (Riyadh. et al., 2014)

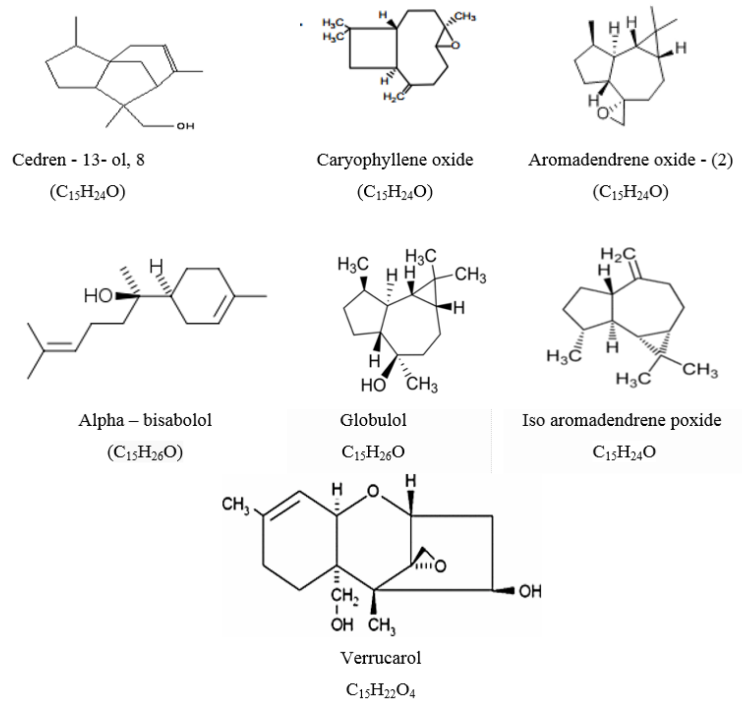


Fig. 2. Chemical structures of sesquiterpene (common compounds) in the essential oil of *C. bonariensis*

(Tian- et al., 2004)

Phenolics Compounds

Phenolic compounds are a key source of antioxidant activity in fruits. Flavonoids, the fraction of phenolics comprised of such compounds as flavones, isoflavones, flavonones and anthocyanins are known to be potent antioxidants in vitro (Moyer, et al., 2002).

Polyphenols are able to act as reducing agents, hydrogen donating antioxidants, as well as singlet oxygen quenchers (Dubost, et al., 2007). Phenolic compounds are known to terminate oxidation by participating in the reactions through resonance stabilized free radical forms, as well as acting as free radical scavengers (Lindsay, 1996). As suggested by Dubost et al. (2007), hydrogen donation may be a key mechanism of action for the antioxidant activity of phenolic compounds.

Polyphenols compounds are an essential part of the defense mechanisms in plants. These compounds protect plants against the attack of environmental stresses such as ultraviolet light, microorganisms and insects.

Simple phenolic acids and flavonoids are the most common phenolic compounds and they generally occur as soluble conjugated (glycosides) and insoluble forms (Nardini & Ghiselli, 2004). In nature, phenolic acids occur mostly in the insoluble or bound forms whereas flavonoids present as glycosides with a single or multiple sugar moieties linked through an OH group (O-glycosides) or through carbon–carbon bonds (C-glycosides).

Polyphenols are extensively studied and around 8000 are characterized although it is possible that over one million molecules possessing protective functions may occur naturally in food plants (Sakakibara et al., 2003; Halvorsen et al, 2006).

In the early 1980, an accurate procedure for the estimation of free, soluble conjugated and insoluble bound phenolics was developed and proved in different foods (Sosulski, & Hogge, 1982). the insoluble bound phenolics have demonstrated a significantly higher antioxidant capacity compared to free and soluble conjugated phenolics (Chandrasekara & Shahidi, 2010).

Nutritionally important bioactive compounds can be divided into more than ten different classes including: phenolic acids, benzoquinones, hydroxycinnamic acids, phenylpropenes, coumarins, chromones, naphthoquinones, xanthones, stilbenes, flavonoids, and lignans (Bravo 1998). In fruits, polyphenols are commonly observed as flavonoids, phenolic acids, and tannins (Figure 3).

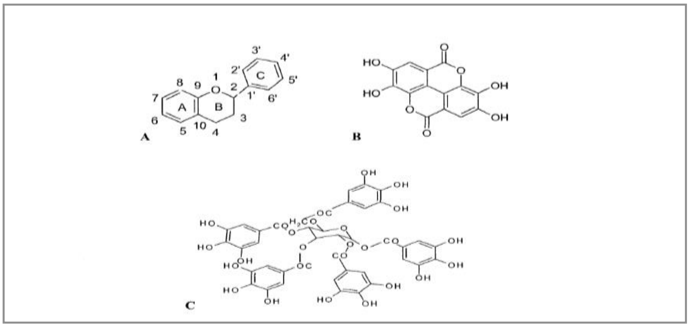


Figure 3 . Common polyphenols in fruits. (A) generic structure of the flavonoid skeletion, (B) Phenolic acid(ellagic acid), (C) tannins (tannic acid)

Flavonoids

Flavonoids are the most abundant polyphenols in our diets. The basic flavonoid structure is the flavan nucleus, containing 15 carbon atoms arranged in three rings (C6–C3–C6).

Flavonoids are themselves divided into six subgroups: flavones, flavonols, flavanols, flavanones, isoflavones, and anthocyanins, according to the degree of oxidation (oxidation state) of the oxygen heterocycle, central third ring. Their structural variation in each subgroup is partly due to the degree and pattern of hydroxylation, methoxylation, or glycosylation (Dai & Mumper, 2010).

Flavonoids are the most abundant phenolic compounds in fruits and vegetables with more than 5000 compounds identified to date (Crespy et al., 2003).

The fiavonoids, widely distributed compounds, are classified into 13 subclasses: chalcones, dihydrochalcones, aurones, flavones, flavonols, dihydroflavonol, flavanones, flavanols, flavandiols, anthocyanidins, isoflavonoids, biflavonoids, and proanthocyanidins (Sakakibara et al., 2003). Fiavonoids have been associated with many physiological properties, including antioxidant, anti-inflammatory, antimicrobial, anti-hyperlipidemic, anticancer, anti-viral, and anti-allergenic, all of which are thought to play a role in reducing the risk of degenerative diseases.

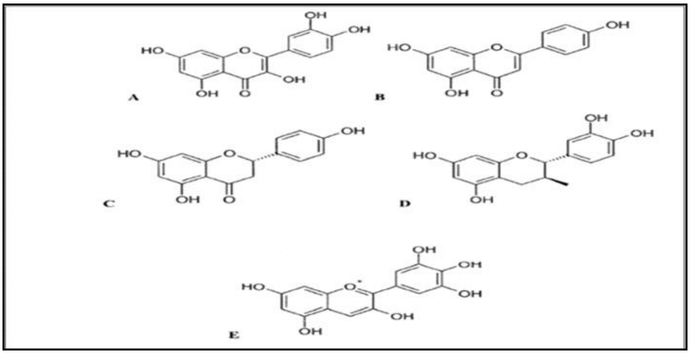


Figure 4. The Most Abundant Flavonoids in Fruits. (A) querectin(flavonol), (B) apigenin (flavone), (C) naringenin (flvanone), (D) (+)- catachin (flavan – 3 – ol), (E) cyanidin (anthocyandins).

Some of the most common flavonoids in medicinal plants include luteolin (flavone), apigenin (flavone), hispidulin (flavone), quercetin (flavonol), and kaempferol (flavonol). Also the most abundant flavonoids in fruits are the flavonols (quercetin, kaempferol and myricetin), flavones (apigenin and luteolin), flavanones (naringin, naringenin, hesperetin, hesperidin), flavan-3-ols (i.e. catechin, catechin gallate, proanthocyanidins), and anthocyanidins (i.e. cyanidin, dephinidin, pelargonidin, and glucosides anthocyanins) (Figure 4.) (Scalbert et al., 2005). Flavonols are the most widely distributed of the flavonoid compounds in fruits and are mainly in the glycosidic form with a hydroxyl group conjugated most commonly at position 3 of the Cring and with possible substitutions at 5, 7, 4', 3' and 5' positions (Figure 1-4A) (Kris-Etherton et al., 2002; Nichenametla et al., 2006). Quercetin, kaempferol and myricetin, the most common flavonols, are quantified in apples, berries, plums, tomatoes, peaches and grapefruit (Harnly et al., 2006).

Tannins

Tannins are polyphenols with high molecular weight which can bind to both proteins and carbohydrates. Tannins are one of the most wide spread polyphenolics in fruits, and are divided in two main classes: hydrolysable tannins and condensed tannins or proanthocyanidins (Singh et al., 2003). Condensed tannins are high molecular weight compounds formed from monomeric units of flavanols or flavan-3-ol, including (+)-catechin, (-)-epicatehin, (+)-gallocatechin, and (-)-epigallocatechin. Oxidative condensation occurs between the heterocyclic carbon C-4 of monomeric units of flavanols and the adjacent positions of carbons C-6 or C-8 of flavanols to create oligomers and polymers proanthocyanidins (Crozier et al., 2006). Condensed tannins are responsible for the astringency of many tannin-rich foods such as red wine and tea, resulting mainly from the precipitation of tannins with salivary proteins. Tannins exist in a wide variety of plant species. In a survey by Bate-Smith and Metcalf (1957), approximately 80% of woody perennial dicots and 15% of annual and herbaceous perennial dicots contained tannins. Considering this information, further studies concerning tannins and their effect on grazing ungulates are necessary.

Anthocyanin

Anthocyanins are responsible for the red and blue coloration of certain fruits, flowers and leaves (McDougall et al., 2005) and are present in high concentrations in blueberries, raspberries, strawberries and red grapes. The popularity of anthocyanin containing foods is increasing tremendously due to recent interest of multiple health promoting features including antioxidant, anti-inflammatory, and anti-cancer activities and more recently due to chemoprotective, vasoprotective and antineoplastic properties (Bae Song-Hwan 2007).

Anthocyanins are commonly identified as an anthocyamdin-sugar conjugate in fruits. Forms include conjugates with hydroxycinnamate and other organic acids, mostly malic and acetic acids such as in red wines (Crozier et al., 2006). Six different anthocyanidins aglycones, including pelargonidin, cyanidin, delphinidin, peonidin, petunidin, and malvidin are the most common constituents (Figure 5). Anthocyanidins are usually conjugated at carbons 3, 5, and 7 of C-ring and, 3'and 5' of B-ring with sugar conjugated at positions 3 and/or 5 of C-ring.

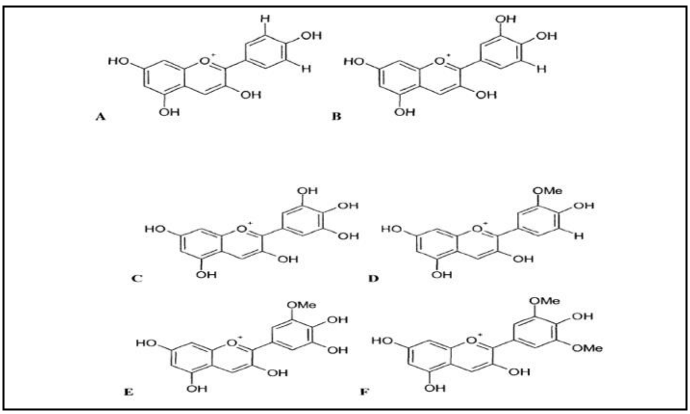


Figure 5 . Anthocyanidins in fruit, (A) pelargonidin, (B) cyaniding, (C) delphinidin , (D) peonidin, (E) petunidin, (F) malvidin. Me,methyl.

Antioxidants activity

Antioxidants are of growing interest in recent years. More and more research is focusing on natural food antioxidants as the public is becoming aware of the importance antioxidants play in a healthy diet.

Historically, antioxidants have been broadly described as "all substances that inhibited oxidation reactions regardless of the mechanism, and narrowly as "those compounds that interrupt the free-radical chain reaction involved in lipid oxidation and those that scavenge singlet oxygen (Lindsay, 1996). It is important to note that "an antioxidant is a reductant, but a reductant is not necessarily and antioxidant (Prior & Cao, 1999). There should, however, be a distinction between the chemical terms of reductant and oxidant as compared to the biological terms of antioxidant and pro-oxidant (Prior & Cao, 1999). The biological term antioxidant refers to any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1995). Reactive species known as pro-oxidants can be defined as a toxic substance that can cause oxidative damage to lipids, proteins and nucleic acids, resulting in various pathologic events and/or diseases (Prior & Cao, 1999). These definitions emphasize that the value of an antioxidant is in its ability to inhibit the free radical chain reaction. This is essential for stability in food systems and for health promotion in vivo.

As foods are extremely complex systems it is very difficult to determine the impact of each antioxidant compound individually. So there are many assays available to measure the antioxidant capacity of a food system. For example used of these is the (DPPH) 2,2-diphenyl-1- picrylhydrazyl and ß-Carotene bleaching assay (BCB).

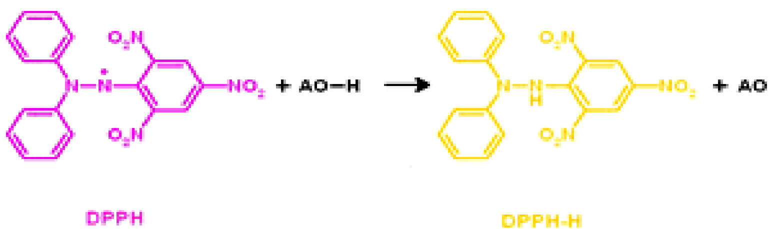
Antioxidant activity using (DPPH) radical scavenging.

The principle of this assay is based on the measurement of scavenging ability of the antioxidants towards the stable radical. The free radical DPPH is reduced to the corresponding hydrazine, when it reacts with hydrogen donors, this stability is evaluated by decolorizing assay which evaluates the decrease in absorbance at 517 nm produced by the addition of antioxidant to DPPH solution in ethanol. The scavenging reaction between (DPPH.) and an antioxidant (H-A) can be written as:

DPPH) + (H-A)) ⟶ DPPH-H + (A)

(Purple) ⟶ (Yellow)

The color turns from purple to yellow as the molar absorptivity of the DPPH radical at 517 nm. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability Reddy et al. (2012). Antioxidants react with DPPH, which is a stable free radical and is reduced to the DPPH H and as consequence the absorbance's decreased from the DPPH radical to the DPPH-H form.



2,2'-diphenyl-1-picrylhydrazyl 2,2'-diphenyl-1-picrylhydrazine

Fig. 6 . Anti-oxidant interaction (DPPH)

Antioxidant activity using β-Carotene bleaching assay.

The antioxidant activity has also been assessed as ability to prevent from oxidation. This method usually used to evaluate the antioxidant activity of compounds in emulsions, accompanied with the coupled oxidation of b-carotene and linoleic acid.In the BCB assay, the oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of a hydrogen atom from diallylic methylene groups of linoleic acid Kumaran and Karunakaran,(2006).

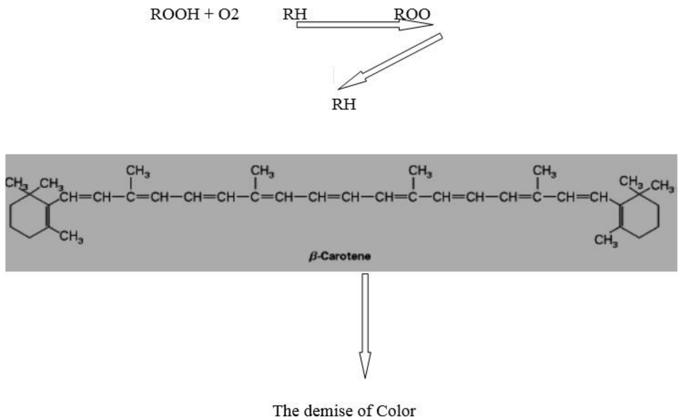


Fig. 7 . Anti-oxidant interaction(BCB)

Mechanism of antioxidants

In food system, the most effective antioxidants function by interrupting the free radical chain mechanism. Antioxidants have been broadly described as “all substances that inhibited oxidation reactions regardless of the mechanism,” and narrowly as “those compounds that interrupt the free-radical chain reaction involved in food oxidation and those that scavenge singlet oxygen (Lindsay, 1996). The biological term antioxidant refers to “any substance that, when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1995).

In order to fully understand the mechanism of action of antioxidants, one must understand the mechanism of chemical oxidation. Oxidation, in the broadest sense, is simply the removal of an electron from an atom or molecule. This is paired with a reduction reaction in which the electron or electrons involved are added to another atom or molecule. In biological, and food systems, ,oxidation reactions can generate a reactive species and initiate a free radical chain reaction. An example of this is illustrated

Initiation :

$$R_2RN_2R \rightarrow 2R\bullet P + N_2$$
$$R\bullet P + OR2R \rightarrow ROOP\bullet$$
$$ROOP\bullet P + LH \rightarrow ROOH + LP\bullet$$

Propagation

$$L\bullet P \quad P + OR2R \rightarrow LOOP\bullet$$
$$LOOP\bullet P + LH \rightarrow LOOH + LP\bullet$$

Inhibition

$$LOO\bullet P \quad P + AH \rightarrow LOOH + A\bullet P$$

Termination

$$AP\bullet P + (n-1)LOOP\bullet P \rightarrow \text{nonradical products}$$
$$LOOP\bullet P + LOOP\bullet P \rightarrow \text{nonradical products}$$

(Huang, et al., 2005)

Antibacterial activity

There is still increasing interest in antimicrobial effects of medicinal plants and their products due to remedies for human diseases and the food microbial safety to reduce the occurrence of microbial (bacteria, yeast, fungi) contamination in foods caused by undesirable pathogenic microorganisms such as Listeria monocytogenes, Escherichia coli, Salmonella typhimurium, Bacillus cereus, and Staphylococcus aureus. Antimicrobially acting compounds and generally could minimize pathogens and toxins produced by microorganisms. In third world countries, including Yemen, where contagious diseases are common, it is important to search out and promote plant-derived medicines. These medicines plants can destroy microbes that cause certain contagious diseases and should be used in conjunction with modern medicines and antibiotics. It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug-resistant microbial pathogens. However, very little information is available on such activity of medicinal plants (Hasegawa et al., 1995; Lee et al., 1998).

Antioxidants and Health

Medicinal plants and their products are used worldwide for thousands of years due to their health effects (anti-inflammatory, antioxidant, antibacterial, digestive, antispasmodic, cholagogue, carminative, diuretic, hypolipidemic, sedative, enhancing the function of the immune system as well as anticancer, antitumor activity, etc.) and a key role in preventing various diseases such as cardiovascular diseases, gastrointestinal disorders, inflammatory diseases, and cancer initiation. According to WHO, as many as 80% of the world's people rely on traditional medicine for their primary health-care needs (WHO, IUCN, & WWF, 1993).

In addition to protecting against quality degradation in food systems, antioxidants can have positive effects on overall human health. The addition of antioxidants into foods can help boost overall nutritive values with often positive effects on sensory quality (Skribic and Filipcev, 2008). In epidemiological studies it has been shown that consumption levels of fruits and vegetables rich in antioxidants and polyphenols is inversely related to a persons risk of having a stroke, death from a stroke, incidence of cardiovascular disease, cardiovascular disease mortality and overall mortality (Bazzano et al., 2002). The health benefits of plant-based antioxidants extend even further.

Common medicinal plants are used in folk medicine because many of them are proved as effective remedies against certain ailments. Whereas they are generally considered to be safe and contain biologically active constituents that have beneficial physiologic effects, some plants are safe in modest amounts. (Craig, 1999).

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