ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF ALOE VERA

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ABSTRAK

AKTIVITI-AKTIVITI ANTIBAKTERIA DAN ANTIOKSIDAN ALOE VERA

Objektif am bagi kajian ini adalah mengaji potensi aktiviti-aktiviti antibakteria dan antioksidan tentang ekstrak aloe vera. Fenolik, flavonoid dan antrakuinon diekstrak dari kedua-dua daun Aloe yuenjiangensis dalam bentuk segar dan kering, masing-masing. Ekstrak fenolik dari daun dalam bentuk segar and bentuk kering tidak menunjukkan sebarang aktiviti antibakteria, manakala ekstraks flavonoid dari daun segar dan antrakuinon dari kedua-dua daun segar and kering menunjukkan aktiviti antibakteria terhadap patogen makanan serta bakteria perosak makanan melalui kaedah penyebaran disk. Kepekatan penyekatan minimum ekstrak ditentukan dengan kaedah dalam kalang dari 3.90 ke 41.1 mg/ml manakala kepekatan pembasmian minimum ekstrak dikalang dari 3.90 ke 50.1mg/ml. Kandungan jumlah fenolik setiap ekstrak dikira dengan reagen Folin-Ciocalteau. Aktiviti antioksidan ekstraks Aloe yuenjiangensis dinilai dengan ujian pembangkaian radikal 2,2-diphenyl-1-picrylhydrazyl (DPPH), kesan chelating ion ferum dan sistem asid linoleik beta karotin. Kandungan jumlah fenolik berbeza di antara daun mentah dan daun kering, dan sama juga dengan kaedah pengekstrakan yang berlainan. Ekstraks anthraquinone daun kering menunjukkan signifikan yang lebih terhadap kedua-dua jenis radikal larut air dan lemak dalam ujian pembangkaian radikal DPPH dan sistem asid linoleik beta karotin, di mana dirujuk kepada kandungan jumlah fenolik yang terdapat dalam ekstraks. Kesan chelating ion ferum menunjukkan perbezaan signifikan dalam ekstraks fenolik kering ($p<0.05$). Dalam kesimpulan, keputusan menunjukkan ektraks Aloe yuenjiangensis mempunyai kapasiti untuk membangkai radikal bebas dan merencat pertumbuhan kedua-dua jenis bakteria patogen dan perosak makanan. Namun, ektraks ini adalah sesuai untuk digunakan sebagai agen antimikrobia dan antioksidan semulajadi yang berasal dari tumbuhan dalam industri makanan.

ABSTRACT

ANTIBACTERIAL AND ANTIOXIDANT ACTIVITY OF ALOE VERA

The main objective of the study was to examine the potential of antibacterial and antioxidant activities of aloe vera extracts. Phenolic, flavonoid and anthraquinone extracted from fresh and dried leaves of *Aloe yuenjiangensis*, respectively. Phenolic extracts from fresh and dry sample show no antibacterial activity, whereas flavonoid extract from fresh form of sample and anthraquinone extracts from fresh and dry form of sample against *Staphylococcus aureus* S277, *Escherichia coli* IMR E91/02C, *Pseudomonas aeruginosa* ATCC 10145 and *Bacillus cereus* exhibited antimicrobial activity through disk diffusion assay. The minimum inhibitory concentrations (MICs) of extracts determined by the broth dilution method ranged from 3.90 to 41.1 mg/ml while the minimum bacteriacidal concentrations (MBCs) of extracts ranged from 3.90 to 50.1mg/ml. Total phenolic content of each extract was quantified with the Folin–Ciocalteau reagent. Antioxidant activities of *Aloe yuenjiangensis* extracts were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, ferrious ion chelating effect and β-carotene linoleic acid system. Total phenolic content varied between fresh and dried leaves, as well as different extraction methods. Dried anthraquinone extracts exhibited significantly ($p<0.05$) greater DPPH radical scavenging assay and beta carotene linoleic acid system towards both lipid- and water-soluble radicals, which was attributed to the total phenolic content. Ferrous ion-chelating effect was significantly ($p<0.05$) greater in the dried phenolic extracts. In conclusion, the results indicate that the extracts of *Aloe yuenjiangensis* have the capacity to scavenge free radicals and to inhibit the growth of both spoilage and pathogenic bacteria. Therefore they could be suitable for using as natural plant-derived antimicrobial and antioxidant agents in the food industry.

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LIST OF ABBREVIATION

AAPH	2,2′-azobis- (2-amidinopropane) dihydrochloride
ABTS	2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic
aw	water activity
BHA	butylated hydroxyanisole
BHT	butylhydroxytoluene
CFU	Colony form unit
DPPH	1,1-Diphenyl-2-picrylhydrazyl or 2,2-Diphenyl-1-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
EPR	electron paramagnetic resonance
ET	electron transfer
Fe^{2+}	ferric ion (II)
$FeCl_2 \cdot 4H_2O$	ferric chloride hydrated
FRAP	ferric ion reducing antioxidant parameter
GC	Gas chromatography
GRAS	Generally recognized as safe
HAT	hydrogen atom transfer
HPLC	high performance liquid chromatography
IASC	Institute Aloe Science Council
IMR	Institute of Medical Research
LDL	low density lipoprotein
MIC	Minimal inhibitory concentrations
ORAC	Oxygen radical absorbance capacity
SPSS	Statistical Packages for Social Science
TBHQ	*tert*-butylhydroquinone
TEAC	Trolox equivalent antioxidant capacity

TSA	tryptic soy agar
TSB	tryptic soy broth
WHO	World Health Organization

LIST OF SYMBOL

%	percent
α	alpha
β	beta
°C	degree Celsius
kg	kilogram
g	gram
mg	microgram
µg	microgram
ml	milliliter
µL	microliter
mM	milimolarity
$A_{control}$	Absorbance of control
A_{test}	absorbance of test
t	time
±	positive and negative
>	more than
<	less than

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CHAPTER 1

INTRODUCTION

The spoilage and poisoning of foods by microorganisms is a problem that has not yet been brought under adequate control despite the range of roust preservation techniques available (Rauha *et al.*, 2000). Foodborne diseases are still a major problem in the world, even in well-developed countries. A variety of microorganisms also lead food spoilage that is encountered as one of the most important matter concerning food industry. So far, many pathogenic microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, or non pathogenic microorganisms such as *Pseudomonas* spp. and *Lactobacillus* spp. have been reported as the causal agents of foodborne diseases and/or food spoilage (Sokmen *et al.*, 2004). Besides microbial spoilage and contamination, chemical spoilage is also another problem occur in food industry. For an example, rancidity rapidly appears in high fat foods such as bakery products and edible oils. Therefore, synthetic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyhydroquinone (TBHQ) have been added as inhibitors of lipid oxidation and stabilizing fat containing food stuffs. However due to possible toxic and carcinogenic effects on health, their usage are being questioned (Amarowiczet *et al.*, 2004; Hu *et al.*, 2003; Sokmen *et al.*, 2004).

Consumers increasingly avoid foods prepared with preservatives of chemical origin. A desire for high quality foods that are more natural, minimally processed and

yet preservative free is highly demanding (Fitzgerald *et al.*, 2003). Natural alternatives are required to prolong shelf life of foods as well as provide a high degree of safety with respect to foodborne pathogenic microorganisms. The application of naturally derived plant extracts to fulfill this aim of growing interest has challenged food industry to increase research on bioactive components from plants (Baydar *et al.*, 2004).

Phytochemicals occurring in herb that play a significant role in disease prevention and health promotion was first recognized as a result of epidemiological studies using both animal and human subjects (Shahidi & Naczk, 2004). This led to an ever-growing interest in herbs or botanicals and nutraceutical products. Bioactives in herbal and nutraceutical products constitute a myriad of chemical compounds, among which phenolic substances often play a primary or a synergistic function. Phytochemicals exhibited various degrees of antimicrobial activity occur in plant after molecular separation techniques to the isolation of various compounds (Vinha *et al.*, 2005; Karadeniz *et al.*, 2000; Yao *et al.*, 2003; Chavan *et al.*, 2003; Tsao & Deng, 2004). The increased demand for minimally processed foods or food with extended shelf-life has further revived interest in exploitation of these natural antimicrobial agents.

Phenolic compounds, at low concentration, may act as an antioxidant in our diet (Blasa *et al.*, 2006) and protect foods from oxidative deterioration. However, at high concentrations, they or their oxidation products may interact with proteins, carbohydrates, and minerals. Phenols are important compounds because of their contribution to human health and their multiple biological effects, such as

antioxidant activity, antimutagenic or anticarcinogenic activities, and antiinflammatory action (Karakaya, 2004).

Antioxidant compounds include vitamins, phenols, carotenoids, and flavonoids. These antioxidants may prevent the incidence of cardiovascular disease (Pastrana-Bonilla *et al.*, 2003). Over 4000 different flavonoids have been described and they are categorized into several subgroups. The four major classes of flavonoid are the 4-oxoflavonoids (flavones, flavonols and others.), anthocyanins, isoflavones, and the flavan-3-ol derivatives (catechin and tannins) and exhibit substantial antioxidant activity (Rhodes & Price, 1996). Flavonoids are a group of C15 aromatic plant pigments, which are biosynthesized via a confluence of the acetate/malonate and shikimate pathways. They are group of natural benzo-γ-pyran derivatives and are ubiquitous in photosynthesizing cells and they occur as aglycones, glycosides and methylated derivatives (Skerget *et al.*, 2005). Flavonols and flavones are usually found in plants bound to sugars as O-glycosides. Flavones may also occur as C-glycosides. The glycosidic form is a general feature of flavonoids, with the exception of flavanols where glycosides are rare. Free flavonoids, flavonoids without their attached sugars, are called aglycones. Aglycones of flavonols and flavones are not present in fresh plants but may be present as a result of food processing (Hollman & Arts, 2000). A single plant may contain different flavonoids, and their distribution within a plant family could be useful in the taxonomy. In plants, the flavonoids occur as white and yellow pigments in flowers, fruits, barks and roots and because of their favorable UV-absorbing properties, they also protect the plant from harmful UV radiation from the sun (Naidu *et al.*, 2000). These polyphenolic compounds are widely found in various types of edible plants, especially in vegetables, fruits, and tea. Flavonols (quercetin, myricetin and kaempferol) and flavones (apigenin and

luteolin) are the most common phenolics in plant-based foods. Flavanones are typically present in citrus fruit, and flavanols in green tea (Puupponen-Pimiä *et al.*, 2001).

The antimutagenic (Park *et al.*, 2004), antibacterial (Turkoglu *et al.*, 2006), antiviral (Du *et al.*, 2003; Evers *et al.*, 2005), anticarcinogenic (Merken & Beecher, 2000), anti-inflammatory and antithrombotic actions (Di Carlo *et al.*, 1999) of flavonoids are well characterised. Flavonoids can act as vasodilators and platelet disaggregators and also possess efficient antioxidant and free radical scavenging abilities (Bahorun *et al.*, 2004). Acylation of anthocyanins with *p*-coumaric and caffeic acids is common in fruits, and it is responsible for a better color stability in food products (Mazza & Miniati, 1993). Among numerous substances identified in medicinal plants, flavonoids represent one of the most interesting classes of bioactive compounds.

Anthraquinone is one of the members in quinine group under the big group of phenolics (Cowan, 1999). Quinones are aromatic rings with two ketone substitutions. They present everywhere in nature and are characteristically highly reactive. These compounds, being colored, are responsible for the browning reaction in cut or injured fruits and vegetables and are an intermediate in the melanin synthesis pathway in human skin (Schmidt, 1988). In addition to provide a source of stable free radicals, quinones are known to irreverse nucleophilic amino acids in proteins (Stern et al., 1996), often leading to inactivation of the protein and loss of function.

The potential range of quinone antimicrobial effects is great. Probable targets in the microbial cell are surface-exposed adhesins, cell wall polypeptides, and membrane-bound enzymes (Cowan, 1999). For all the plant-derived antimicrobials included quinone, the possibility of toxic effects must be thoroughly examined before it is applied in food system. Anthraquinone included emodin, rhein and physcion that isolated from *Cassia tora* seed inhibited phytopathogenic fungi such as *Pyricularia grisea, Botrytis cinerea, Phytophythora infestans* and *Erysiphe graminis* (Kim *et al.*, 2004). Since anthraquinone was proofed to exhibit anti-inflammatory and immunomodulatory effects of anthraquinones, the mechanism was believed involves antioxidant. Therefore, anthraquinones may act as antioxidants and radical scavengers (Choi & Chung, 2003).

Aloe vera is a member of liliaceae family and has been widely applied for centuries as traditional garden herbs. There are more than 360 different species of aloes grown in the dry regions of North American, Europe, and Asia (Hu *et al.*, 2003). Aloe is a succulent plant which is xerophytes and is adapted to live in low water availability area and is characterized by possessing a large water storage tissue. Aloe succulents is one of the xerophytates use crassulacean acid metabolism, an additional photosynthetic pathway involving malic acid (Ni *et al.*, 2004) that commonly found in cacti to store simple sugar and organic compounds in stomata. Of over 300 Aloe species, Aloe vera Linn. is most widely accepted and used for various medical, cosmetic and nutraceutical purposes. In Japan, *Aloe arborescens* Miller *var. natalensis* Berger is used as a folk remedy, and *Aloe barbadensis* Miller attracts much attention as a health food (Okamura *et al.*, 1996). Aloe Vera products have long been used in health foods for medical and preservative purposes. In the food industry, Aloe vera has been utilized as a source of functional food, especially

for the preparation of health drinks and other beverages, including tea yogurt and fruit juice. Bottled aloe vera juice or gel is widely available for internal consumption as a tonic, and it has been claimed to cure many illness, such as gout, constipation and arthritis (Eshun & He, 2004). It has been also applied in pharmaceutical industry due to its positive effect on the inhibition of tumor growth and fight against various skin disorders. An extensive application of aloe vera gel in the cosmetic and toiletry industries was found because of its valuable moisturizing emollient effect and widely applied in creams, cleansers and soups (Eshun & He, 2004). It has been reported to posses immunomodulatory, antiimflammatory, UV protective, antiprotozoal and wound and burn healing promoting properties (Choi & Chung, 2003).

Aloe vera is a rich source of polysaccharides and has various carbohydrate constituents. Mannose-6-phosphate is the major sugar in aloe vera gel. As it is linked to a protein and form mucopolysaccharide, it may produce even greater wound-healing effects (Grey *et al.*, 1991). Aloe vera contains anthraquinone as its major active principles hydroxyanthrone derivatives, mainly of the aloe-emodin-anthrone 10-*C*-glucoside type. The major constituent is known as barbaloin (aloin) (15–40%). It also contains hydroxyaloin (about 3%). Barbaloin (= aloin) is in fact a mixture of aloin A (10*S*) and B (10*R*). Another aloe species origin from Japan, *Aloe ferox* also contains aloinoside A and B. Aloin A and B interconvert through the anthranol form as do aloinoside A and B. The yellow exudates from the inner epidermal cell layers are known for its purgative activity. Purgative principles from aloe have been identified as an anthrone-C-glucosyl, barbaloin (aloin A) and homonataloin (Okamura *et al.*, 1996).

Hu *et al.* (2003) suggest that growth stage plays a vital role in the composition and antioxidant activity of Aloe vera. Free radical-scavenging activity of Aloe vera (*Aloe barbadensis* Miller) extracts in aloatic epidermis is stronger than pulp as more active free radical scavengers were in the skin (Hu *et al.*, 2005). Components in the rind of Aloe vera are responsible for the higher antioxidant activity of Aloe vera extracts. In fact, there are many cases reported that rind and pomace of plant are higher in phenolic content than pulp or flesh that commonly served as edible part during processing. They can be utilized as good source to recycle for extraction of polyphenol. Grape seed and skin (Baydar *et. al.* 2004), apple pomace (Lu & Foo, 2000), almond skin (Sang *et al.*, 2002), peanut skin (Nepote *et al.*, 2005) are examples of it.

Previous studies on aloe vera are focused on their health contribution to human body (Borrelli & Izzo, 2000; Choi *et al.*, 2001; Schiller, 2001; Eshun & He, 2004) but potential of aloe vera as preservative for food safety is lacking of focus (Hu *et al.*, 2003; Valverde *et al.*, 2005). Freeze dried aloe vera extracts are high in total flavonoid content and exhibit strong antioxidant activity (Hu *et al.*, 2003). Flavonoid of aloe vera plant has not been determined for its potential to exhibit antioxidant activities as well as antimicrobial activities. Anthraquinone give a promising effect against fungi (Jasso de Rodriguez *et al.*, 2005; Ali et al., 1999) and inhibit a possible causative factor of gastric cancer, *Helicobacter pylori* (Choi & Chung, 2003). Information of aloe vera against food spoilage bacteria and food-borne pathogen is still not available. Thus, in this study was carried out to determine antibacterial as well as antioxidant activities of targeted groups of compounds of aloe vera.

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