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Antioxidant and anti-quorum sensing activities of green pod of *Acacia nilotica* L.

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ABSTRACT

The antioxidant and anti-quorum sensing activities of eight extracts were studied in green pods of *Acacia nilotica*. The specific phenolic compositions and their quantifications were performed by HPLC and MS/MS, which showed that the HEF (pH 4) was higher in gallic acid, ellagic acid, epicatechin, rutin, and GTs. In order to find antioxidant potential of various extracts, their activities were studied for TPC, AOA, FRSA, RP, inhibition of LPO, FIC activity, $HO^{\cdot}$ and $O_2^{\cdot-}$ scavenging activities. Among them HEF (pH 4) has shown potent antioxidant activity. HEF (pH 4) was also found effective in protecting plasmid DNA and HAS protein oxidation induced by $HO^{\cdot}$. Pre-treatment of HEF (pH 4) at 75 and 150 mg/kg body weight for 6 days caused a significant increase in the levels of CAT and SOD and decrease in the level of MDA content in liver, lungs, kidneys and blood when compared to CCl_4-intoxicated rats. Eventually, the extracts were also screened for anti-QS activity. Of these extracts two showed QS inhibition: HEF (pH 4) and HCE. The results obtained strongly indicate that green pod of *A. nilotica* are important source of natural antioxidants.

1. Introduction

Acacia nilotica Lamarack belongs to the Mimosaceae family, and is widely distributed in tropical and subtropical countries. Ayurvedic medicine practices use of natural medicinal plants to promote self healing, good health and longevity, and have declared that *A. nilotica* can provide the nutrients and therapeutic ingredients to prevent, mitigate or treat many diseases or conditions (Biswas et al., 2002). According to Hartwell, traditionally the bark, leaves, pods and flowers are used against cancer, cold, congestion, cough, diarrhea, dysentery, fever, gall bladder, hemorrhoid, ophthalmia, sclerosis, small pox, tuberculosis, leprosy, bleeding piles, leucoderma and menstrual problems (Ambasta, 1994). This plant offers variety of bioactive components such as gallic acid, ellagic acid, isoquercitin, leucocyanadin, kaempferol-7-diglucoside, naringenin-7-0-β-D-(6′-0-galloyl) glucopyranoside, rutin, derivatives of (+)-catechin-5-gallate, apigenin-6,8-bis-C-glucopyranoside, m-catechol and their derivatives. They have spasmogenic, vasoconstrictor, anti/-hypertensive, -mutagenic, -carcinogenic, -spasmodic, -inflammatory, -oxidant and -platelet aggregatory properties (Biswas et al., 2002; Malan, 1991; Rastogi and Mehrotra, 1991; Singh et al., 2007).

Aerobic organisms are continually subjected to reactive oxygen species (ROS), the derivatives of oxygen generated as by-products during cellular metabolism and other exogenous environmental factors such as UV light, ozone, tobacco smoke, different xenobiotics, ionizing radiation herbicides, pesticides (Cadenas et al., 1997; Halliwell and Gutteridge, 1999). Oxidative stress, a result of imbalance between the antioxidant defence system and the formation of ROS, may induce damage to cellular biomolecules such as DNA, RNA, proteins, enzymes, carbohydrates, and lipids through oxidative modification and contributing to the pathogenesis of human diseases (Halliwell and Gutteridge, 1999; Gülçin et al., 2006; Prakash et al., 2007b). As a result, ROS have been implicated in many diseases, including acquired immunodeficiency syndrome, malaria, cardiovascular disease, gastric ulcer, diabetes, malignant tumors, rheumatic joint inflammation, cataracts, Parkinson's and Alzheimer's disease, etc. (Moskovitz et al., 2002; Halliwell and Gutteridge, 1984). Living systems have specific pathways to overcome these repair mechanisms and fail to keep pace with such deleterious effects.

Abbreviations: AOA, antioxidant activity; ARP, anti-radical power; ASE, ascorbic acid equivalent; BHA, butylated hyroxyanisole; BHT, butylated hydroxytoluene; CAT, catalase; CCl_4, carbon tetrachloride; DNPH, 2,4-dinitrophenylhydrazine; DPPH, 1,1-diphenyl-2-picrylhydrazyl; FIC, ferrous ion chelation; FRSA, free radical scavenging activity; H_2O_2, hydrogen peroxide; LPO, lipid peroxidation; HSA, human serum albumin; MDA, malondialdehyde; NADH, nicotinamide adenine dinucleotide; NBT, nitro blue tetrazolium; $O_2^{\cdot-}$, superoxide radical(s); $HO^{\cdot}$, hydroxyl radical(s); PCO, protein carbonyl; PMS, phenazine methosulphate; RP, reducing power; SDS, sodium dodecyl sulphate; SOD, superoxide dismutase; TBA, thiobarbituric acid; TCA, trichloroacetic acid; TPC, total phenolic content.

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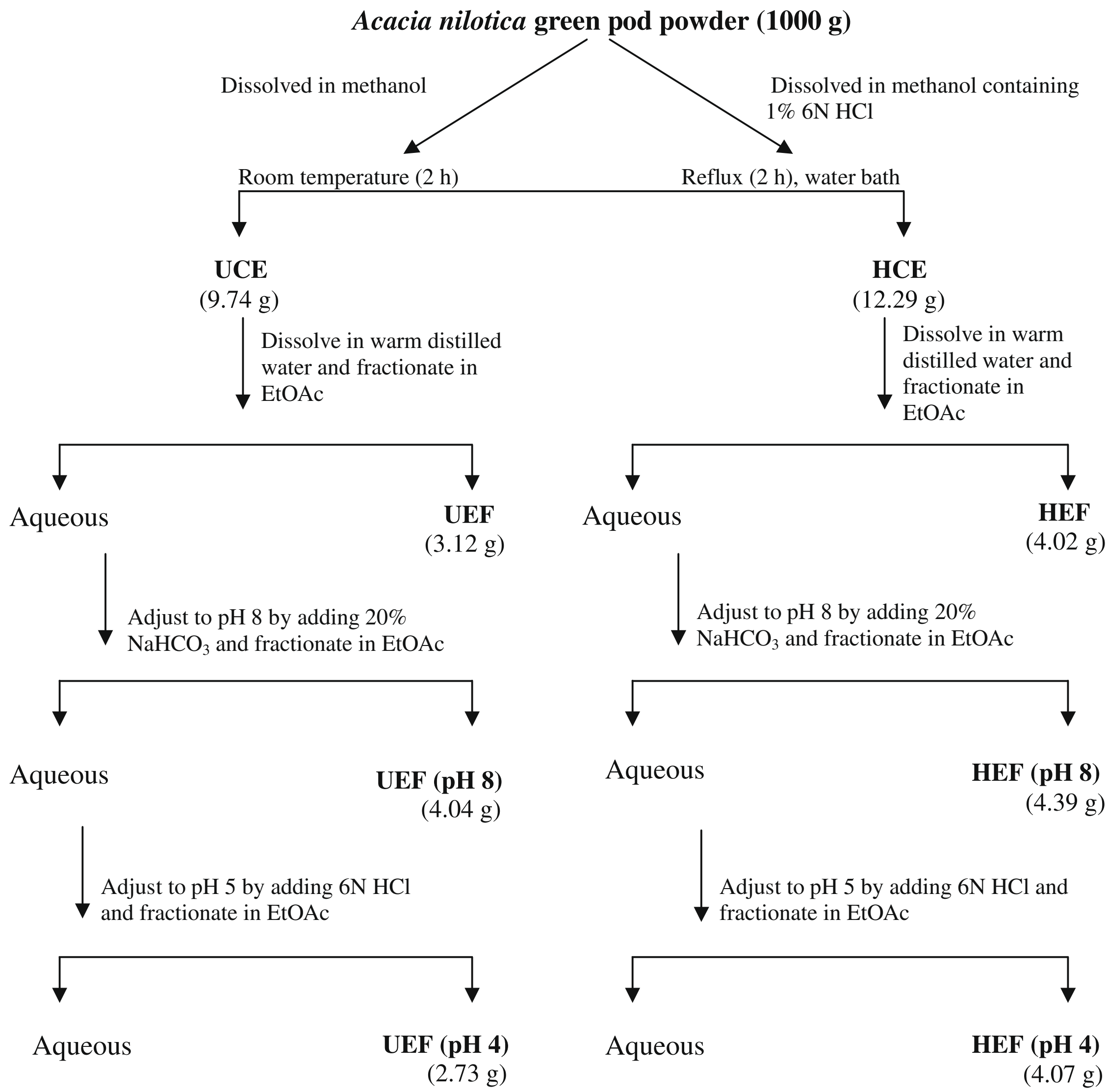

Chart 1. Systematic representation of preparation of various extract/fraction(s) of green pod of *A. nilotica*. UCE, unhydrolysed crude extract; HCE, hydrolysed crude extract; UEF, unhydrolysed ethyl acetate fraction and HEF, hydrolysed ethyl acetate fraction.

Natural antioxidants such as flavonoids, phenolics, tannins, curcumin and terpenoids are found in various plants (Fritz et al., 2003; Prakash et al., 2007a; Jayaprakasha et al., 2002). They can reduce the access of oxidants and other deleterious molecules due to their ability to scavenge oxygen-nitrogen-derived free radicals by donating hydrogen atom or an electron, chelating metal catalysts, activating antioxidant enzymes, and inhibiting oxidases (Ames et al., 1993; Ardestani and Yazdanparast, 2007; Patricia et al., 2005; Robak and Gryglewski, 1988). Based on accumulative evidence, in recent decades, tremendous interest has considerably increased in finding natural substances (i.e. antioxidants) present in foods or medicinal plants to replace synthetic antioxidants, which are being restricted due to their side effects. On the other hand, polyphenols, used as natural antioxidants, are gaining importance, due to their health benefits for humans, decreasing the risk of cardiovascular and degenerative diseases by reduction of oxidative stress and counteraction of macromolecular oxidation (Bingham et al., 2003; Silva et al., 2004). Natural antioxidants are also in high demand for application as nutraceuticals/functional food/bio-pharmaceuticals because of consumer preferences.

Many gram negative bacteria, including *Erwinia carotovora*, *Enterobacter agglomerans*, *Chromobacterium violaceum* and *Pseudomonas aeruginosa* use *N*-acyl homoserine lactones (AHLs), signal molecules to monitor their own population density. At a threshold population density, AHLs interact with cellular receptors and trigger the expression of a set of target genes, including virulence, antibiotic production, biofilm formation, bioluminescence, mobility and swarming, in a process called “quorum sensing” (QS) (Manefield et al., 2002). They represent highly attractive targets for the development of novel therapeutics. The major advantage of this novel strategy for anti-infective therapy is that it circumvents the problem of resistance, which is intimately connected to the use of conventional antibacterial agents, as it specifically interferes with the expression of pathogenic traits rather than to impede growth of the bacteria. In addition to antioxidant activity, several studies demonstrated the anti-QS activity of medicinal and dietary plants and/or their active molecules (Adonizio et al., 2006; Bosgelmez-Tinaz et al., 2007; Manefield et al., 2002; Rasmussen et al., 2005). The efficacy and toxicity of previously reported QS-inhibitors (i.e. halogenated furanones) have been important concern; and hence attention has been focused on identification of such QS blockers from natural and non-toxic sources for the development of novel non-antibiotic drugs for treating bacterial diseases in humans as well as in other animals. Therefore, the