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Effect of different processing methods on antioxidant activity of underutilized legumes, *Entada scandens* seed kernel and *Canavalia gladiata* seeds

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ABSTRACT

The present study is proposed to determine the antioxidant activity of raw and processed samples of underutilized legumes, *Entada scandens* seed kernel and *Canavalia gladiata* seeds. The indigenous processing methods like dry heating, autoclaving and soaking followed by autoclaving in different solutions (plain water, ash, sugar and sodium bicarbonate) were adopted to seed samples. All other processing methods than dry heat showed significant reduction in phenolics (2.9–63%), tannins (26–100%) and flavonoids (14–67%). However, in processed samples of *E. scandens*, the hydroxyl radical scavenging activity and β-carotene bleaching inhibition activity were increased, whereas, 2,2-azinobis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\cdot+}$), ferric reducing antioxidant power (FRAP), metal chelating and superoxide anion scavenging activity were similar to unprocessed ones. In contrary, except dry heating in *C. gladiata*, all other processing methods significantly ($P < 0.05$) reduced the 2,2′-diphenyl-1-picryl-hydrazyl ($DPPH^{\cdot}$) (20–35%), $ABTS^{\cdot+}$ (22–75%), FRAP (34–74%), metal chelating (30–41%), superoxide anion radical scavenging (8–80%), hydroxyl radical scavenging (20–40%) and β-carotene bleaching inhibition activity (15–69%). In addition, the sample extracts of raw and dry heated samples protected DNA damage at 10 μg. All processing methods in *E. scandens* and dry heating in *C. gladiata* would be a suitable method for adopting in domestic or industrial processing.

1. Introduction

Oxidative stress is defined as an imbalance between production of free radicals and reactive metabolites, so-called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants (Durackova, 2010). The continued oxidative stress can lead to chronic inflammation, which in turn could mediate most chronic diseases including cancer, diabetes, and cardiovascular, neurological, aging and pulmonary diseases. Oxidative stress can activate a variety of transcription factors can lead to the expression of over 500 different genes, including those for growth factors, inflammatory cytokines, chemokines, cell cycle regulatory molecules, and anti-inflammatory molecules (Reuter et al., 2010). ROS can control the expression of various tumor suppressor genes and also implicated in the chemopreventive and anti-tumor action of nutraceuticals derived from fruits, vegetables, spices, and other natural products used in traditional medicine (Gupta et al., 2012). During endogenous metabolic reactions, aerobic cells produce ROS such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), and organic peroxides as normal products of the biological reduction of molecular oxygen (Fridovich, 1978). In such conditions, external supply of antioxidants is essential to countervail the deleterious consequences of oxidative stress (Reuter et al., 2010). The bioactive compounds derived from legumes, fruit and vegetables can modulate inflammatory pathways and thus affect the survival, proliferation, invasion, angiogenesis and metastasis of the tumor (Gupta et al., 2010).

The consumption of plant foods like fruits, vegetables, legumes and cereals has been linked with reduction in the risk of developing chronic diseases, such as cancer, diabetes, obesity and cardiovascular diseases (Adams and Standridge, 2006). It is becoming clear that the regular intake of these plant foods in our daily life can be able to prevent free radical mediated degenerative diseases. Among them, legumes (Family: Fabaceae), the third largest family is recognized as a second most valuable plant source for human and animal nutrition (Doyle, 1994). They are excellent sources of protein, dietary fiber, starch, micronutrients and bioactive compounds with low level of fat. In addition to their nutritive value, legumes contain significant quantities of polyphenolic compounds such as flavonoids, isoflavones, phenolic acids and lignans (Lin and Lai, 2006). In fact, 650 genera and 20,000 species of legumes are available, but only few species like common beans (*Phaseolus vulgaris*), soya bean (*Glycine max*) and cowpea (*Vigna unguiculata*)

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are commonly available in the market. Due to their low production rate compared with consumption, an ever increasing demand has been witnessed (Ali and Kumar, 2000). The dependence on these certain plant species can lead to high price, scarcity and cause malnutrition in developing countries. Thus, many researchers are interested in the exploration of cost effective underutilized legumes.

Entada phaseoloides Merrill (Gila bean), is an important tribal pulse with a variety of medicinal uses. It occurs throughout the sub-Himalayan tract, from Nepal eastwards ascending to 4000 ft. in Sikkim, Assam, Bihar and Orissa, and in the monsoon forest of Western and Eastern Ghats and it is abundant in Andaman Islands. The soaked and boiled seed kernels are consumed by tribal peoples in India (Janardhanan and Nalini, 1991). Due to its wide array of chemical compounds in the seeds, it is used as an alexiteric, narcotic, tonic, emetic, anthelmintic, antipyretic, stomachache, edema and diabetes mellitus (Das, 1994) (Zheng et al., 2012). A potent Kunitz type trypsin inhibitor from seed acts as a potential candidate molecule for the development of insect resistant transgenic plants (Lingaraju and Gowda, 2008).

Canavalia gladiata (sword bean), is a leguminous plant originated in the Asian continent and spread throughout the tropics. They are cultivated on a limited scale throughout Asia, the West Indies, Africa and South America and have been introduced into tropical parts of Australia (Herklots, 1972). Their immature pod is consumed as a vegetable and the mature dry beans may be consumed after cooking because of their presence of antinutritional factors (Purseglove, 1968). The nutritional and antinutritional properties of *Entada scandens* and *C. gladiata* were reported by Siddhuraju et al. (2002), Sridhar and Seena (2006) and Vadivel et al. (2008). In spite of these health benefits, the utilization of these raw legumes is limited by the presence of antinutrients like phenolics, tannins, phytic acid, protease inhibitors, lectins, α-amylase inhibitors, canavanine and saponins and their consumption can lead to nausea, vomiting and diarrhea. The removal of undesirable components is therefore essential to improve the nutritional quality of legumes and effectively utilize their full potential as human food. In order to inactivate or reduce the above mentioned antinutrients, various conventional, simple processing methods have been used such as dry heating, roasting, boiling, soaking in water, alkali and acid, solvent extraction, germination and fermentation (Khokhar and Apenten, 2003; Siddhuraju and Becker, 2003). Heat treatment has been used to deactivate the thermo-labile antinutritional factors and soaking could be one of the processes for removal of soluble antinutritional compounds, which can be eliminated with the discarded soaking solution (Vidal-Valverde et al., 1992). The nutritive quality of most tropical legume grains, particularly cowpea, soybean, pigeon pea, lima bean and winged beans was notably improved by heat treatment (Akande and Fabiyi, 2010) and the amino acid, canavanine content in *C. gladiata* was reduced in overnight soaking and boiling in excess water (Ekanayake et al., 2007). Moreover, the processing methods like dry heating, autoclaving, soaking following by autoclaving in plain water, ash and sodium bicarbonate solution were found to improve the nutritional value of *Mucuna pruriens* by reducing maximum level of antinutrients present in it (Siddhuraju and Becker, 2003; Vadivel et al., 2010; Emenalom et al., 2005). In other parts of Asia, sword beans are often soaked in water over-night, boiled in water to which a small quantity of sodium bicarbonate has been added, rinsed, boiled, pounded and used in curries, or as a substitute for mashed potato (Eknayake et al., 1999). Moreover, the processing (dry heating, soaking and autoclaving in various solutions like plain water, ash and sugar solution) of *C. ensiformis* seeds showed higher antioxidant activity than raw seeds (Sowndhararajan et al., 2011). The increase or decrease in antioxidant activity of samples will depend upon our method of processing. This study is therefore centered to evaluate the antioxidant and free radical scavenging activity of raw and traditionally processed *E. scandens* and *C. gladiata* seeds.

2. Materials and methods

2.1. Sample collection

The mature and dry raw seeds of *E. scandens* were collected from Kolli hills, Tamil Nadu, India and *C. gladiata* (red color) was purchased from Bangalore, India. Botanical identity of both the samples was established based on the morphology of the seeds, vegetative and floral parts. The identity was confirmed by comparing voucher specimens available in the botanical Survey of India, Coimbatore. After collection and purchase, the immature and damaged seeds were removed. The kernel was separated from whole seed of *E. scandens* and the following processing methods were adopted.

2.2. Processing methods

E. scandens kernel and whole seed of *C. gladiata* were cracked into similar size. Both of the samples were randomly divided into 7 batches. The first batch was kept raw without any treatment. The second batch of seeds was dry heated in a Petri plate containing sand in a hot air oven at 120 °C for 30 min. The third batch of seeds was autoclaved using sample: water in the ratio of 1:5 (W/V) at a 15 lbs pressure for 15 min. The other four batches were subsequently soaked separately in various solutions like water, ash (Dried male inflorescences of sugar palm tree were ignited in a muffle furnace at 650 °C for 4 h, cooled and 0.1% of this ash solution was prepared using water and filtered), sodium bicarbonate (0.1%) and palm sugar solution (Bud of a coconut tree was made into slit and sap was collected, boiled and poured into bamboo tubes and left to solidify. Then, 1% of this sugar solution was prepared using water) at a ratio of 1:7 (w/v) for 12 h at room temperature. After 12 h of soaking, the samples were autoclaved with freshly prepared respective solutions (1:5) (w/v) at 15 lbs pressure for 15 min. After discarding the autoclaved liquid, the samples were dried, powdered and stored separately at 4 °C until further analysis.

2.3. Sample extraction

The raw and all processed samples were subjected to extraction. Before extraction, the samples were defatted with petroleum ether. Then the samples were extracted with 80% methanol (1:5 w/v) for 48 h at room temperature. The extract were filtered, air dried and stored at 4 °C for further analysis.

2.4. Chemicals

2,2′-Azobis (2-amidinopropane) dihydrochloride (AAPH), Butylated hydroxyanisole (BHA), 2,2′-diphenyl-1-picryl-hydrazyl (DPPH·), β-carotene, α-tocopherol, catechin, linoleic acid, 2,4,6-tripyridyl-*s*-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) and 2,2-azinobis (3-ethyl benzothiazoline-6-sulfonic acid) diammonium salt (ABTS) were procured from Sigma (Bangalore, Karnataka, India). All other chemicals like nitro blue tetrazolium (NBT), ethylenediamine tetra acetic acid (EDTA), pBR 322, Ferrozine and linoleic acid were purchased from HiMedia (Mumbai, Maharastra, India).

2.5. Determination of total phenolics and tannin contents

Total phenolics and tannins were measured as tannic acid equivalents (Makkar et al., 2007) from tannic acid standard curve (3–15 μg range). One milliliter of the sample extract was transferred to a test tube and 0.5 mL of Folin–Ciocalteu reagent and 2.5 mL of sodium carbonate solution (20% w/v) were added. After an incubation period of 40 min in dark, the absorbance was recorded at 725 nm with UV–visible spectrophotometer (Cyberlab-UV100, USA) against the reagent blank. Using the same extracts and method, the tannins were estimated after treatment with polyvinylpolypyrrolidone (PVPP).

2.6. Estimation of total flavonoids

Total flavonoid content was measured according to the method of Zhishen et al. (1999). Sample extract was added with 0.3 mL of 5% sodium nitrite and well mixed. After 5 min of incubation, 0.3 mL of 10% aluminum chloride solution was added. Then, after 6 min, 2 mL of 1 M sodium hydroxide was added to the mixture and made up the volume to 10 mL with water. The absorbance was measured at 510 nm with UV–visible spectrophotometer. Total flavonoids were measured from rutin (20–100 μg) standard curve and expressed as mg rutin equivalents/g extract.

2.7. Free radical scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH·)

The antioxidant activity of extracts and standards (BHA, rutin and tannic acid) was measured in terms of hydrogen donating ability using a stable, commercially available organic and nitrogen centered DPPH· radical by the method of Brand-Wil-