BIOLOGICAL ACTIVITIES OF *CAJANUS CAJAN* ETHANOLIC EXTRACTS

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INTRODUCTION

Pigeon pea (*Cajanus cajan* (L) Millsp) is among the dry leguminous seed which important source of proteins in human diet food, especially in the vegetarian population. Pigeon pea has high levels of proteins and important amino acids such as methionine, lysine and tryptophan. The Extraction of seed and leaves are widely used in Indian and Chinese traditional folk medicine [1]. The chemical compounds of pigeon peas compose of isoguercitrin, quercetin, quercetin-3-methyl ether and 3-hydroxy-4-prenyl-5-methoxystilbene-2-carboxylic acid [2]. The rich content of phenolic and flavonoids contained in leaves extracts were promising their bioactivity such as antipasmodic, anti-imflammatory, antimicrobial and antioxidant activities [3]. Fungal endophyte MD98 extracts from leaves was produced phenolic and flavonoids compounds which have antioxidant properties, superoxide dismutase and glutathione reductase activities in HepG2 cell [4]. The ethanolic extract of leaves contain pinostrobin, cajanin, longstylin C, longistylin A and cajaninstilbene, there was potent to antioxidant, antiplasmodical, anti-inflammatory and hypocholesterolemic activities. While, the crude ethanolic extracts of roots contain genistein, genistin, longstylin C, longistylin A and cajanol, which were antioxidant and anticancer activities [5]. The present study was taken up to evaluate antioxidant and anti-tyrosinase activities of *Cajanus cajan* seed ethanolic extract. The antioxidant is claimed that it is biologically active in protecting the body whereas the skin collagen and elastic tissue against damaging by reactive oxygen species. Tyrosinase is known to play a role as key enzyme for melanin biosynthesis in plants and animals. Tyrosinase inhibitors therefore can be clinically useful for the treatment of some dermatological disorders that associate with melanin hyperpigmentation.

MATERIALS AND METHODS

Preparation of Plant Extracts The dried seed of Pigeon pea were ground into powder and extracted with 70 % ethanol at room temperature for 10 times. The combined filtrate of ethanol solution was evaporated under reduced pressure at room temperature.

Scavenging of Diphenyl-picrylhydrazyl (DPPH) Radicals Assay The free radical scavenging activity of ethanolic extracts was analyzed by the DPPH assay [6]. The amount of 100 µL of various concentrations sample (20, 40, 60, 80 and 100 µg/mL) were reacted with 100 µL of $6x10^{-3}$ M DPPH ethanolic solution in a 96-well plate, incubated at 37 ^{o}C for 30 min. The absorbance was measured at 517 nm using a UV–VIS microplate reader. All experiments were carried out in triplicates.

Lipid Peroxidation (β-Carotene Bleaching Model) The antioxidant activity of crude extract was measured by β-carotene bleaching model system with slight modification [7]. Emulsion I was prepared by dissolving 10 mg of β-carotene in 10 ml of chloroform. Four milliliters of β-carotene solution, 40 mg of linoleic acid and 400 mg of Tween 40 were mixed and removed chloroform at 50 ^{o}C under vacuum by rotary evaporator. The emulsion was further made up to 100 ml with MillQ water. Emulsion II was prepared same as emulsion I with out β-carotene. Test sample, 50 µl of varied concentration sample were mixed with emulsion I. Blank sample, 50 µl of varied concentration sample were mixed with emulsion II. Absorbance was measured at 450 nm after mixture incubation in oven at 50 ^{o}C and the reaction reading will reccord from zero time (t=0) till 60 min.

Inhibition of tyrosinase activity Determination of tyrosinase inhibition activity was performed by the dopachrome method using L-DOPA as the substrate and described by Iida *et al* [8] with slight modifications. Briefly, 50 µL of various sample concentration, 50 µL of phosphate buffer (pH 6.8) and 50 µL of 787 units/mL of mushroom tyrosinase solution were mixed in 96-well plate. After pre-incubation at 37 ^{o}C for 10 min, 50 µL of 160 µg/mL L-Dopa was added and incubated at 37 ^{o}C for 2 min. The amount of dopachrome was measured at 492 nm using a UV–VIS microplate reader.

RESULTS AND DISCUSSION

The biological activity of *C. cajan* seed ethanolic extracts was showed in Table 1 and 2.

Table 1 Antioxidant activity (EC_{50}) of crude ethanolic extract of *C. cajan* seed, compared with standard.

Sample	DPPH assay (μg/ml)	Lipid peroxidation (μg/ml)
Crude ethanolic extract of *C. cajan* seed	44.36	25.97
Trolox	1.90	1.20

The DPPH radical was used to evaluate the free radical scavenging capacity of antioxidants extensively. The concentration of *C. cajan* seed ethanolic extract to quench DPPH radical (EC_{50}) was 44.36 μg/ml. Lipid peroxidation was estimated of capacity of crude extract to protect β-carotene color change from yellow to colorless. The activity (EC_{50}) was 25.97 μg/ml. The results show that the antioxidant activity of *C. cajan* seed ethanolic extract lower activity than Trolox, that know compound had high antioxidant activity.

Table 2 Anti-tyrosinase activity (EC_{50}) of crude ethanolic extract of *C. cajan* seed, compared with standard.

Sample	Anti-tyrosinase (μg/ml)
Crude ethanolic extract of *C. cajan* seed	267
Kojic acid	2.31

For the activity of *C. cajan* seed ethanolic extracts to inhibit on dopa oxidase of mushroom tyrosinase. These extracts showed lower activity than Kojic acid, that know compound had high anti-tyrosinase activity and used in whitening agent.

CONCLUSION

The results reveal that *C. cajan* seed ethanolic extracts had high antioxidant activity and slightly anti-tyrosinase activity. Although less activity than Trolox and Kojic acid. These crude ethanolic extracts could be potential sources of antioxidant and anti-tyrosinase activity. It would be promissing to do more studies as skin-whitening and anti-wrinkle agents. Future biological investigations on human melanocytes and dermal fibroblast need to further confirm, including safety evaluation.

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