

## Evaluation of *In Vitro* Antioxidant Capacities of Six Accessions of Winged Beans (*Psophocarpus tetragonolobus*)

Charles O Olaiya<sup>1\*</sup>, Kehinde O Soetan<sup>2</sup> and Kayode O Karigidi<sup>3</sup>

<sup>1</sup>Department of Biochemistry, College of Medicine, University of Ibadan, Oyo State, Nigeria

<sup>2</sup>Department of Veterinary Physiology and Biochemistry, University of Ibadan, Oyo State, Nigeria

<sup>3</sup>Department of Chemical Sciences, Ondo State University of Science and Technology, Okitipupa, Ondo State, Nigeria

\*Corresponding Author: Charles O Olaiya, Department of Biochemistry, College of Medicine, University of Ibadan, Oyo State, Nigeria.

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### Abstract

Winged beans (*Psophocarpus tetragonolobus*), a member of legume family does not only contain nutrients, but also bioactive compounds with antioxidant potential. The aim of this present study is to evaluate the antioxidant capacities of six accessions of the seeds of winged beans, namely TPT-3, TPT-6, TPT-12, TPT-30, TPT-43 and TPT-48, from the Genetic Resources Unit, International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. Antioxidant capacity was assessed by determining Total phenolics (TPC), total flavonoids content (TFC), total antioxidant capacity (TAC), reducing power and 2, 2-diphenyl -1- picrylhydrazyl (DPPH) scavenging power using standard methods. The results showed that TPC and TFC ranged from (0.853 to 0.941 mgGAE/g) and (0.678 to 1.169 mg QUE/g), respectively with TPT-3 having the highest TPC content and TPT-30 recording the highest TFC content. Total antioxidant capacity (TAC) ranged from (1.278 to 1.806 mg AsA/g) with TPT-43 having the highest content. Also, TPT-43 exhibited the highest DPPH scavenging activity (37.71%) and the highest reducing power (2.143). These findings indicate that the winged beans investigated can serve as good sources of dietary antioxidants and free radical scavengers in man and animals.

**Keywords:** Winged Beans; Antioxidants; Phenolics; Flavonoids; DPPH

### Introduction

Legume is one of the most important food crops in the tropics, they are the next important food crops after cereals [1]. They are rich in lysine and tryptophan but low in the sulphur-containing amino acids such as methionine and cysteine. They are cheaper than animal protein and therefore serve as an important source of affordable protein especially to people with low income in many tropical countries in Africa [2]. Recently the consumption of legume has been correlated with reduce risk of diabetes and obesity [3]. This physiological role of legume may be due to vast presence of polyphenol with numerous biological roles such as anticancer, antioxidant, antimicrobial and anti-inflammatory abilities. Natural polyphenols exert their beneficial health effects by their antioxidant activity; they scavenge free radicals and reactive oxygen species (ROS), chelate metal catalysts, reduce  $\alpha$ -tocopherol radicals, inhibit lipid peroxidation and activate antioxidant enzymes [4].

Winged bean (*Psophocarpus tetragonolobus*) is a tropical food crop that is listed as one of the underutilized legumes and it is an underexploited food source for the tropics. It produces edible leaves, shoots, flowers, pods, and tubers as well as seeds [5]. Winged bean is a very remarkable and exceptional crop, in that all its parts except the stalks and roots are edible, palatable, and valuable nutritionally. The

most prominent of its parts are its ripe seeds, which may be only consumed after processing; which can be cooked, simmered, or roasted. The seeds contain about 35 per cent of carbohydrates. Soluble sugars account for 9.8 to 13.8 percent of the carbohydrates composition, and starch is absent [6].

The seeds are highly rich in fibre with its fibre content three times higher than that of common bean (*Phaseolus vulgaris* L). The seeds are also very important as animal feed. The seed is valuable because of its high protein content which is similar amino acids composition to soybeans, and may be substituted for the soybeans meal in the diets of monogastric domestic animals [7].

Genetic variation in legume species and their wild relatives is of prime importance to the successful breeding of improved crop cultivars with added value and durable resistance to pests. The collection and preservation of legume germplasm has been established to ensure that scientists have access to as many genes as possible [8]. Studies on the antioxidant activities of natural substances have been of interest in recent years [9].

Despite all these important qualities of winged beans, there is dearth of information on the antioxidant activities of different accessions of Winged beans (*Psophocarpus tetragonolobus*). This study therefore aims to evaluate the antioxidant potentials of six accessions of *Psophocarpus tetragonolobus*.

## Materials and Methods

### Sample materials

The six accessions of winged beans used in this work were provided by the Genetic Resources Center (GRC) of International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. The accessions named as TPT-3, TPT-6, TPT-12, TPT-30, TPT-43 and TPT-48.

### Sample preparation

Dried Winged bean seeds were milled with laboratory mill. One (1g) of legume accessions was soaked in 10 ml distilled water. The solution was left overnight for 12 hours and centrifuged at 3000 rpm for 10 minutes. The supernatant was decanted, stored in refrigerator and used for the analysis.

### Antioxidant assays

#### Total phenolic content

The total phenolic content of the legume samples was determined by Folin-Ciocalteu phenol reagent method [10]. 1.0 ml of the samples was mixed with 1 ml (10%) of Folin-Ciocalteu phenol reagent After 5 minutes, 5 ml of 7% Na<sub>2</sub>CO<sub>3</sub> solution was added to the mixture followed by addition of 5 ml of distilled water. This was mixed thoroughly. The mixture was kept in the dark for 90 minutes at 25°C after which the absorbance was read at 750 nm. The total phenolic content was evaluated from a Gallic acid standard curve and expressed as gallic acid equivalent (mgGAE/g).

#### Total Flavonoid content

The total flavonoid content of the legume sample was determined using the method of Park, *et al* [11]. 1.0 ml of the sample was mixed with 3.4 ml (30%) of methanol, 0.15 ml (0.5M) of NaNO<sub>2</sub> and 0.15 ml (0.3M) of AlCl<sub>3</sub>·6H<sub>2</sub>O. After 5 minutes, 1 ml of 1M NaOH was added. The absorbance was read at 506 nm and determined from quercetin calibration curve and expressed as quercetin equivalent (mgQUE/g).

#### DPPH radical scavenging activity

DPPH (2,2-diphenyl -1- picrylhydrazyl) radical scavenging activity of the legume was estimated according to the method of Gyamfi, *et al* [12]. 1.0 ml (100 µg/ml – 400 µg/ml) was added to 4.0 ml of DPPH (25 mg/l). The samples were mixed thoroughly and allowed to stand in dark room for 30 minutes and absorbance was read at 520 nm. Percentage inhibition of DPPH scavenging ability was calculated using:

$$\text{Percentage inhibition of DPPH} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{(\text{Absorbance of control})}$$

DPPH solution without sample served as control.

**The reducing power**

The reducing power was determined according to the method of Oyaizu [13]. 1.0 ml (100 µg/ml - 500 µg/ml) of sample was mixed with 1.0 ml phosphate buffer (0.2M, pH 6.6) and 0.5 ml (0.1%), potassium ferricyanide followed by incubation at 50°C for 20 minutes. After which 0.5 ml of 10% trichloroacetic acid was added to terminate the reaction. Upper portion of the solution (1 ml) was taken, mixed with 1 ml distilled water and 0.1 ml (0.01%) of Iron(III) chloride solution was added. The reaction mixture was allowed to stand 10 minutes at room temperature before the absorbance was read at 700 nm.

**Total antioxidant capacity**

The total antioxidant capacity (TAC) of the legume samples was determined by the method of phosphomolybdate [14]. 0.2 ml of the sample was mixed with 2 ml of reagent solution (0.6M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The mixtures were incubated in a water bath at 95°C for 90 minutes. The samples were allowed to cool and absorbance was read at 765 nm against a reagent blank. The TAC was calculated from ascorbic acid calibration curve and expressed as Ascorbic acid equivalent.

**Statistical Analysis**

All data are given as the mean ± SD of three measurements. The significance of the differences between the means of the samples were established by the analysis of variance (P < 0.05) and charts were drawn with graph pad prism 5.

**Results**

The results of total phenolic contents (Figure 1) of this legume accessions expressed as GAE/g showed that it is rich in phenolics. Phenolics serve as major index of antioxidant in food. The TPC is not significantly different across legume accessions. However, TPT-3 has the highest value (0.941 mg GAE/g) of TPC while TPT-12 (0.852 mg GAE/g) has the lowest in all the accessions examined.

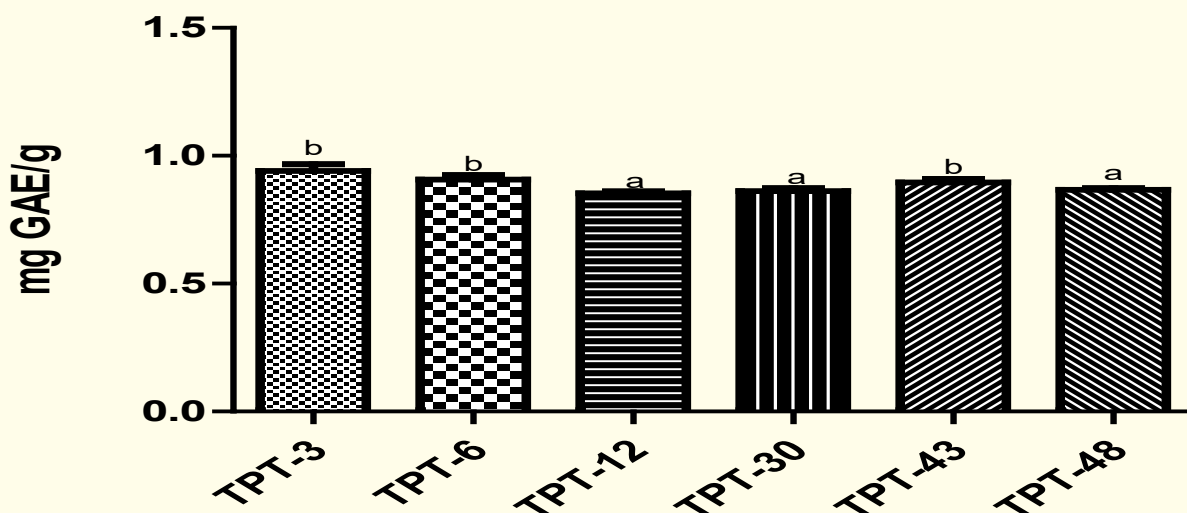


Figure 1: Total Phenolics Content (TPC) of winged beans.

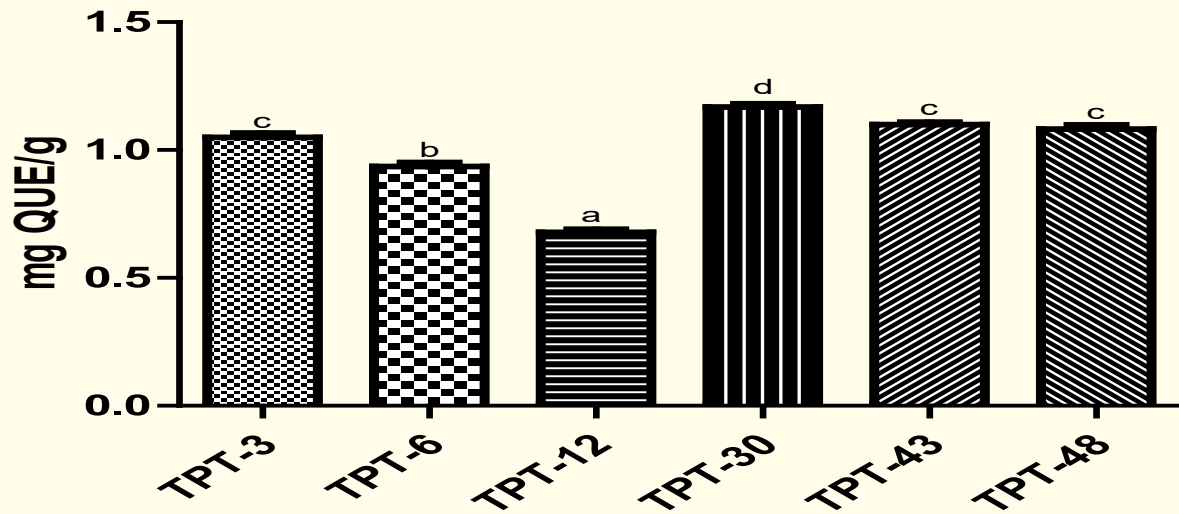


Figure 2: Total Flavonoids Content (TFC) of winged beans.

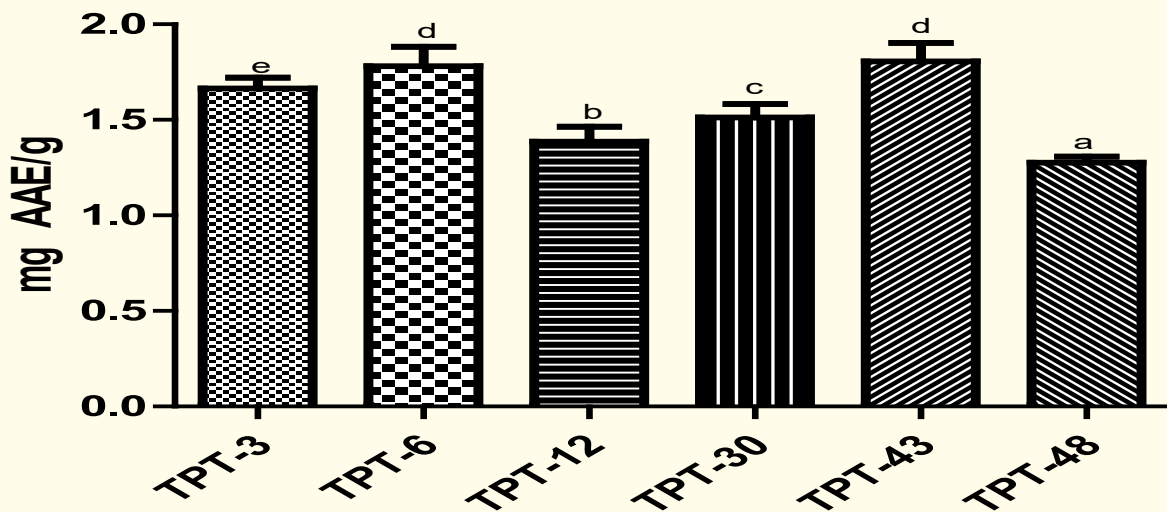


Figure 3: Total Antioxidant Capacity (TAC) of winged beans.

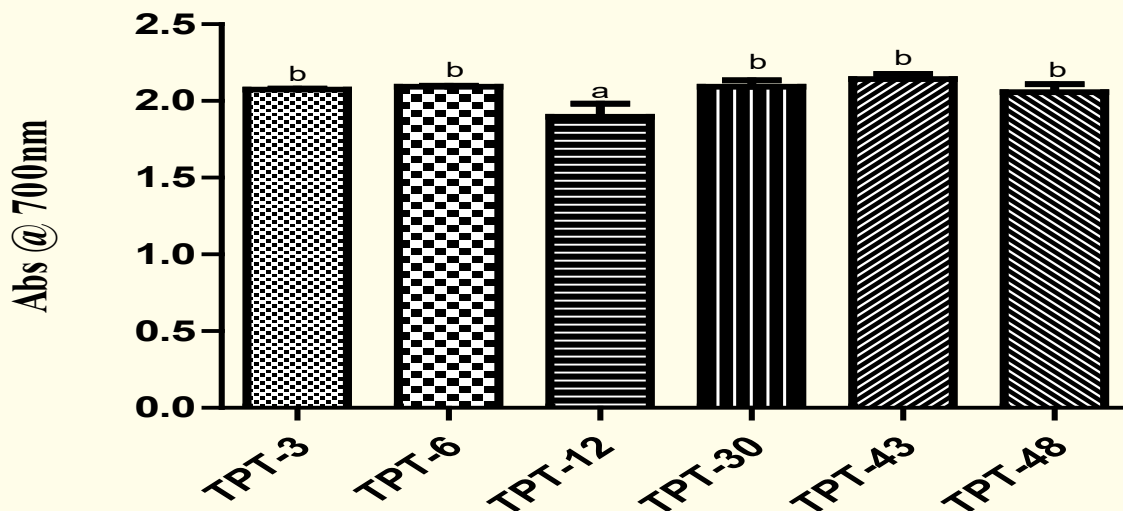


Figure 4: Reducing Power (RP) of winged beans.

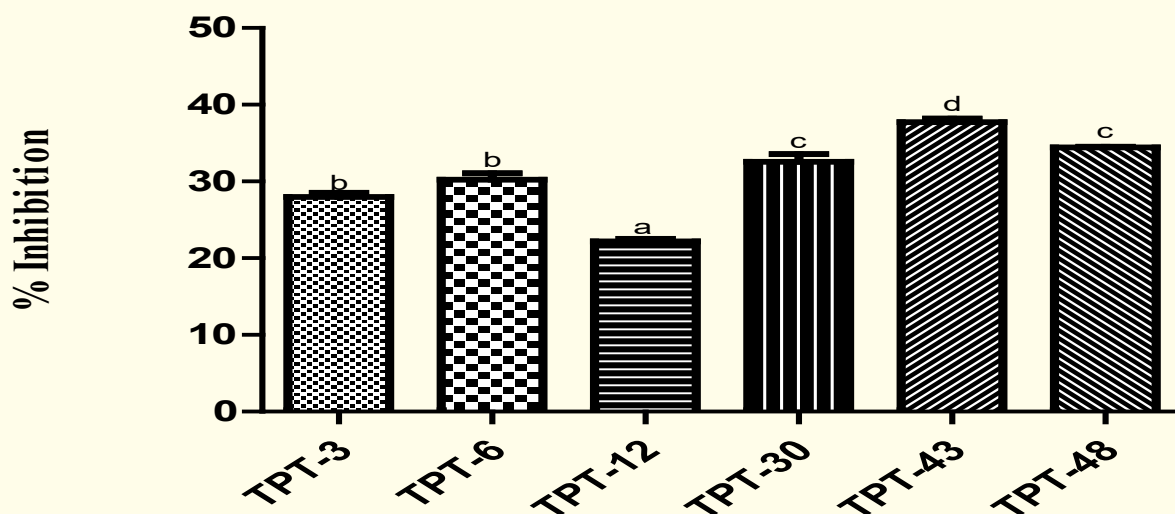


Figure 5: DPPH scavenging activity of winged beans.

### Discussion

Phytochemicals reduce the risk of free radicals that cause oxidative damage to living cells, which result into common degenerative disorders like cancer and cardiovascular diseases [15]. Phytochemicals play important roles in the free radical scavenging activities of plants [16]. The antioxidant properties of plants are attributed to their rich phytochemical composition.

Phenolic compounds are very important as antioxidants. Their antioxidant effects anti-carcinogenic, anti-oxidant, anti-inflammatory, anti-tumoral, anti-microbial, anti-mutagenic, anti-aggregant, anti-ischaemic and anti-allergic. Phenolics also alleviate cardiovascular diseases [10,17].

Phenolic compounds are responsible for the antioxidant activity of fruits as a result of their redox properties that allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [18]. The result from this study showed that the selected winged bean accessions are rich in TPC (Figure 1).

Flavonoids are secondary metabolites widely found in fruits, vegetables and legumes. They have been linked with several biological activities which include antioxidant, anti-inflammatory, antiviral and anticancer effects [19]. Several studies have showed that the consumption of flavonoid-rich foods protect against diseases associated with oxidative stress [20,21]. The result of this study showed that the selected winged bean accessions are rich in TFC (Figure 2). The flavonoid content is significantly difference among the accessions with TPT-30 has the highest content 1.169 mg QUE/g while TPT-12 has the lowest 0.679 mg QUE/g.

DPPH is regarded as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule does not dimerize, as would be the case with most other free radicals. However when a solution of DPPH is mixed with that of a compound that can donate a hydrogen atom, it is converted to its reduced form with the loss of this violet colour. The loss of its violet colour is directly proportional to the antioxidant power of the extract. The result of DPPH scavenging ability of our legume samples reported as percentage inhibition was showed in figure 4. TPT-43 exhibited the highest ability to scavenge DPPH while TPT-12 exhibited the lowest. The differences in their ability to scavenge DPPH are significantly different.

The principle of reducing power is based on the reduction of  $Fe^{3+}$  to  $Fe^{2+}$  which will result in formation of coloured complex. Higher absorbance indicates a higher antioxidant activity. The data obtained revealed that TPT-43 has the highest reducing ability which is significantly higher than that of TPT-12 which exhibited the lowest ability. The results showed that the legume accessions have high reducing ability when compared with previously reported underutilized legumes [4].

## Conclusion

From the results obtained in this study, it was clearly shown that the different accessions of winged beans examined possess bioactive phytochemicals with antioxidant properties. Thus, they can serve as sources of health-promoting nutrients and phytochemicals for human and animals.

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