COMPARATIVE PHYTOCHEMICALS AND *IN VITRO* ANTIOXIDATIVE EFFECTS OF JACK BEANS (*CANAVALIA ENSIFORMIS*) AND SWORD BEANS (*CANAVALIA GLADIATA*)

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Abstract
Jack Bean (JB) (Canavalia ensiformis) and Sword Bean (SB) (Canavalia gladiata) are lesser-known and underutilized legumes with many food and medicinal benefits. Phytochemical screening and the in vitro antioxidant assays of aqueous extracts of jack bean and sword bean were carried out using standard methods. DPPH (2, 2-diphenyl -1-picrylhydrazyl) scavenging activity, total phenolic content (TPC), total flavonoids contents (TFC) and reducing power were assayed as determinant of in vitro antioxidant effects. Phytochemical screening showed the presence of saponins, tannins, flavonoids, steroids and alkaloids in both JB and SB while cardiac glycosides and anthraquinones were absent in both JB and SB. However, SB contained terpenoids which were absent in JB. The results of in vitro antioxidant assays showed that aqueous extract of sword bean had a significantly higher (P<0.05) DPPH (2, 2-diphenyl -1-picrylhydrazyl) scavenging activity than aqueous extract of jack bean. The total phenolic content (TPC) of sword bean was not different from the TPC of jack bean. Aqueous extract of sword bean had a significantly higher (P<0.05) total flavonoid content (TFC) than aqueous extract of jack bean and sword bean had a significantly higher (P<0.05) reducing power effects than the jack bean. The study concluded that jack bean and sword bean exhibited in vitro antioxidative effects and both JB and SB should be exploited as functional foods for the benefit of human and animals.

Key words: Jack bean, sword bean, phytochemical, antioxidant

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1. INTRODUCTION

Phytochemicals are compounds that reduce the risk of free radicals that are responsible for the oxidative damage to living cells, resulting in degenerative disorders like cancer and cardiovascular diseases (Liu, 2003; Boyer and Liu, 2004).
Antioxidants are substances which inhibit oxidative damage by preventing the action of free radicals and reactive oxygen species (ROS) and also delay the initiation or slow down the rate of lipid oxidation reaction (Boskou, 2006; Duaqan *et al*. 2011).
Studies of the antioxidant properties of natural substances have been on the increase in recent years (Enujiugha *et al*., 2012).
Legumes are sources of natural antioxidants that can protect against oxidative stress and play vital roles in the chemoprevention of extract is reported to have hepatoprotective activity and anticancer effect (Nair, 2014).
diseases having their aetiology and pathophysiology in reactive oxygen species (Dragland *et al.*, 2003; Atawodi, 2005).
Jack bean (*Canavalia ensiformis)* and sword bean (*Canavalia gladiata*) are among the underutilised legumes in Nigeria (Ojo *et al.*, 2016). They are classified as lesser-known legumes largely due to a seemingly lack of awareness on its nutritional potentials (Odoemelam, 2007). The nutritive and protein quality of the seeds as shown by some studies seems to be similar to that of most of the edible legume grains and hence, they are advocated to be a good source for extending protein sources (Moreina *et al*., 1993). There is also a pharmaceutical interest in the use of jack bean (*C. ensiformis)* as a source for the anti-cancer agents trigonelline and canavanine (Morris, 1999). Sword bean (*C. gladiata*) root

The aim of this study is to investigate the *in vitro* antioxidant effects of jack bean and sword

bean, as a way of exploiting the utilization of these lesser-known legumes for the health benefits of humans and animals.

2. MATERIALS AND METHODS

Source of the Jack beans and Sword beans

The Jack bean (*Canavalia ensiformis*) (Tce-5) and Sword bean (*Canavalia gladiata*) (Tcg-4) seeds used for these studies were obtained from the Genetic Resources Center of the International Institute for Tropical Agriculture (I.I.T.A) in Ibadan, Oyo State. The pictures of the jack beans and sword beans are shown in Figures 1 and 2.

Phytochemical screening

Phytochemical analyses of the aqueous extracts of jack bean and sword bean were conducted using the method of (Trease and Evans, 1983).

Sample Preparation

Dried JB and SB seeds were ground into powdery form with a Blender/Miller III Model The total flavonoid content of JB and SB seeds was determined using the method of Park *et al.* (1999).

The reducing power

The reducing power of JB and SB seeds was determined according to the method of Oyaizu (1986).

MS-223 Taiwan, China. Two aqueous extracts were prepared from 1g each of the powdered sample of JB and SB. 1g of each legume accessions was soaked differently in 10ml extraction solvent. The solution was left overnight for 12 hours and centrifuged at 3000 rpm for 10 minutes. The supernatant was stored in the refrigerator and later used for the analysis.

Antioxidant Assays

DPPH radical scavenging activity

DPPH (2, 2-diphenyl -1- picrylhydrazyl) radical scavenging activity of JB and SB seeds was estimated according to the method of (Gyamfi *et al.*, 1999).

Total phenolic content

The total phenolic contents of JB and SB seeds were determined by spectrophotometric method (Kim *et al.*, 2003).

Total flavonoid content

3. RESULTS AND DISCUSSION

Phytochemical screening results of jack bean and sword bean are shown in Table 1.

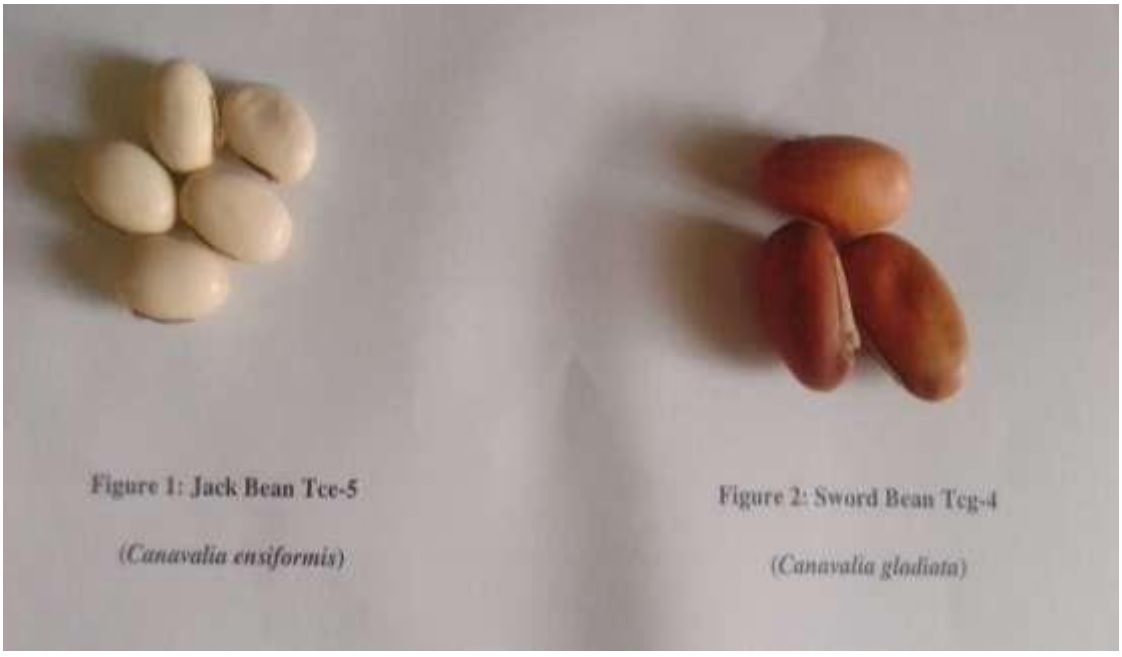

TABLE 1: Phytochemical Screening of Jack bean and Sword Bean

	Saponins	Tannins	Flavonoids	Cardiac glycoside	Terpenoids	Steroids	Anthraquinones	Alkaloids
Jack bean	++	+	+	-	-	+	-	+
Sword bean	++	+	++	-	+	+	-	+

+=Present ++ = Strongly present - = Absent

Tannins, steroids and alkaloids were present in both jack bean and sword bean, saponins were strongly present in jack bean and sword bean, flavonoids were strongly present in sword bean and present in jack bean. Terpenoids were present in sword bean but absent in jack bean while cardiac glycoside and anthraquinones were absent in jack bean and sword bean.

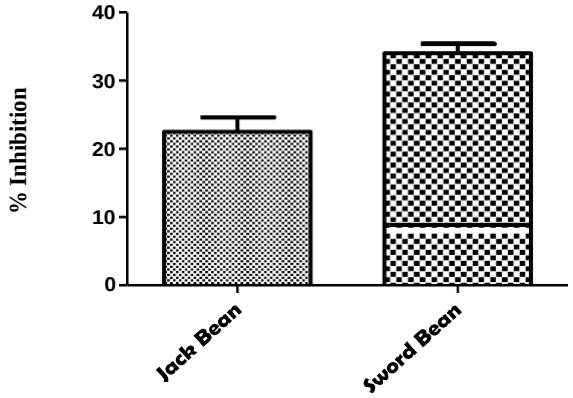

Figure 3: DPPH (2, 2-diphenyl -1- picrylhydrazyl) scavenging activities of jack bean and sword bean

This result showed that the aqueous extract of sword bean had a significantly higher ($P<0.05$) DPPH scavenging activity than the aqueous extract of jack bean (Figure 3)

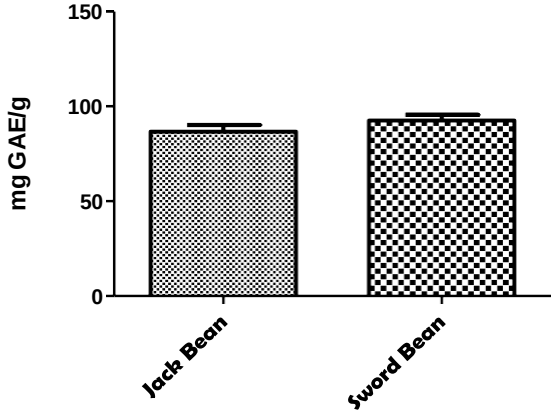

Figure 4: Total Phenolics Contents (TPC) of jack bean and sword bean

The results of total phenolic contents (TPC) expressed as GAE of the jack bean and sword bean seeds showed that these legumes are both rich in total phenolics (Figure 4). The levels of total phenolics in aqueous extracts of sword bean and jack bean were not significantly different.

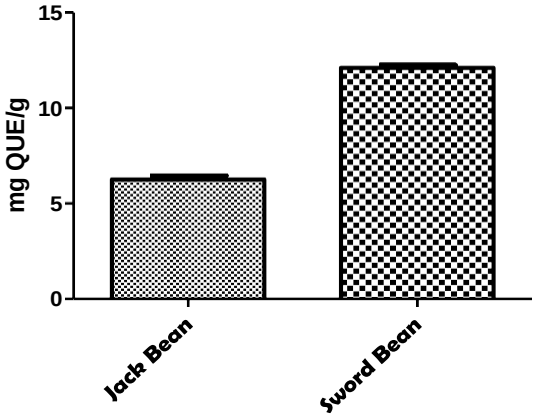

Figure 5: Total Flavonoids Contents (TFC) of jack bean and sword bean

The result of total flavonoids content (TFC) expressed as QUE of the jack bean and sword bean is shown in Figure 5.

It was observed that the aqueous extract of sword bean had a significantly higher ($P<0.05$) TFC than the aqueous extract of jack bean.

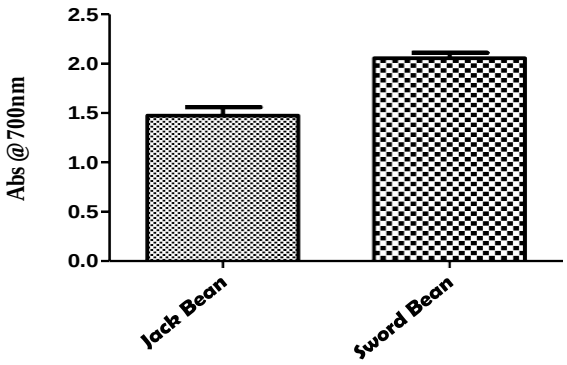

Figure 6: Reducing power capacity of jack bean and sword bean

The result of reducing power capacity is shown in Figure 6. The aqueous extract of sword bean had a significantly higher ($P<0.05$) reducing power capacity than the jack bean.

Phytochemicals are compounds that alleviate the risk of free radicals that cause oxidative damage to the living cells and result in common degenerative disorders like cancer and cardiovascular diseases (Liu, 2003; Boyer and Liu, 2004). Phenolic compounds are considered to be very important as antioxidants. Their antioxidant properties include; anti-carcinogenic, anti-oxidant, anti-tumoral, anti-microbial, anti-aggregant, anti-ischemic, anti-allergic, anti-mutagenic and anti-inflammatory effects, as well as being effective in alleviating cardiovascular diseases (De Rigal *et al.*, 2000; Kim *et al.*, 2003).

Results of phytochemical screening in this study revealed the presence of saponins, tannins, flavonoids, steroids and alkaloids in both jack bean and sword bean while cardiac glycosides and anthraquinones were absent in both jack bean and sword bean (Table 3). However, sword bean contains terpenoids which is absent in jack bean.

This result is similar to the work of Oke, (2014) who reported the phytochemicals constituents of pigeon peas (also a food legume) to include alkaloids, flavonoids, saponins, tannins and terpenes but anthraquinones, phlobatannins and sterols were not detected. Phytochemical screening is a very important tool in determining some biologically active and medicinal components of vegetables and plants.

These phytochemicals contribute to the free radical scavenging ability of plants. It has been hypothesized that bioactive components with antioxidant capacities present in foods may contribute to lower incidence of cardiovascular disease (Wang *et al.*, 2011). The presence of some phytochemicals supports the ethnomedicinal use of legumes in the treatment of diseases.

Phenolic compounds are responsible for the antioxidant activity of fruits due to their redox properties that allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Macheix *et al.*, 1999).

Foods rich in antioxidant activity are generally believed to be the best option in combating such disorders and health risks. The antioxidant properties of plants are attributed to their rich phytochemical composition.

Saponins exhibit antioxidant activities (Chan *et al.*, 2014; Chen *et al.*, 2014; Soetan *et al.*, 2015). Phenolic compounds are applied in the control of human pathogenic infections (Doughari, 2012).

Tannins are produced by condensation of simple phenolics having several molecular structures and are generally divided into hydrolysable and condensed proantho-cyanidins (polymers of flavan-3-ols) (Haslam, 1989). Tannins are biologically active compounds with beneficial nutritional effects. Phenolic substances can be found primarily in the seeds of certain pigmented cultivars of sorghum, millets and legumes (Salunkhe *et al.*, 1982). Condensed tannins, which are the predominant phenolic compounds in legume seeds occur widely in lentil, pea, coloured soybean and common bean and condensed tannins play important roles in the defense system of seeds that are at risk of oxidative damage by several environmental forces (Takahata *et al.*, 2001; Troszynska *et al.*, 2002; Amarowicz *et al.*, 2004).

Flavonoids are widely distributed in plants and their use as antioxidants or free radical scavengers have been widely documented (Kar, 2007; Ghasemzadeh and Ghasemzadeh, 2011).

Alkaloids play active roles in plants' defense against herbivores and pathogens, and are utilized as phytomedicines due to their biological activities (Madziga *et al.*, 2010; Doughari, 2012).

Antioxidant-rich foods have been reported to inhibit the formation of cell-to-cell adhesion factors (Beretz and Cazenave, 1988).

DPPH Scavenging Activity (2, 2-diphenyl -1-picrylhydrazyl)

DPPH is a free radical which is stable at room temperature, producing a violet solution in ethanol (Xu and Chang, 2007). DPPH assay

shows the ability of jack bean and sword bean extracts to convert 2, 2-diphenyl -1-picrylhydrazyl to its reduced form Diphenylpicrylhydrazine with the loss of its violet colour. The violet colour of DPPH is lost whenever it is mixed with a sample that has the ability to donate a hydrogen atom. It is converted to its reduced form with the loss of this violet colour (Alam *et al*., 2013; Olaiya *et al*., 2016). DPPH is widely applied in evaluating the antioxidant effects of natural compounds (Udenigwe *et al*., 2009). The use of DPPH radical scavenging activity has provided a rapid and easy method to assess antioxidant activity.

Total Phenolics Contents

Phenolics present in food perform their health benefits majorly through their antioxidant capacities (Fang *et al*., 2002). Phenolics can reduce oxygen concentration, scavenge singlet oxygen and hydroxyl radicals, bind metal ion catalysts, decompose primary oxidation products to non-radical species and are powerful chain breaking antioxidants (Shahidi and Naczk, 2003). Phenolic compounds play a contributory role in the overall antioxidant activities of plant foods.

Total Flavonoids Contents

Flavonoids are plant secondary metabolites found widespread in human and animals diets. They have many health benefits like antioxidant and anticancer effects (Umamaheswari and Chatterjee, 2008). Flavonoids from many plants have exhibited free-radical scavenging activities and protection against oxidative stress *in vitro* (Enujiugha *et al*., 2012). Flavonoids carry out their antioxidative abilities through free radical scavenging and inhibition of enzymes implicated in free radical generation (Benavente-Garcia *et al*., 1997).

Reducing Power

Reducing power activity is commonly used to assess the ability of natural antioxidants to donate electrons (Dorman *et al*., 2003), and reducing power activity is an important indicator of antioxidant capacity. It is based on the ability of antioxidants to donate electron, which results in the reduction of Fe^{3+} to Fe^{2+} (Ebrahimzadeh *et al*., 2008).

Gan *et al*. (2015) reported that legumes with red and black bean coats possess higher phenolic contents and hence higher antioxidant capacities than those with white bean coats. This is similar to the results in this study, with sword bean, possessing a red bean coat exhibiting higher antioxidant capacities than the jack bean with white bean coat. Gan *et al*. (2015) documented that

the red and black sword beans, particularly their bean coats, are good sources of antioxidant phenolics with potential health benefits. However, bioactive phytochemicals inherent in plant-based foods have many other powerful biological properties which may not be correlated with their antioxidant property, including induction of antioxidant defense systems *in vivo* or as modulators of gene expression (Carlsen *et al*. 2010).

4.CONCLUSIONS

This study concluded that jack bean and sword bean are rich in phytochemicals like flavonoids and saponins which should be exploited as medicinal foods for the benefits of human and animals. Jack bean and sword bean could be used as potential ingredients to formulate nutraceutical products for medical and veterinary applications. The *in vivo* antioxidant activities of jack bean and sword bean and their mechanism of actions need to be investigated.

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