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Evaluation of bioactive components in seeds of *Phaseolus vulgaris* L. (fabaceae) cultivated in Côte d'Ivoire

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ABSTRACT
Objectives: The aim of this study is to determine the active phytochemical constituents in common beans seeds (*Phaseolus vulgaris* L.) to ascertain the rationale for its use in traditional medicine. Likewise, the role of the bioactive components was related with a substantial decrease in chronically degenerative diseases.
Methodology and results: Aqueous extract of *Phaseolus vulgaris* seeds was concentrated using rotary evaporator. Phytochemical screening comprising chemical tests were realized to identify the functional groups, which have active principles. Bioactive components contents and glycemic index have been determined. Results indicated that seeds of *Phaseolus vulgaris* contain alkaloids, flavonoids, glycosides, polyphenols, saponins, steroids, tannins and terpenoids that are the main phytochemical groups with biological activities. Therefore, *Phaseolus vulgaris* seeds can be associated with a decrease risk for a wide variety of chronic and degenerative diseases such as cancer, obesity, cardiovascular diseases and diabetes. *Phaseolus vulgaris* seeds are considered as a good dietary source.
Conclusions and application of findings: Phytochemical screening showed that *Phaseolus vulgaris* seeds have some bioactive components such as alkaloids, anthocyanin, carbohydrate, catechin, fibers, flavonoids, phasine, phytic acid, quercetin, saponins, steroids, tannins and terpenoids and trypsin inhibit. Important biological activities have been described for these metabolites like enhancement of the bifidogenic, antioxidant, antimutagenic, anticarcinogenic and antihyperglycemic effects. Thus, we understand its regular consumption in many regions of world for food and medical uses. They could constitute an interesting alternative in fight against the diseases such as diabetes. Therefore, exploitation of the potentials of *Phaseolus vulgaris* seeds especially in areas of traditional medicine and pharmaceutical industries could be necessary. Likewise, the use of *Phaseolus vulgaris* seeds like food complement or diet food is to be wished. The role of some bioactive compounds present in *Phaseolus vulgaris* seeds related with a substantial decrease in metabolic diseases such as diabetes must be analyzed.
Key words: Active principles, Common beans, *Phaseolus vulgaris* L., Seeds, phytochemical constituents, phytochemical screening, functional groups.

INTRODUCTION

Since the beginning of human civilization, medicinal plants have been used by humanity for its therapeutic value. According to the World Health Organization in 2008, more than 80% of the world's population relied on traditional medicine for their primary healthcare needs. Plant compounds are of interest as a source of safer or more effective substitutes than synthetically produced bioactive component. Phytochemical progress was aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals. These procedures have shown that many substances originally thought to be rather rare in occurrence are of almost universal distribution in the plant kingdom. The drugs contained in medicinal plants are known as active principles. The bioactive principles are divided chemically into a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenoids (Ferreira et al., 2008). Medicinal plants produce bioactive compounds used mainly for medicinal purposes. These compounds either act on different systems of animals including man, and/or act through interfering in the metabolism of various affections. In either way the bioactive compounds from plants play a determining role in treatment of the various diseases. Therefore, the identification of bioactive compound in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods is important.

The genus *Phaseolus vulgaris* includes all species of legume seeds normally known as common beans. Archeological investigations showed that common beans originated on the American Continent, specifically in southern United States, Mexico, Central America, and the northern part of South America (Gepts & Dpbouk, 1991). In particular, the species *P. vulgaris* was introduced into Europe in the sixteenth century and since then it has become a very important crop in many regions of the world such as Africa. Dry common bean is a legume widely consumed throughout the world and it is recognized as the major source of dietary protein in many African countries (Guzman-Maldonado & Paredes-Lopez, 1998). A large variability exists in common bean seeds; color and size are two important quality characteristics for the consumers. Seed size and weight depend on genetic variations, cultivar and environmental conditions (Gonzalez de Mejia et al., 2005). The seed color of beans is determined by the presence and concentration of flavonol glycosides, anthocyanins, and tannins (Beninger & Hosfield, 2003; Aparicio-Fernandez et al., 2005). Recently, *P. vulgaris* is gaining increasing attention as a functional or nutraceutical food, due to its rich variety of phytochemicals with potential health benefits such as proteins, amino acids, complex carbohydrates, dietary fibers, oligosaccharides, phenols, saponins, flavonoids, alkaloids, tannins, among others (Geil & Anderson, 1994; Mishra et al., 2010). Important biological activities have been described for fibers, phenolic compounds, lectins, trypsin inhibitors, and phytic acid from common beans like enhancement of the bifidogenic effect (Queiroz-Monici et al., 2005); antioxidant (Heimler et al., 2005); anticarcinogenic (Hangen & Bennink, 2002) effects. *P. vulgaris* seeds have a notable place in the folklore throughout the world and in the traditions of many cultures such as pharmacotherapeutic effects (Hangen & Bennink, 2002; Mishra et al., 2010). Preclinical investigations have unanimously reported how the acute, repeated administration of extracts of *P.* vulgaris, as well as some of their isolated ingredient reduced food intake, body weight, and lipid accumulation in lean, diabetes and obese laboratory animals have been carried out on this plant. Thereby, the mode action of the *P. vulgaris* seeds producing the therapeutic effect can also be better investigated if the bioactive ingredients are characterized. Hence, the aim of this study is to determine the active phytochemical constituents in *P. vulgaris* and their role related with a substantial decrease in chronically-degenerative diseases.

MATERIALS AND METHODS

Sample collection and preparation: Samples of common beans (*Phaseolus vulgaris* L.) seeds were purchased from Abidjan (Côte d'Ivoire) markets. The seeds were air-dried for a period of seven days at room temperature. The air-dried stem-bark and leaves were ground to 0.50 mm mesh size.

Sample extraction: Powdered common beans seeds were weighed 100 g. About was kept in 300 ml distilled water were added. The mixture was left for 3 h with occasional shaking. The samples were taken in triplicate. The extracts (100 ml) were obtained using a sieve cloth and concentrated to 5-10 ml using rotary evaporator.

Phytochemical analysis of seeds extract: Phytochemical screening comprising chemical tests to detect the presence of alkaloids, flavonoids, glycosides, polyphenols, saponins, steroids, tannins and terpenoids were realized according to the methods described by several authors (Eogona et al., 2005; Konkon et al., 2010). We also realized the determination of some bioactive compounds contents by standard procedures (Henningson et al., 2001; Sharma et al., 2003). Glycemic index (GI) has been achieved according to Jenkins et al. (1981) method. Wistar rats of 4-5 weeks old rats fed with seeds of common beans and with glucose as food reference were used for this study.

Statistical Analysis: Data were analyzed using Statistica software (release 7.5). Differences in mean values were tested by analysis of variance, and significance levels were obtained with Duncan's test. A significance level of <0.05 was used. Data are the means of three replicates.

RESULTS AND DISCUSSION

The result of the phytochemical screening indicated that the seeds of *Phaseolus vulgaris* contain alkaloids, flavonoids, glycosides, polyphenols, saponins, steroids, tannins and terpenoids which are the main phytochemical groups with biological activities (Table 1).

Table 1: Detection of phytochemical constituents of extracts of *Phaseolus vulgaris* seeds

Phytochemicals	Presence / Absence
Alkaloids	+
Flavonoids	+
Glycosides	+
Polyphenols	+
Saponins	+
Steroids	+
Tannins	+
Terpenoids	+

Alkaloids, comprising a large group of nitrogenous compounds are widely used as cancer chemotherapeutic agents (Chabner & Horwitz, 1990). Alkaloids also interfere with cell division; hence the presence of alkaloids in the plant makes it a possible remedy in the treatment of cancer. Glycosides have been found to be effective in congestive heart failure, regardless of the cardiac rhythm and that the beneficial effect is brought about by its direct action to increase the force of myocardial contraction. It also acts directly on the smooth muscles of the vascular system. They exert a number of effects on neural tissues and this indirectly influence the mechanism and electrical activities of the heart, and modify vascular resistance and capacitance (Branndwald et al., 1961). The fore-going would suggest the use of *P. vulgaris* as a cardiac tonic. Saponins are glycosides of both triterpenes and sterols having hypertensive and cardiac depressant properties (Trease & Evans, 1985), hence the presence of these metabolites in *P. vulgaris* seeds tend to support its medical uses. The result of the phytochemical screening revealed that tannins, flavonoids are present in the seeds. This could be responsible for their antibacterial properties as reported by Sofowora (1993). The presence of tannins also, showed that the seed could be used as purgative, cough, asthma and hay fever according Gills (1992). Furthermore, Tsuda et al. (1994) reported the antioxidant activity of extract from *P. vulgaris* seeds. By using a fluorescence assay with liposomes and 3-[4-(6-phenyl)-1,3,5-hexatrienyl]phenylpropionic acid, Beninger & Hosfield (2003) showed that pure flavonoid

compounds such as anthocyanins, quercetin glycosides and tannins, present in the seed displayed antioxidant activity.
Afterwards, the extraction of the secondary metabolites in *P. vulgaris* seeds showed that phytoconstituents content were significantly different. Carbohydrate (46%) was the highest followed by starch resistant (32.31%), proteins (16.5%) and fibers (5%). They are the major compounds, while catechin, saponins, anthocyanin, quercetin have low contents, they can be regarded as intermediate compounds. Trypsin inhibitor (0.05%), phasine and phytic acid (0.02%) were significantly low; they are the minor *P. vulgaris* seeds compounds. Carbohydrates constitute the main fraction of *P. vulgaris* seeds. Of these, starch and non- starch polysaccharides (dietary fiber) are the major constituents, with smaller but significant amounts of mono, di and oligosaccharides (Bravo et al., 1998). *Phaseolus vulgaris* seeds contain slow digested carbohydrates and high proportion of non-digested carbohydrates that might be fermented in the large intestine. Non-digested carbohydrates reaching the colon include mainly resistant starch, soluble and insoluble dietary fiber, and non-digestible oligosaccharides (Henningson et al., 2001). The non-digested carbohydrates are associated with a low glycemic response, low serum cholesterol levels, and a decrease of colon cancer risk factors (Serrano & Goni, 2004). The physiological effects of non-digested carbohydrates from common beans may be related to colonic fermentation end products, short chain fatty acids, such as acetic, propionic and butyric acids, and the content and distribution of short chain fatty acids are dependent on the microflora and the carbohydrate substrate at the intestinal tract (Cumming & Macfarlane, 1997). It has been suggested that resistant starch exerts a sparing effect on dietary fiber on fermentation in the colon, which means that bacteria prefer to ferment resistant starch than to ferment fiber (Henningson et al., 2001). Several studies indicate that the long-term consumption of a diet with a high glycemic load is a significant independent predictor of the risk of developing type 2 diabetes. More recently, evidence has been accumulating that a low glycemic index (GI) diet might also protect against the development of obesity and therefore of diabetes (Ludwig, 2000). The glycemic index (GI) was introduced by Jenkins *et al*. (1981) to express the rise of blood glucose after eating a meal against a standard blood glucose curve after glucose or white bread in the same subject (Table 2).

Table 2: Compounds content determination of *Phaseolus vulgaris* seeds

Compounds	Content (mg/100 g of dry seeds)	Rate (%)
Carbohydrates	62,900.0 [a]	46.07 [j]
Proteins	22,400.0 [b]	16.50 [a]
Starch resistant	44,120.0 [c]	32.31 [b]
Fiber	6,900.0 [d]	5.05 [c]
Catechin	61.0 [e]	0.040 [d]
Saponins	56.0 [ef]	0.040 [f]
Anthocyanins	45.0 [f]	0.030 [i]
Quercetin	31.0 [g]	0.030 [i]
Trypsin inhibitor	07.0 [h]	0.005 [e]
Phasine	04.0 [i]	0.002 [n]
Phytic acid	03.0 [i]	0.002 [n]

Values are means of three independent measurements. In column values with different Superscripts (lowercase letters) in a row are significantly different at the level of $p<0.05$.

In this regard, beans have a low GI 30 related to glucose (Boby & Leelamma, 2003), whereas those of rice, whole meal bread and baked potatoes reach 50, 77 and 85, respectively (Foster-Powell et al., 2005). Low-GI diets improved adipocyte insulin-mediated glucose uptake *in vitro* and was found to be useful in normalizing diet-insulin responses of hyperinsulinaemic subjects (Behall & Howe, 1995). The literature suggests that a 10% fall in the GI of a diet would result in a 30% increase in insulin sensitivity. Low-GI diets have been shown to reduce fasting triacylglycerol and non-esterified fatty acid concentrations (Jenkins et al., 2002). In addition, these diets increase HDL-cholesterol and decrease total cholesterol, while improving *in vivo* and *in vitro* insulin-mediated glucose uptake. Prospective studies have demonstrated that low-GI carbohydrates improve insulin sensitivity in subjects with diabetes, obesity and cardiovascular disease (Rizkalla et al., 2002). Important biological activities have been also described for polyphenolic compounds such as catechin, anthocyanin and quercetin, proteins and phytic acid like enhancement of the bifidogenic, antioxidant, antimutagenic, anticarcinogenic effects; as well as an antihyperglycemic effect (Hangen & Bennink, 2002; Sharma et al., 2003; Heimler et al., 2005; Queiroz-Monici et al., 2005; Mishra et al., 2010).

These various assertions reveal that *P. vulgaris* seeds can intervene in several diseases treatment. Thus, regularly consummation of *P. vulgaris* seeds could have beneficial effects on human health. However, although trypsin inhibitors are protease inhibitors that are considered as antinutritional factors, to our knowledge there is no scientific evidence about their role in human health benefits. Most of the biological benefits are reported for other legumes such as soybean, lentil, and tepary beans (*Phaseolus acutifolius*). The content of trypsin inhibitors in different cultivars of *P. vulgaris* depends of the genotype, growing site and/or environmental conditions (Gonzalez de Mejia et al., 2005). However, trypsin inhibitors are heat sensitive and some different technological methods have been development to inactive them, such as the traditional or home cooking, autoclaving, extrusion, toasting and microwave (Sayeed & Njaa, 1985). Trypsin inhibitors have bifidogenic effect according (Queiroz-Monici et al., 2005), so its play an important role in human health.

CONCLUSION

Common beans (*Phaseolus vulgaris* L.) seeds have some bioactive components related with health benefits, such as alkaloids, anthocyanin, carbohydrate, catechin, fibers, flavonoids, phasine, phytic acid, quercetin, saponins, steroids, tannins and terpenoids and trypsin inhibitors; however, there is still more to learn about the mechanism of those bioactive compounds on chronic-degenerative diseases. Together, these data suggest that seeds of *P. vulgaris* may constitute potentially interesting, novel remedies for the treatment of overweight and metabolic syndrome such as diabetes. Undoubtedly, this area of research holds considerable potential on nutraceutical foods. Future studies, designed to confirm and extend those currently available in literature, are needed. Likewise, the need to exploit the potentials of *Phaseolus vulgaris* seeds especially in areas of traditional medicine and pharmaceutical industries arises.

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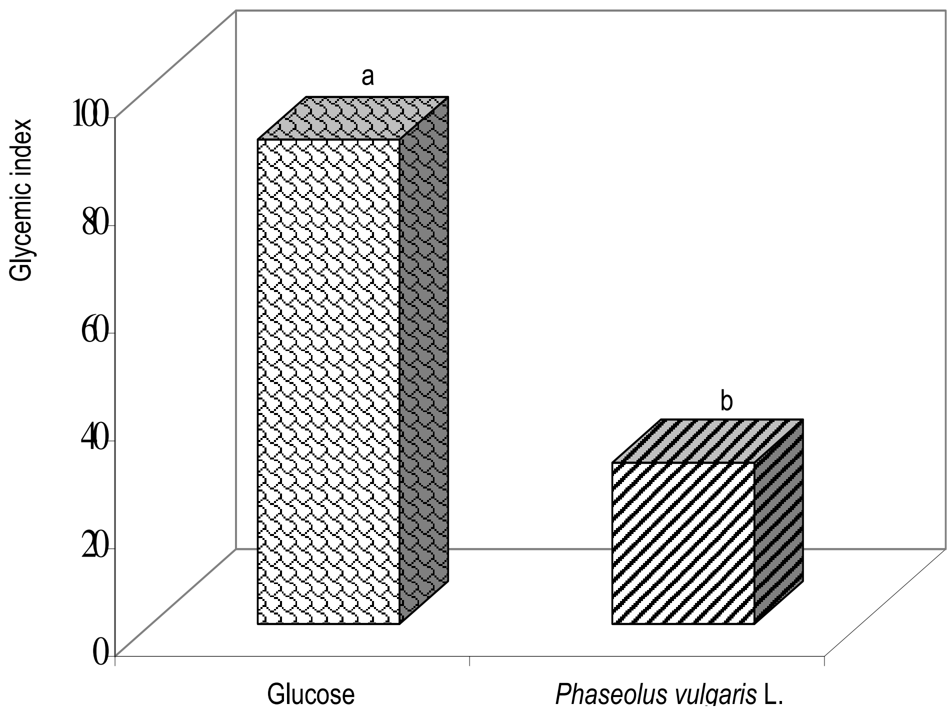

Figure 1: Glycemic index of glucose and extract of *Phaseolus vulgaris* seeds

Values are means of three independent measurements. Values with different superscripts (lowercase letters) in a row are significantly different at the level of $p<0.05$.