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Original Research Paper

Screening of phytochemicals in bean grains (*Phaseolus and Vigna spp.)* methanolic extracts by thin layer chromatography technique

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ABSTRACT

The aim of the study was to screen the phytochemicals of bean grains *Phaseolus vulgaris* (common bean), *Phaseolus lunatus* (black-eyed), *Phaseolus lunatus* (large seed with white coat), *Vigna unguiculata* (Cowpea) and *Vigna sesquipedalis* (Yardlong). Total phenol content ranged between 2.84 and 13.04 [mg GAE/g] about a mean of 11.27 [mg GAE/g]. Total flavonoids content also ranged from 2.30 to 14.30 mgRUE/g with a mean of 13.41 mgRUE/g. Alkaloids were present in all the extracts of the samples with exception of white seeded P*haseoluslunatus* which did not show any activity. Flavonoids, saponin and terpene were present in all methanolic extracts of all the four samples. A total of 17 different bands were recorded for flavonoids with R_f values ranging between 0.06 and 0.82. Saponins gave a total of 19 different bands with R_f values ranging from 0.08 to 0.93. A total of 15 different bands were recorded for terpenes with R_f values ranging from 0.05 to 0.91. From the phytochemical analysis and the thin layer chromatograph profiles the methanolic extracts of the grain legumes investigated are of medicinal values.

Key words: *Grain Legumes, Alkaloids, Flavonoids, Saponins, Terpenes, Chromatography*.

INTRODUCTION

Legumes are second to the gramineae in their importance to humans (Graham and Vance, 2003). Generally legume seeds contain 20 to 30% protein and they are rich in essential amino acids. Legumes have the ability to develop nodules and to fix atmospheric nitrogen in symbiosis with compatible rhizobia. They complement the nutritional profiles of cereals and tubers in diet of humans (Durant and Gius, 1997). Legumes can be used in traditional food and forage. They also have industrial uses for the preparation of biodegradable plastics (Paettau *et al.,* 1994).

The separation and purification of plant constituents are mainly carried out on chromatographic techniques based on their size, shape, or charge (Adejumo and Awosanya, 2005). Thin layer chromatography (TLC) is the most widely used for all the simple chromatographic methods for the analysis of mixtures (Hahn-Deinstrop, 2000). It is widely adopted for the rapid and positive analysis of drugs and drug preparations. TLC provides a chromatographic drug fingerprint in very short time. It is therefore suitable

for monitoring the identity and purity of drugs, and for detecting adulterations and substitutions. It is also used to analyze drug combinations and phytochemical preparations (Wagner, *et al.,* 1984).The TLC is also very useful for preliminary study before other instrumental techniques (Mohammad *et al.,* 2010; Braz *et al.,* 2012).

Grain legumes are known for their role as food for humans or feed for animals. Though some have been useful in folk medicine, for instance, isoflavones from soybeans and other legumes have been suggested to reduce the risks of cancer and lower serum cholesterol (Kennedy, 1995), there is still need to exploit grain legumes on the basis of their medicinal properties.

The study aims at the determination of the total phenol and flavonoid contents of five grain legumes and also assess their medicinal properties by the application of their thin layer chromatography.

MATERIALS AND METHODS

Materials

The five experimental materials for the study were *Phaeolus vulagaris* (with red seed coat), Phaseolus lunatus (black-eyed), Phaseolus lunatus (large seed with white coat), Vigna unguiculata (black eyed) and Vigna sesquipedalis (red seed coat). The seeds of these five legumes were collected from the seed bank of the Department of Botany, University of Ghana. Standards of the flavonoids and phenols were obtained from Sigma Aldrich Co. (St. Louis, USA). All other chemicals were of the analytical grade.

Preparation of sample

Samples were prepared by following a modified approach by Tsai *et al.* (2009). The samples were pulverized into fine powder. Ten grams of the pulverized samples were extracted with 100 ml of methanol at 25°C for 24 hours and filtered through Whatman No. 1 filter paper. The residue was extracted with two additional 100 ml portions of methanol as described above and combined methanolic extracts were concentrated under reduced pressure below 40°C to obtain the crude extract. The crude extracts were dissolved again in methanol at a concentration of 20 mg/ml and stored at 4°C for further analyses.

Determination of total phenol content

Total phenolic content in the methanolic extracts was determined by using Folin-Ciocalteau reagent based on modified version of the method by Harborne (1989). Each sample (150 µl, 10 mg/ml) was added with 1200 µl distilled water and 450 µl aqueous sodium carbonate solution. One hundred microliters of Folin-Ciocalteu reagent was added to the mixture and agitated. The mixture was allowed to stand for 90 minutes and the absorbance was measured at 760 nm by using UV/visible spectrophotometer (SpectraMax Plus384, United States, CA). The concentration of total phenolic compounds was calculated based on standard curve of gallic acid (0.2-1.0 mg/ml) with the linear equation, $y = 0.624x - 0.939$, where $R2 = 0.995$. The results were expressed as µg of gallic acid equivalent (µg GAE) per gram of the extracts.

Determination of total flavonoid content

The modified aluminium chloride colorimetric method by Barros *et al.,* (2007) was used to determine flavonoid content. Extract (100 µl, 10 mg/ml) of the test legumes was mixed with distilled 500 µ water and sodium nitrite, NaNO2 (5%, 30 µl). The mixture was allowed to stand for 5 minutes. Aluminium chloride solution, $AlCl3.H_2O$ (10%, 60 µl) was added to the mixture and left for 6 minutes. Sodium hydroxide, NaOH (1M, 200 µl) and 110 µl distilled water were added to the solution and mixed well. Absorbance of the solution was measured at 510 nm (SpectraMax Plus384, United States) and the concentration of total flavonoids content was calculated based on standard curve of rutin (0.2-1.0 mg/ml) with the linear equation $y = 0.0101x + 0.2238$, where $R^2 = 0.9563$. The results were expressed as µg of rutin equivalent (µg /RE) per gram of the extracts.

Thin layer chromatography (TLC)

Methanolic extracts of the samples were separated on silica gel thin layer aluminium plates of 15x5 cm with 3 mm thickness. Extracts were spotted manually using capillary tube. Solvent systems used for the separation of the following phytochemical compounds alkaloids, flavonoids, saponins and terpenes were a mixture of chloroform and diethylamine (9 : 1), a mixture of chloroform and ethylacetate (6 : 4), a mixture of chloroform : methanol : distilled water : toluene (8 : 1 : 0.5 : 0.5) and toluene, respectively.

After separation of the phytochemicals, the various compounds were identified by using the following spray reagents: 10% ethanolic sulphuric acid (for alkaloids detection), 5% ferric chloride solution (for flavonoids detection) and vanillin sulphuric acid (for saponin and terpene detection). Colour of the spots was noted and retention factor (R_f) values calculated by using the following formula:

$$R_f = \frac{Distance\ travelled\ by\ solute}{Distance\ travelled\ by\ solvent} x\ 100$$

RESULTS AND DISCUSSION

Total phenols and flavonoids content

The colour of band for alkaloids was grey. Estimates for

Table 1. Mean concentration of total flavonoids and total phenols of five legumes.

Legume	Polyphenols	
	Total Flavonoids (mg RUE /g extract)	**Total Phenols (mg GAE/g extract)**
Phaseolus vulgaris.	14.33	4.67
Vigna unguiculata (black-eyed)	3.56	3.77
Vigna sesquipedalis	12.57	13.04
Phaseolus lunatus (black-eyed)	2.38	3.47
Phaseolus lunatus (large seed with white coat)	9.37	2.84
Lsd	0.011	0.141

Table 2. TLC fingerprint for alkaloids in methanolic extracts of five legumes.

		LEGUMES				
Fraction	**R_f values**	***Phaseolus vulgaris***	***Vigna unguiculata***	***Vigna sesquipedalis***	***Phaseolus lunatus***	***Phaseolus lunatus***
A	0.13	+	+	+	-	-
B	0.30	-	-	-	+	-
C	0.51	+	+	+	+	-
D	0.66	-	+	+	-	
E	0.67	+	-	-	-	-
F	0.95	+	+	+	-	-
Total		4	4	4	2	0

'+' present; '-'absent

total phenol content and total flavonoid content for the species are presented in Table 1. The highest content of 13.04 mg GAE / g of phenol was recorded for Vigna sesquipedalis. Extracts from white large seeded P. lunatus had the lowest value of 2.84 mg GAE / g. The highest flavonoid content of 14.33 mg/g RUE was recorded by *Phaseolus vulgaris*, while the lowest of 2.38 mg GAE / g was recorded in black-eyed P. lunatus.

TLC profile for alkaloid

TLC fingerprint for alkaloid is presented in Table 2. The band was brown in colour. A total of six different bands were present in the four Phaseolus species. Out of the total of six bands four different types were present in *Phaseolus vulgaris,* black-eyed P. lunatus and V. sesquipedalis; two were present in black-eyed V. unguiculata. No alkaloid activity was detected in large white seeded P. lunatus. The six different bands ranged between Rf values of 0.13 and 0.95. The band with Rf value of 0.30 was only present in black-eyed Phaseolus lunatus. The band with R_f value of 0.67 was also present in only *P. vulgaris*.

TLC profile for flavonoid

Table 3 shows the TLC profile for flavonoids. A total of 17 different bands were recorded with R_f values that ranged between 0.06 and 0.82. Six different bands were recorded by *P. vulgaris,* four bands with the following R_f values were unique to *Phaseolus vulgaris*, 0.25, 0.32, 0.47 and 0.82. Extracts from *V. unguiculata* recorded a total of seven bands with five unique bands with the following R_f values: 0.06, 0.18, 0.22, 0.31 and 0.46. Extracts of *V. sesquipedalis* recorded a total of four bands while extracts of black-eyed P. lunatus recorded a total of five bands with two unique bands of Rf values 0.40 and 0.76, respectively. Extracts of large white seeded coat P. lunatus recorded a total of bands with two unique bands with Rf values of 0.28 and 0.77, respectively.

TLC profile for saponin

Table 4 shows the TLC fingerprint profile for saponins. A total of 19 different bands were recorded with R_f values ranging from 0.08 to 0.93. Extracts of *Phaseolus vulgaris* recorded a total of five different bands with three unique bands with R_f values 0.25, 0.61 and 0.86, respectively. A total of six different bands were recorded by extracts of black-eyed *V. unguiculata* with two unique bands with R_f values of 0.09 and 0.58, respectively. Extracts of V. *sesquipedalis* recorded a total of seven bands three of which are unique with the following R_f values: 0.08, 0.13

Table 3. TLC fingerprint for flavonoid in methanolic extracts of five legumes.

		LEGUMES				
Fraction	**R_f values**	***Phaseolus vulgaris***	***Vigna unguiculata***	***Vigna sesquipedalis***	***Phaseolus lunatus***	***Phaseolus lunatus***
A	0.06	-	+	-	-	-
B	0.18	-	+	-	-	-
C	0.21	+	-	+	+	+
D	0.22	-	+	-	-	-
E	0.25	+	-	-	-	-
F	0.28	-	-	-	-	+
G	0.31	-	+	-	-	-
H	0.32	+	-	-	-	-
I	0.33	-	-	+	+	-
J	0.35	+	+	-	-	+
K	0.40	-	-	-	+	-
L	0.46	-	+	+	+	-
M	0.47	+	-	-	-	-
N	0.76	-	-	-	+	-
O	0.77	-	-	-	-	+
P	0.81	-	+	+	-	-
I	0.82	+			-	-
Total		6	7	4	5	4

'+' present; '-'absent

Table 4. TLC fingerprint for saponins in methanolic extracts of five legumes.

		LEGUMES				
Fraction	**$R_f$values**	***Phaseolus vulgaris***	***Vigna unguiculata***	***Vigna sesquipedalis***	**Lima bean Blackeyed**	**Lima bean white (large)**
A	0.08	-	-	+	-	-
B	0.09	-	+	-	-	-
C	0.13	-	-	+	-	-
D	0.15	-	-		+	-
E	0.21	-	+	+	+	-
F	0.22	-	-	-	-	-
G	0.25	+	-	-	-	-
H	0.36	-	-	-	-	+
I	0.41	-	-	-	-	+
J	0.57	-	-	+	-	-
K	0.58	-	+	-	-	-
L	0.59	-	-	-	+	-
M	0.61	+	-	-	-	-
N	0.63	-	-	-	-	+
O	0.64	-	-	-	+	-
P	0.72	+	+	+	+	-
Q	0.84	-	+	+	-	+
R	0.86	+	-	-	-	-
S	0.93	+	+	+	+	-
Total		5	6	7	6	4

'+' present ; '-' absent

and 0.57. Extracts of black-eyed *P. lunatus* recorded a total of six different bands three of which were unique and had R_f values of 0.15, 0.59 and 0.64, respectively. A total of four different bands were recorded by extracts from large white seeded *P. lunatus* three of which were unique with R_f values 0.36, 0.41 and 0.63, respectively.

TLC profile for terpene

Table 5 shows the TLC fingerprint profile for terpenes. A

Table 5. TLC fingerprint for terpene in methanolic extracts of five legumes.

		LEGUMES				
Fraction	**R_f values**	***Phaseolus vulgaris***	***Vigna unguiculata***	***Vigna sesquipedalis***	***Phaseolus lunatus***	***Phaseolus lunatus***
A	0.05	-	-	+	-	-
B	0.07	-	+	-	+	+
C	0.08	+	-	-	-	-
D	0.12	-	-	-	-	+
E	0.13	+	+	+	+	-
F	0.16	-	-	-	+	-
G	0.17	+	-	-	-	-
H	0.20	-	-	+	-	-
CI	0.21	+	+	-	-	-
J	0.44	+	-	-	-	-
K	0.46	-	+	+	+	-
L	0.49	-	-	-	-	+
M	0.64	-	-	-	+	-
N	0.65	+	+	+	-	-
O	0.91	-	+	-	-	-
Total		6	6	5	5	3

'+' present ; '-' absent

total of 15 different bands were recorded with R_f values ranging from 0.05 to 0.91. Extracts of *Phaseolus vulgaris* recorded a total of six different bands with three unique bands with R_f values 0.08, 0.17 and 0.44, respectively. A total of six different bands were recorded by extracts of black-eyed *V. unguiculata* with two unique bands with R_f values of 0.07 and 0.91, respectively. Extracts of *V. sesquipedalis* recorded a total of five bands two of which are unique with the following R_f values: 0.05 and 0.20, respectively. Extracts of black-eyed *P. lunatus* recorded a total of five different bands two of which were unique and had R_f values of 0.16 and 0.64, respectively. A total of three different bands were recorded by extracts from large white seeded *P. lunatus* two of which were unique with R_f values 0.12 and 0.49, respectively.

Alkaloids are synthesized form of amino acids. They contain one or more N atoms as constituents of heterocytes. They are stored in protonated form and are mostly found in the vacuole, which is acidic. They have multiplicity of host-mediated biological activities, including antimicrobial (Deepak *et al.,* 2012; Tylor, 2011), cytotoxic (Hanita *et al.,* 2013), analgesic and antipyretic activities (Semwal *et al.,* 2011). Flavonoids are phenolic compounds. They consist of 15 carbon atoms in C6-C3-C6 basic carbon skeleton. They exist in the form of glycosides and are accumulated in vacuole and chromoplast of plants. They are responsible for the colouration of flowers and leaves. They have the properties of antibacterial (Elzbieta *et al.,* 2013), antioxidant (Magdalena *et al.,* 2013), anticancer (Ali *et al.,* 2013), anti-inflammatory, antipyretic and analgesic activities (Emad, 2013). Saponins are glycosidic triterpenoids and they are widely found in plants. They have foaming properties. Saponins consist of polycyclic aglycones attached to one or more sugar side chains. They are water soluble and have bitter taste. There are three major classes of saponins and they are steroid glycosides, steroid alkaloid glycosides and triterpene glycosides the largest group. They are found in most vegetables, beans and herbs. They have the pharmacological properties like anti-inflammatory and antipyretic activities (Emmanuel *et al.,* 2012; Adiukwu *et al.,* 2013). Terpenes have been found in resins, latex, waxes and oils and they have the property that makes plants toxic or indigestible as a defense measure against herbivores. They also serve as antibiotics that protect the plants from pathogenic microbes. Terpenes are used commercially as aroma substances for food beverages and cosmetics, vitamins (A,D and E), natural insecticides (example, pyretrin), solvents (example, turpentine) and as rubber and gutta-percha. They are reported to have antimicrobial, antioxidant (Sankhadip *et al.,* 2010), anticancer (Chonthicha *et al.,* 2013) and antiparasitic activities (Ifedaya *et al.,* 2013).

Conclusion

Generally, alkaloids gave the lowest number of bands. No alkaloid activity was observed in the white seeded *Phaseolus lunatus*, while only 2 bands were observed in the black-eyed *Phaseolus lunatus*. Relatively, *P. lunatus* seeds gave lowest alkaloid activities. For each of the experimental materials, unique bands were recorded for each of the phytochemical activities. The TLC study has shown that the experimental materials have considerable levels of phytochemical activities for alkaloids, flavonoids, saponins and terpenes which have proven to have

medicinal values.

It is recommended that seeds of the grain legumes must be cultivated and incorporated into the human diets for the improvement of their health status.

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