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Phytochemical investigation and thin layer chromatography of methanolic extracts of some selected grain legumes

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Abstract
The experiment was conducted on the following grain legumes: *Phaseolus vulgaris*, two varieties of *Phaseolus lunatus*, *Vigna unguiculata* and *Vigna sesquipedalis*. It was conducted to characterize them on the basis of their phytochemical properties. Total phenol content ranged between 2.84 and 13.04 mg/g GAE about a mean of 11.27 mg/g GAE. Total flavonoid content also ranged from 2.30 to 14.30 mg/g RUE with a mean of 13.41 mg/g RUE. Alkaloid was present in all the extracts of the samples with exception of white seeded *Phaseolus lunatus* which did not show any activity. Flavonoid, saponin and terpene were present in all methanolic extracts of all the four samples. A total of 17 different bands were recorded for flavonoid with R_f values ranging between 0.06 and 0.82. Saponin 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.

Keywords: Grain legumes, alkaloids, flavonoids, saponins, terpenes, thin layer chromatograph profile.

Introduction
Legumes are second to the Gramineae in their importance to humans (Graham and Vance, 2003) [12]. Generally legume seeds contain 20% to 30% protein and they are rich in lysine. 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) [8]. Legumes can be used in traditional food and forage. They also have industrial uses for the preparation of biodegradable plastics (Paettau *et al*., 1994) [20].
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) [1]. Thin layer chromatography (TLC) is the most widely used for all the simple chromatographic methods for the analysis of mixtures (Hahn-Deinstrop, 2000) [13]. 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) [25]. The TLC is also very useful for preliminary study before other instrumental techniques (Mohammad *et al*., 2010; Braz *et al*., 2012) [19, 5].
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) [17], there is still the need to exploit grain legumes on the basis of their medicinal properties.
The aim of this study was to characterize four different legumes to assess their level of medicinal properties by the application of the thin layer chromatography.

Materials and methods
Preparation of sample
Samples were prepared by following a modified approach by Tsai *et al*. (2009) [23]. The samples were pulverized into fine powder. Ten grams of the pulverized samples was extracted with 100 ml of methanol at 25 °C at 20g 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-Ciocalteu reagent based on modified version of the method by Harborne (1989) [26]. 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 (Spectra Max Plus384, United States). 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 $R^2 = 0.995$. The results were expressed as µg of gallic acid equivalent (GAE/µg) per gram of the extracts.

Determination of total flavonoid content

The modified aluminium choride colorimetric method by Barros *et al*. (2007) [4] was used to determine flavonoid content. Mushroom extract (100 µl, 10 mg/ml) was mixed with distilled 500 µ water and sodium nitrite, $NaNO_2$ (5%, 30 µl). The mixture was allowed to stand for 5 minutes. Aluminium chloride solution, $AlCl_3.H_2O$ (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 (Spectra Max 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 (RE/µg) 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 compounds, reagents such as iodine and Dragendorff were used to identify the compounds. 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 phenol and flavonoids content

Estimates for total phenol content and total flavonoid content for the species are presented in Table 1.The highest content of 13.04 mg/g GAE of phenol was recorded for *Vigna sesquipedalis*. Extracts from *P. lunatus* (large seed with white coat) had the lowest value of 2.84 mg/g GAE. The highest flavonoid content of 14.33mg/g RUE was recorded by *Phaseolus vulgaris*, while the lowest of 2.38 mg/g RUE was recorded by *P. lunatus*. All the four legumes differed significantly among themselves.

TLC profile for alkaloid

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

TLC profile for flavonoid

Table 3 shows the TLC profile for flavonoid. 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. sp, 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 *P. lunatus* (black-eyed) recorded a total of five bands with two unique bands of R_f values 0.40 and 0.76, respectively. Extracts of *Phaseolus lunatus* (large seed with white coat) recorded a total of four different bands with two unique bands with R_f values of 0.28 and 0.77, respectively.

TLC profile for saponin

Table 4 shows the TLC fingerprint profile for saponin. 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* (black-eyed) 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 and 0.57. Extracts of *P. lunatus* (black-eyed) 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 *P. lunatus* (large seed with white coat) 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 terpene. A 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 *V. unguiculata* (black-eyed) 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 *P. lunatus* (black-eyed) 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 *P. lunatus* (large seed with white coat) 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 (Tylor, 2011) [24], cytotoxic (Hanita *et al*., 2013) [14], analgesic and antipyretic activities (Semwal *et al*., 2011) [22]. 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) [9], antioxidant (Magdelena *et al*., 2013), anticancer (Ali *et al*., 2013) [3], anti-inflammatory, antipyretic and analgesic activities (Emad, 2013) [10]. 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. There 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) [11, 2]. 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 (e.g. pyretrin), solvents (e.g. turpentine) and as rubber and gutta-percha. They are reported to have antimicrobial, antioxidant (Sankhadip *et al*., 2010) [21], anticancer (Chonthicha *et al*., 2013) [8] and antiparasitic activities (Ifedaya *et al*., 2013) [16].
In conclusion, the grain legumes investigated may have some level of medicinal activities.

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

Legume	Polyphenols	
	Total Flavonoids (mg/g extract) in RUE	**Total Phenols (mg/g extract) in GAE**
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* (black-eyed)**	***Phaseolus lunatus* (black-eyed)**	**Lima bean Blackeyed**	***Phaseolus lunatus* (large seed with white coat)**
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

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

			Legumes			
Fraction	**R_f values**	***Phaseolus vulgaris***	***Vigna unguiculata* (black-eyed)**	***Phaseolus lunatus* black-eyed**	**Lima bean Blackeyed**	***Phaseolus lunatus* (large seed with white coat)**
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* (black-eyed)**	***Vigna sesquipedalis***	***Phaseolus lunatus* black-eyed**	***Phaseolus lunatus* white (large seed with white coat)**
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

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

			Legumes			
Fraction	**R_f values**	***Phaseolus vulgaris***	***Vigna unguiculata* (black-eyed)**	***Vigna sesquipedalis***	***Phaseolus lunatus* black-eyed**	***Phaseolus lunatus* white (large)**
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

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