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Full Length Research Paper

Comparative evaluation of phytochemicals in the raw and aqueous crude extracts from seeds of three *Lablab purpureus* varieties

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The phytochemicals in the raw and aqueous crude extracts from seed of three varieties of *Lablab purpureus* (LP), including Rongai brown, Rongai white and Highworth black were comparatively evaluated as a preliminary study for their pharmacological potential. The phytochemicals analyzed were trypsin inhibitors, hydrogen cyanide, oxalate, haemagglutinin units, phytate, tannin, saponin and alkaloids. The study concluded that raw (LP) contained higher concentration of phytochemicals than the aqueous crude extracts of (LP) in the three varieties studied.

Key words: Phytochemicals, *Lablab purpureus*, pharmacological, crude extract.

INTRODUCTION

Lablab purpureus (lablab beans) is an under-utilized legume that has potential as human food (Bawa et al., 2003). It is a legume that thrives well during the dry season between November and February in Northern Nigeria. It is drought resistant and is usually sown after the normal cropping season, thereby acting as a buffer crop for ruminant feeding during the dry season (Adu et al., 1992). *L. purpureus* is reported to have certain medicinal properties (Handa et al., 1989; Adeleke et al., 2012). Phytochemicals include secondary metabolites of plants, and other antinutritional factors, which are reported to be pharmacologically active (Zank, 1991). Several reports have provided evidence for the pharmacological effects of plant phytochemicals. Tannins are reported to have anthelmintic effects (Molan et al., 2000a, b) and useful in the treatment of inflamed or ulcerated tissues and they also have remarkable activities in the cancer prevention and anticancer activity (Akinpelu et al., 2009). Flavonoids, phenols and saponins have been reported to exhibit their actions through effects on membrane permeability, antioxidative action and anti-inflammatory effects (Olayinka and Okoh, 2010). Many triterpene saponins and their aglycones have varied uses including anti-inflammatory, antipyretic, fibrinolytic, analgesic, anti-ulcerogenic, anti-oedema and antimicrobial agents (Hostettmann and Martson, 1995; Soetan et al., 2006; Ndukwe et al., 2007). Alkaloids are haemolytically active, toxic to micro-organisms and are widely used as therapeutic agents in the management of cancer (Viji and Parvatham, 2011). Glycosides are reported to inhibit tumour growth and to also protect against gastrointestinal infections (Adeshina et al., 2010). Terpenoids have been reported to be active against bacteria, fungi, protozoa and virus (Maiyo et al., 2010). El-Mahmood et al. (2008) linked antimicrobial properties of plants to bioactive secondary metabolites (saponins, tannins, alkaloids, flavonoids, phenols, glycosides and diterpenes).

Due to the discovery of the beneficial effects of phytochemicals in food plants, this study was designed to evaluate the concentration of various phytochemicals in *L. purpureus*, which could be a guide to ascertain their pharmacological potentials. The phytochemicals to be evaluated are trypsin inhibitors, haemagglutinins, cyanogenic glycosides, oxalates, phytates, tannins, saponins and alkaloids.

MATERIALS AND METHODS

Source of *L. purpureus*

The three varieties of raw *L. purpureus* seeds used for this study were Rongai white (NAPRI 4), Rongai brown ($P_1$509114) and Highworth black (Grif 12293). They were obtained from the

International Institute of Tropical Agriculture (I.I.T.A.) Ibadan, Nigeria.

Extraction of the aqueous crude extract

Dried raw lablab seeds (150 g) were weighed and ground using a clean electronic grinder. Then 400 ml of distilled water was added to the ground seeds. The solution was allowed to stand for 6 h in distilled water, with regular stirring and was filtered using cheese cloth. The filtrate was then placed in a water bath at 60°C for 6 h to allow for evaporation of the water in the extract. The phytochemicals in the extract were then evaluated.

Quantification of the phytochemicals

The phytochemicals in the three varieties of the raw and aqueous crude extract of *L. purpureus* were quantified using standard methods.

Determination of trypsin inhibitor activity

Trypsin inhibitor activator of the samples were determined by the method of Liener (1979). 0.2 g of each sample was weighed into a screw-cap centrifuge tube. 10 ml of 0.1 M phosphate buffer was added and the contents were shaken at room temperature for 1 h on a UDY shaker. The suspension obtained was centrifuged at 5000 rpm for 5 min and filtered through Whatman No.42 filter paper. The volume of each was adjusted to 2 ml with phosphate buffer. The test tubes were placed in water bath, maintained at 37°C and 6 ml of 5% tricarboxylic acid (TCA) solution was added to one of the tubes to serve as a blank. 2 ml of casein solution was added to all the tubes previously kept at 37°C and were incubated for 20 min. The reaction was stopped after 20 min by adding 6 ml of TCA solution to the experimental tubes and then shaken. The reaction was allowed to proceed for 1 h at room temperature. The mixture was filtered through Whatman No.42 filter paper and the absorbance of filtrate from sample and trypsin standard solutions were read at 280 nm.

Determination of haemagglutinin level

Haemagglutinatinin level of the samples were determined by the method of Jaffe (1979). 2 g of each sample was weighed and 50 ml of solvent of mixture of isobutyalcohol and trichloroacetic acid were added and allowed to shake on a UDY shaker for 6 h to extract the haemagglutinin. The mixture was filtered through a double layer filter paper and maintained in a water bath for 2 h at 80°C and the filtrate was allowed to cool. A set of standard solutions of haemagglutinin ranging from 0 to 10 ppm were prepared from the haemagglutinin stock solution. The absorbances of the standard solution as well as that of the filtrate were read at 220 nm on a digital spectrophotometer 21D.

Determination of hydrocyanic acid

The hydrocyanic acids (HCN) of the samples were determined using the procedure of Bradbury et al. (1999). 5 g of each sample was weighed and each sample was incubated for another 16 h at a temperature of 38°C. After the extraction, the filtration was done using a double layer of hardened filter paper. The distillation was done with Markham distillation apparatus. Each sample extracted was transferred into a two-necked 500 ml flask connected with a steam generator. This was steam-distilled with saturated sodium bicarbonate solution contained in a 50 ml conical flask for 60 min. 1 ml of starch indicator was added to 20 ml of each distillate and was titrated with 0.2 N of iodine solution.

Determination of oxalate

The total oxalates were determined according to the procedure of Fasset (1996). The extraction was done by weighing 1 g of each sample and soaked with 100 ml of distilled water. These were allowed to stand for 3 h and each was filtered through a double layer of filter paper. 10, 20, 30, 40 and 50 ppm standard solution of oxalic acid were prepared and read on the spectrophotometer at 420 nm for the absorbance. The absorbances of filtrate from each sample were also read on the spectronic 20.

Determination of phytate

Phytates were determined using the method of Maga (1983). 2 g of each sample was weighed. 100 ml of 2% concentrated hydrochloric acid was used to soak each sample into conical flask for 3 h and filtered through a double layer of hardened filter paper. 50 ml of each filtrate was placed in 250 ml beaker and 107 ml of distilled water was added in each case to give proper acidity. 10 ml of 0.3% ammonium thiocyanate solution was added into each solution as indicator. This was titrated with standard iron (III) chloride solution which contained 0.00495 g iron per ml. The end point was slightly brownish-yellow which persisted for 5 min.

Determination of tannin content

Tannins were determined using the method of Dawra et al. (1988). 0.2 g of each sample was weighed into a beaker. Each was soaked with solvent mixture (80 ml of acetone and 20 ml of glacial acetic acid) for 5 h to extract tannin. The filtrates were removed and the samples were filtered through a double layer filter paper to obtain the filtrate. A set of standard solution of tannic acid was prepared ranging from 0 to 10 ppm. The absorbances of the standard solution as well as that of the filtrates were read at 720 nm on a spectronic 20.

Determination of saponin

Saponins were determined using the procedure of Brunner (1984). 2 g of sample was weighed and 100 ml of isobutylalcohol (octanol) was added and left for 5 h on a UDY shaker for uniform mixing to obtain a uniform solution. The mixture was then filtered through a No.1 Whatman filter paper. The filtrate was transferred and was saturated with magnesium carbonate solution. The mixture was transferred into 100 ml volume flask and made up to mark with distilled water. The mixture obtained here was then filtered to obtain a clear colourless solution to be read on a spectrophotometer at 380 nm. 0 to 5 ppm of standard saponin solutions were prepared from 1000 ppm saponin stock standard solution and saturated with magnesium carbonate as above and also filtered. The absorbances of the saponin standard solution (that is, 0-5 ppm) were also read at 380 ppm to obtain the gradient of plotted curve.

Determination of alkaloids

Alkaloids were determined using the procedure of Henry (1973). 2 g of sample was weighed and 20 ml of 80% alcohol was added to give a smooth paste. The mixture was transferred and more alcohol was added to give up to 100 ml. 1 g of magnesium oxide was

Table 1. Phytochemicals in the three varieties of raw *L. purpureus* seeds.

Sample	Trypsin inhibitors (U/mg) protein	Haemagglutinins (U/mg)	Cyanogenic glycosides (mg/kg)	Oxalates (mg/g)	Phytates (mg/g)	Tannins (mg/g)	Saponins (mg/g)	Alkaloids (mg/g)
Rongai brown	44.8±0.57*(**)	23.7±0.10*	185±0.89*	9.3±0.36*	13.6±0.27*	4.7±0.06	11.3±0.17*(**)	4.8±0.12*
Rongai white	31.6±0.21*(**)	18.7±0.17*(**)	175±0.00*(**)	8.2±0.12(**)	14.4±0.06(**)	3.5±0.84	11.6±0.06*	3.7±0.15*(**)
Highworth black	39.5±0.29	28.6±0.06*(**)	195±0.57*(**)	9.8±0.17	14.0±0.00	4.2±0.81	12.1±0.06*(**)	6.8±0.15*(**)

Most of the values were significant at p<0.05*, *Statistical significance among groups using F test, **Statistical significance between groups at p<0.05 using 't' test.

Table 2. Phytochemicals in the aqueous crude extracts of three varieties of *L. purpureus* seeds.

Sample	Trypsin inhibitors (U/mg) protein	Haemagglutinins (U/mg)	Cyanogenic glycosides (mg/kg)	Oxalates (mg/g)	Phytates (mg/g)	Tannins (mg/g)	Saponins (mg/g)	Alkaloids (mg/g)
Rongai brown	2.40±0.03*(**)	1.00±0.03*	1.40±0.03*	0.40±0.01*(**)	5.20±0.01*	0.20±0.01*	3.7±0.02*(**)	3.9±0.02*
Rongai white	2.11±0.03(**)	1.05±0.01	1.30±0.03(**)	0.50±0.01	2.80±0.01(**)	0.10±0.01(**)	4.4±0.02	3.0±0.04(**)
Highworth black	2.17±0.01	1.13±0.01(**)	1.50±0.02(**)	0.60±0.01(**)	6.50±0.03*(**)	0.30±0.01(**)	4.9±0.02*(**)	4.1±0.02*(**)

Most of the values were significant at p<0.05, *Statistical significance among groups using F test, **Statistical significance between groups at P<0.05 using 't' test.

added and the mixture was digested in a boiling water bath for 1.5 h under a reflux air condenser with occasional shaking. The mixture was filtered white hot through a small Buchner funnel. The residue was returned to the flask and redigested for 30 min with 50 ml alcohol after which the alcohol will be evaporated, adding hot water to replace the alcohol lost. When all the alcohol have been removed, 2 to 3 drops of 10% HCl was added and the whole solution was later transferred into a 150 ml volumetric flask. 5 ml of zinc acetate solution and 5 ml of potassium ferrocyanide solution was added, thoroughly mixed to give a homogenous solution.

The flask was allowed to stand for a few minutes, filtered through a dry filter paper and 10 ml of the filtrate was transferred into a separating funnel and the alkaloids present were extracted vigorously by shaking with five successive 30 ml portions of chloroform. The residue obtained was dissolved in hot water and transferred into a Kjeldahl flask with the addition of 0.2 g sucrose and 10 ml conc. H_2SO_4 and 0.02 g selenium for digestion to a colourless solution. To determine the percentage of NH_3 by Kjeldahl distillation method percentage nitrogen got is converted to a percentage total alkaloid by multiplying by a factor of 3.26.

Statistical analysis

The experiments were performed in triplicates and the results expressed as mean ± standard deviation and compared among the groups using the analysis of variance (ANOVA). Statistical difference was also considered between groups using student "t" test for 2 different means. The value of p<0.05 was regarded as significant for statistical comparison in all cases. GraphPad Prism, Version 5.0, San Diego, Ca was the statistical package used.

RESULTS

The results of the phytochemicals in the raw and aqueous crude extracts of the three varieties of Lablab purpureus are presented in Tables 1 and 2 respectively. In the raw *L. purpureus* seeds, for the trypsin inhibitor activities, the raw Rongai brown has the highest value while the raw Rongai white recorded the least value. The result of the Heamagglutinin content reveals that raw Highworth black had the highest value while raw Rongai white recorded the least. For the cyanogenic glycosides, raw Highworth black has the highest value with raw Rongai white recording the least. For the oxalates, raw Highworth black recorded the highest while raw Rongai white has the least. For the phytates, raw Rongai white had the highest value while raw Rongai brown had the least. For the tannins, raw Rongai brown recorded the highest value while raw Rongai white recorded the lowest value. For the saponins, raw Highworth

black has the highest value while raw Rongai brown had the lowest value. Lastly, for the alkaloids raw Highworth black had the highest value while raw Rongai white recorded the least value.

In the raw *L. purpuresus* seed varieties, the differences in the phytochemicals among the groups were significant at $p<0.05$ except for tannins. The differences between the groups were also statistically significant ($p<0.05$) except in oxalates levels between Rongai brown and Highworth black, in phytate levels between Rongai brown and Highworth black and in tannin in all the three varieties of the *L. purpureus*.

In the aqueous crude extracts of the *L. purpureus* seeds, for the trypsin inhibitors, Rongai brown recorded the highest value while Rongai white had the least value. The result of the Heamagglutinin content reveals that Highworth black had the highest value while Rongai brown recorded the least. For the cyanogenic glycosides, Highworth black had the highest value with Rongai white recording the least. For the oxalates, Highworth black recorded the highest value while Rongai brown had the least. For the phytates, Highworth black had the highest value while Rongai white had the least value. For the tannins, Highworth black recorded the highest value while raw Rongai white recorded the lowest value. For the saponins, Highworth black had the highest value while Rongai brown had the lowest value. Lastly, for the alkaloids Highworth black had the highest value while Rongai white recorded the least value. The differences in the phytochemicals among all the groups were significant at $p<0.05$. The differences between the groups were also statistically significant ($p<0.05$) except in Haemagglutinin level between Rongai brown and Rongai white varieties.

DISCUSSION

These trypsin inhibitor values recorded for the raw *L. purpureus* seeds compare favourably with the values obtained for soyabean ranging from 35.30 and 36.90 tiu/mg protein and lima bean ranging from 29.43 to 36.65 tiu/mg protein as reported by Ologhobo (1980) but exceed those obtained for cowpea ranging from 19.60 to 28.20 tiu/mg protein as reported by Ologhobo and Fetuga (1983). The results are also consistent with that reported for cowpea ranging from 19.0 to 46.7 tiu/mg protein by (Marconi et al., 1993). The trypsin inhibitors are reported to be one of the major toxic components of legumes (Liener and Kakade, 1980; Akinyele, 1989).

The trypsin inhibitor activities obtained for the raw lablab beans are contrary to the results reported by Lambert (1972) for 26 varieties of cowpea (*Vigna sinensis*). The authors obtained a range of 15.5 to 23.80 tiu/mg protein and went on to show that environmental and genetic factors affect the trypsin inhibitor activities of legumes. These results compare well with that recorded for some varieties of cowpea TVNU66 having 13 HU/mg and TVNU226 having 27 HU/mg as reported by Marconi et al. (1993). The results, however, contradict those of Ologhobo and Fetuga (1983) reporting a mean value of 49.6 HU/mg protein for cowpea (*Vigna unguiculata*). However, there appears to be no basis for comparison in as much as the isolation procedures and methods employed differ. Furthermore, the varieties of legumes used and even the environment in which these legumes were grown might have some modifying influences, as reported by Swaminathan and Jain (1973). The variability within species and between locations may be as high as 10%. The occurrence of cyanogenic glycosides in legumes was reported by Okolie and Ugochukwu (1989). The cyanogenic glycosides on hydrolysis yield toxic HCN (Osuntokun, 1972; Fernando, 1987). The cyanide ions inhibit several enzyme systems, depress growth through interference with certain essential amino acids and the utilization of essential nutrients. The results obtained for the raw lablab beans is lower than that reported by Egbe and Akinyele (1990) reporting 420 mg/kg cyanogenic glycoside in lima bean (*Phaseolus lunatus*). Okolie and Ugochukwu (1989) showed total cyanide contents of 381-1095 mg/kg for seeds of Nigerian varieties of *Phaseolus aureus*, *V. unguiculata*, *Cajanus cajan* and *Canavalia gladiatus*. These were higher than that reported for raw *L. purpureus* seeds. The differences observed in the varieties of lablab beans and different species of legumes could be attributed to the genetic variations and different chemical composition of the soils on which they were cultivated. Similarly, macro and micro mineral deficiencies in the soil have been found to result in the non-protein nitrogen pool being large and this could result in elevated cyanogenic glycoside levels (Butler et al., 1973).

Oxalates (C_2 dicarboxylic acid anion) are produced and accumulated in many crop plants and pasture seeds. Oxalates may be present in plants as the soluble salts, potassium, sodium or ammonium oxalate as oxalic acid or as insoluble calcium oxalate. Oxalate is a concern because high oxalate diets can increase the risk of renal calcium absorption. Although there were no values in literature with which to compare the results as regards oxalates in legumes, the results agree with total oxalates reported by Aremu (1989) for *Lophira alata* (9.85 mg/g), *Hyphaene thebaica* (9.57 mg/g) and *Bixa orellana* (8.44 mg/g) which are wild underutilized crop seeds in Nigeria. Phytic acid, a hexaphosphate derivative of inositol is an important storage form of phosphorus in plants. It is insoluble and cannot be absorbed in the human intestines. Phytates can render metals like calcium, iron, zinc and magnesium unavailable into the body by forming insoluble salts with these metals (Edman and Forbes, 1977). However, the results contradict that obtained by Ologhobo (1980), recording a range of 2.90 to 3.25 mg/g phytate for cowpea.

The results compare well with that recorded for lima beans tannin ranging between 0.32 to 0.93 mg/g by

Ologhobo (1980) but it is higher than that recorded for cowpea ranging between 0.24 and 0.58 mg/g and soyabean varieties ranging between 0.34 and 0.37 mg/g by Ologhobo (1980). Some studies revealed that the occurrence of tannins in plants are of no nutritional significance unless at very high levels often 10% or more of the dry weight. Butler (1989) reported that tannins may decrease protein quality by decreasing digestibility and palatability. Egbe and Akinyele (1990) found 0.59mg/g tannins in raw lima beans (*Phaseolus lunatus*). The results obtained for lablab beans suggest that they may very insignificantly affect the nutritional potentials of these legumes.

The result of the levels of saponins in this study is similar to that reported by Achinewhu (1983) recording 11.8 mg/g saponins for rubber seed (*Hevea brasiliensis*). The results are also similar to that reported by Osagie et al. (1996), that cowpea contains 11.18 mg/g saponin. Sodipo and Arinze (1985) reported beans to contain a considerable amount of saponins of about 245.0 mg/kg. While there are suggestions that the consumption of saponins should be encouraged because of their hypocholesterolaemic activity, forage saponins have been reported by Cheeke et al. (1978) to cause toxic and anorexic effects in the rat and swine, thereby limiting the feeding value of high-saponin animal feeds such as alfalfa.

Alkaloids are basic natural products occurring primarily in plants. It has been reported that probably 10 to 20% of all higher plants contain alkaloids (Wink, 1993). Literature is scarce on the levels of alkaloids in food legumes. Osagie et al. (1996) reported that alkaloid-containing species and varieties may have been eliminated by choice from Nigeria's staple foods because of the bitter taste associated with alkaloids. The values recorded for the alkaloids were low when compared with that of other anti-nutritional factors in lablab beans. This may be an advantage to the nutritional significance of the lablab beans.

The levels of each of the phytochemicals contained in the raw *L. purpureus* seeds were significantly higher than the levels found in the aqueous crude extracts. This could be due to the processes involved in the isolation of the aqueous crude extracts which involves soaking, heating and boiling in water for an extended period of time. It has been reported that different processing methods such as autoclaving, boiling, cooking, fermentation, germination (sprouting), heating and soaking reduce the levels of anti-nutritional factors in plants (Egbe and Akinyele, 1990; Osagie, 1998; Balogun et al., 2001). Akanji et al. (2003) reported that aqueous heating destroyed all haemagglutinins and trypsin inhibitor activities in the seeds of jackbean (*Canavalia ensiformis* L), dry heating gave percentage losses in the haemagglutinin and trypsin inhibitor activities, while phytate and tannin contents were partially affected by heat treatments. Bawa et al. (2003) also reported that cooking of lablab seeds significantly ($p<0.05$) decreased the levels of trypsin inhibitor activity, phytic acid, tannin and cyanide. The reduction in the level of the antinutritional factors in the aqueous crude extracts of *L. purpureus* seeds is also similar to report by Esenwah and Ikenebomeh (2008) that soaking and boiling significantly reduced the levels of the anti-nutritional factors (trypsin inhibitor, tannin and phytic acid) of the African Locust Bean (*Parkia biglobosa* Benth) seed. Abeke et al. (2010) also reported a decrease in the level of trypsin inhibitors, phytic acids, tannins and hydrocyanic acids in the Rongai variety of *L. purpureus* seeds, with increase in duration of cooking.

Conclusion

It is concluded that *L. purpureus* contain significant concentration of several phytochemicals with good potentials for use as phytomedicines. More work is required to describe the best isolation method for the phytochemicals in LP. The pharmacological effects of the individual phytochemicals in LP also need to be assessed against micro-organisms and parasites. The presence of other secondary metabolites in *L. purpureus* seeds, apart from those reported in this study also require more in-depth study. It is recommended that beyond general phytochemical analysis of plants, further studies should focus on quantitation of phytochemicals and their biosafety evaluation to identify those with the low or high levels.

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