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Full Length Research Paper

Flavonoid profile, anthocyanin, carotenoid, sugar and vitamin compositions of *Lonchocarpus sericeus* seeds

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ABSTRACT

This study reports analyses of flavonoid profile, anthocyanin, sugar, carotenoid and vitamin compositions in *Lonchocarpus sericeus (L. sericeus*) seeds. Flavonoid profile, anthocyanin, sugar, carotenoid were determined with gas chromatography coupled with flame ionization detector (GC-FID) while vitamins were determined using pulse flame photometric detector (GC-PTPD). Total flavonoid concentration of *L. sericeus* seeds was 815.86 mg/100g and its profile consists of flavonol (72.5 %), flavanones (19.3 %), flavones (7.25 %), flavanol (0.93 %) and isoflavones (0.02 %). Quercetin was the most abundant with 28.8 %. *L. sericeus* seeds composed of 80.5 % disaccharides and 19.5 % monosaccharide with sucrose having 80.4 % abundance. Total vitamin, carotenoid and anthocyanin contents of the seeds were 70.32 mg/100g, 102.18 µg/100g and 12.18mg/100g respectively. Vitamin E (33.3 %), β-carotene (65.4 %) and cyanin (30.3 %) had highest abundance in vitamins, carotenoids and anthocyanins respectively. These results show that the seeds of *L. sericeus* have intrinsic sweetness and can scavenge for free radicals.

Keywords: flavonols, disaccharides, fat soluble vitamins, quercetin, anthocyanins

INTRODUCTION

Lonchocarpus sericeus (L. sericeus) is a leguminous plant commonly called a cube root or Senegal lilac. It is a dry deciduous tree that can grow from 10 to 16 meters high and flowers with dense hanging racemes of purple flowers which makes it perfect for display purposes. The flowers have a marked smell similar to vanilla. It is frequently planted in villages as a shade tree and in gardens and commentaries. The wood is clear yellow, sometimes marbled, with heart-wood and olive-green (Kojs et al, 2004; Musa et al, 2006; Adewuyi et al, 2012). The bark strips easily and is a good source of fiber. *L. sericeus* has been reported to possess antioxidant, antifungi and insect-repellant properties. It is used in the treatment of epilepsy, laxative, to stimulate appetite and to treat stomach disorder in Africa. It also has common application in treatment convulsions and back aches (Kojs et al, 2004; Fontenele et al, 2005; Ezeagu and Gowda). Phytochemicals such as alkaloids, saponins, carotenoids, flavonoids, tannins, triterpenes and steroids have been detected in the methanol extracts of the stem bark, seeds and root (Musa et al, 2006) but anthocyanin, vitamins and carotenoids which have been shown to be hepatoprotective, anti-inflammatory, antiviral, free radical scanvengers have not been reported (Lohachoompo et al, 2004; Kelebek et al, 2009; Okiei et al, 2009; Afify et al, 2012). Study conducted by Ezeagu and Gowda (2006) showed that *L. sericeus* is a source of leucine and lysine which are essential amino acids. Physicochemical properties and fatty acid composition of *L. sericeus* seed oil reveled high quantity of oil and unsaturated fatty acids

which make the oil from the seeds a suitable feed stock for the production of lubricants (Adewuyi et al, 2012). Despite these, little or no study has reported flavonoid profile, anthocyanin, sugar, carotenoid and vitamin compositions of *L. sericeus* seeds. These amongst other compositions are important parameters to establish health functions food products such as seeds. Therefore, this study was designed to determine the nutritional compositions and phytochemicals of *L. sericeus* seeds.

MATERIALS AND METHODS

Seed Samples

Mature seeds were collected from a tree within the premises of the Federal Polytechnic, Ilaro(6.89^{o}N,3.02^{o}E) between April and August, 2013. The seeds were washed with deionized-distilled water and then oven-dried at 60 ^{o}C. The dried seeds were pulverized using a blender, sieved and kept in an air tight container for further analysis.

Determination of flavonoid composition

The procedures described by Whitehead et al. (1983) and Provan et al. (1994) were used for the extraction of flavonoids from L.sericeus. Briefly, 50 mg of the sample was extracted with 5 ml of 1 M NaOH for 16h on a shaker at ambient temperature. After this, the extract was centrifuged (5000 g). The residue was rinsed with deionized water, centrifuged again and the combined supernatants were placed in a disposable glass test tube which was heated at 90 ^{o}C for 2h to release conjugated flavonoids. The heated extract was cooled, titrated with 4 M HCL to pH < 2.0, diluted to 10 ml, with deionised water, and centrifuged to remove the precipitate. 15 ml of the supernatant obtained was passed through a conditioned Varian (Varian Assoc., Habor City, CA) Bond Elut PPL (3-ml size with 200mg packing) solid- phase extraction tube at 5 ml min^{-1} attached to a Visiprep (Supelco, Bellefonte, PA). The tubes were then placed under vacuum (-60 kPa) until the resin was thoroughly dried after which the flavonoids were eluted with 1mL of ethyl vials. The PPL tubes were conditioned by first passing 2mL of ethyl acetate followed by 2 ml water (pH < 2.0).

The composition of flavonoid in purified *L. sericeus* extract was analyzed using gas chromatography coupled with flame ionization detector (GC-FID). 1 µl of each solution was injected into GC (Hewlett-Packard Model 5890, USA) with FID which has HP-1 column (30 m x 0.25 µm x 0.25 mm id), nitrogen carrier gas, a detector section temperature of 320 ^{o}C and a split ratio (20:1) mode inlet section (250 ^{o}C). The column was initially at 60 ^{o}C held for 5 min and increased at 15 °C/ min for 15 min, maintained for 1 min and at 10 °C/mi for 4 min held for 2 min. Flavonoids obtained were compared with their standards which were analyzed before the samples.

Determination of anthocyanin composition

5 g of *L. sericeus* was extracted with 100 ml of 1 % HCl in MeOH and was placed in an orbital shaker for 4 hr. The solution was filtered with Whatman No. 4 filter paper and filtrate was evaporated at 40 ^{o}C to dryness using a rotary evaporator. 2 g each of *L. sericeus* extract was dissolved in 100 ml methanol. The mixture was stirred thoroughly for gas chromatography coupled with flame ionization detector (GC-FID) analysis. 1µl of each solution was injected into GC (Hewlett-Packard Model 5890, USA) with FID which has SupelCoWax 10 column (30 m x 0.25 µm x 0.25 mm id), hydrogen carrier gas, a detector section temperature of 320 ^{o}C and a split ratio (20:1) mode inlet section (250 ^{o}C). The column was initially at 45 ^{o}C held for 2 min and increased at 30 °C/ min to 60 ^{o}C for 2 min, at 2 °C/min to 160 ^{o}C and at 5 ^{o}C/min to 230 ^{o}C for 20 min. Different anthocyanins were identified by their peaks.

Determination of carotenoid composition

The carotenoid composition was analyzed using modified method of extraction of Takagi (1985). 5 g of the pulverized sample was homogenized in 75ml of acetone and kept at room temperature for 1h in the dark. The homogenate was filtered through Whatman No. 4 filter paper by suction. Extraction was repeated three times with the same volume of acetone. The extracts were combined and evaporated under reduced pressure and the residue was re- extracted by a mixture of diethyl ether and petroleum ether in equal ratio. The extract was evaporated using rotary evaporator. Then the concentrated extract was dried of water by using the anhydrous sodium sulphate before gas chromatography analysis. Dried extract was dissolved in methanol for gas chromatography coupled with flame ionization detector (GC-FID). 1µl of the methanolic extract was injected into GC (Hewlett-Packard Model 5890, USA) with FID which has AC- 5 column (30 m x 0.25 µm x 0.25 mm id), nitrogen carrier gas, a detector section temperature of 320 ^{o}C and a split ratio (20:1) mode inlet section (250 ^{o}C). The column was initially at 60 ^{o}C, increased at 10 °C/min for 20 min, maintained for 20 min and at 15 ^{o}C for 4 min and maintained for 4 min

Determination of sugar composition

50mg of the dried extract of *L. sericeus* was derivatised using Silylation process. Tetramethyl silyl (TMS) group replaced active hydrogen on the compounds after the treatment with the derivatization reagent. The content was concentrated to 1 ml for gas

Table 1. Flavonoid composition of *L. serceus* seed

Flavonoids	Composition (mg/100g)
Flavonol	
Quercetin	234.67
Kaempferol	172.39
Galangin	145.30
Myricetin	32.74
Isorhamnetin	6.08
Morin	0.04
Flavones	
Apigenin	59.06
Luteolin	0.08
Acacetin	0.02
Isoflavones	
Genistein	0.2
Daidzein	0.19
Biochanin	0.07
Flavanones	
Naringenin	98.26
Naringenin glycoside	59.09
Nobiletin	0.05
Hesperetin	0.04
Flavanol	
(-)-Epicatechin-3-gallate	1.31
(-)-Epigallocatechin-3-gallate	1.01
(+)-Catechin	5.11
(+)-Gallocatechin	0.02
(-)-Epicatechin	0.13

chromatography analysis and 1µl was injected into the injection port of gas chromatography (GC Hewlett-Packard Model 5890, USA) with FID which has BPX70 column (12m x 0.32mm x 0.25µm i.d) isothermally set at 210 ^{o}C with hydogen carrier gas. A detector section temperature of 325 ^{o}C, split ratio (50:1) and inlet temperature (250 ^{o}C) were used.

Vitamin Composition

Vitamins (A, D, E, K, B1, B2, B3, B5, B6, B9 and C) in *L. sericeus* seeds were determined accordingto Association of Analytical Chemists' [15] method. 1 µl of the extract was injected into the injection port of gas chromatography (HP 6890) coupled with pulse flame photometric detector (GC-PFPD) with a column HP (30m x 0.25µm x 0.255 mm id) to obtain individual peaks of each vitamin. The carrier gas was nitrogen and the inlet temperature was 250 ^{o}C while the detector temperature was 320 ^{o}C. The column temperature was initially at 50 ^{o}C held for 2 min and increased at the rate of 10 ^{o}C/min for 20 min, maintained for 4min and held at the rate of 20 ^{o}C/min to 320 ^{o}C for 2 min.

RESULTS AND DISCUSSION

Flavononoid composition

Table 1 shows the composition of flavonoids in *L. sericeus* seeds. Total flavonoid concentration of *L. sericeus* seeds was 815.86 mg/100g and their relative abundance followed; flavonol (72.5 %), flavanones (19.3 %), flavones (7.25 %), flavanol (0.93 %) and isoflavones (0.02 %). Quercetin was the most abundant with 28.8 % while the least abundant were acacetin and (+)-gallocatechin (0.002 %). High abundance of kaemferol, galangin, naringenin, naringenin glucoside, apigenin and myricetin was also obtained in this order.

Quercetin which is the most abundant flavonoid in the seeds has been reported to be the most biologically, pharmaceutically active and most common dietary flavonoid (Malešev and Kunti, 2007; Bukhari et al, 2008). Quercetin is an effective metal chelator and contributes significantly to the anti-bacterial and anti-inflammatory actions of flavonoids. Also, it has been reported to be antioxidative, anticarcinogenic, anti-aggregatory and vasolidating (Erlund, 2004). This shows that *L. sericeus* seeds can act as sources for free radicals scavengers (Azeez et al, 2012).

Table 2 presents the composition of anthocyanin in *L. sericeus* seeds. The total anthocyanins determined were 12.18 mg/100g with cyanidin having highest abundance (30.3 %). The relative abundance of anthocyanin components followed cyaniding > delphinidin > peonidin >pelargonidin > malvidin > petunidin. Cyanidin has been reported to be the most common anthocyanin in red flowers (Harborne and Williams, 2000), thus, *L. sericeus* seeds could have intrinsic red colour.

Table 3 presents the results of carotenoid composition of *L. sericeus* seeds. Total carotenoid content was 102.18 µg/100g with β-carotene having the highest abundance of 65.4 %. The relative abundance of carotenoids followed β-carotene (65.4 %) > β-cryptoxanthin (26.6 %) >

Table 2. Anthocyanin composition of *L. serceus* seed

Anthocyanin	Composition (mg/100g)
Cyanidin	3.69
Delphinidin	2.73
Malvidin	0.97
Peonidin	2.24
Petunidin	0.32
Pelargonidin	2.23

Table 3. Carotenoid composition of *L. serceus* seed

Carotenoid	Composition (µg/100g)
β-cryptoxanthin	27.22
Lycopene	3.32
β-carotene	66.81
Anthera-xanthin	2.05
Asta-xanthin	2.20
Neo-xanthin	0.05
Viola-xanthin	0.50
Xanthophyll	0.03

Table 4. Sugar composition of *L. serceus* seed

Sugar	Composition (mg/100g)
Monosaccharide	
Ribose	0.21
Xylose	0.12
Arabinose	0.22
Rhamnose	0.07
Fructose	7.20
Glucose	75.17
Disaccharide	
Maltose	0.21
Lactose	0.17
Sucrose	342.08

Table 5. Vitamin composition of *L. serceus* seed

Vitamins	Composition (mg/100g)
Fat-Soluble	
A	3.89
D	12.04
E	23.42
K	4.07
Water Soluble	
B3	5.33
B6	4.16
B5	8.20
B1	0.29
B2	0.07
B9	0.71
C	8.14

lycopene (3.2%) > asta-xanthin (2.2%) > anther-xanthin (2.01%) > neo-xanthin (0.49%) > viola-xanthin = xanthophyl (0.05%).

Carotenoids such as lycopene and β-carotene have shown inverse relationship with incidence of cancer and have been proved to possess antioxidant activity due to their ability to quench singlet oxygen, detoxify free radicals and inhibit lipid peroxidation (Sarada et al, 2002; Erlund, 2004). High concentration of β-carotene shows that *L. sericeus* seeds are capable of scavenging singlet oxygen and detoxify radicals (Azeez et al, 2012).

The results of sugar compositions in *L. sericeus* seeds are presented in table 4. The total amount of sugar in *L. sericeus* seeds was 425.45 mg/100g. The seeds

composed of 80.5 % disaccharides and 19.5 % monosaccharide with sucrose having 80.4 % abundance. The ratio of reducing sugars to non-reducing sugars is 1: 4. Also, the ratio of sucrose : glucose : fructose is 47.5 : 10.4 : 1. With high content of sucrose in *L. sericeus* seeds, it shows that the seeds are a good source of energy and contain intrinsic sweetness (Kelebek et al, 2009).

Table 5 shows the composition of vitamins in L. sericeus. Total vitamin content of the seeds was 70.32 mg/100g having 61.7 % fat-soluble vitamins and 38.3 % water-soluble vitamins. Vitamin E was the most abundant with 33.3 % and vitamin B2 was the least abundant with 0.1 %.

Vitamin E is a fat soluble vitamin which is known to have several biological functions of which the most important are its antioxidant function and prevention of lipid peroxidation (Shin et al, 2003). Vitamins C and E have been reported to help body fight against free radicals (Okiei et al, 2009; Azeez et al, 2012).

CONCLUSION

This study has reported the analyses of flavonoid profile, anthocyanin, carotenoid, sugar and vitamin compoistions of *L. sericeus* seeds. The seeds are a good source of antioxidant with high free radical scavenging ability due to high concentrations of flavonoids, β-carotene and vitamin E obtained. The seeds could also serve as sources of nutrients and energy as shown by their sygar contents and compositions

REFERENCES

Adewuyi A, Oderinde RA, Rao BVSK, Prasad RBN (2012). Chemical Composition and Molecular Speciation of the Triacylglycerol of the Oils of Lonchocarpus sericeus and Lonchocarpus cyanescens. Nat. Prod. Res. **26**(20), 1954-1956.

Afify AMR, El-Beltagi HS, Abd El-Salam SM, Omran AA (2012). Biochemical changes in phenols, flavonoids, tannins, vitamin E, β-carotene and antioxidant activity during soaking of three white sorghum varieties. Asian Pac. J. Trop. Biomed. 203-209

AOAC (2006). Official Methods of Analysis. 18th Edition. The Association of Official Analytical Chemists, Arlington, USA.

Azeez L, Adeoye M.D, Majolagbe TA, Lawal AT, Badiru R(2012). Antioxidantactivity and phytochemical contents of some selected Nigerian fruits and vegetables. Ame. J. Chem.2(4), 209-213

Azeez L, Adeoye MD, Ganiyu OT, Abdul-salami IO, Majolagbe TA, Lawal AT (2012). Influence of microbial contamination on antioxidant composition and free radicals scavenging effects of fresh and decaying spices. Fountain J. Nat. Appl. Sci.1(1), 55-64Bukhari SB, Memona S, Tahir MM, Bhanger MI (2008). Synthesis, characterization and antioxidant activity of cobalt-quercetin complex. J. Mol. Str. **892**, 39-46

Erlund I (2004). Review of the flavonoids quercetin, hesperetin, and naringenin, Dietary sources, bioactivities, bioavailability and epidemiology. Nutr. Res. **24**, 851-874

Ezeagu IE, Gowda LR (2006). Protein extractability, fractionation and amino acid composition of some leguminous seeds Found in Nigeria. J. Food Biochem. **30**, 1-11

Fontenele JB, Leal LKAM, Ferreira MAD, Silveira ER, Viana GSB (2005). Antiplatelet Effect of Lonchocarpin and Derricin Isolated from Lonchocarpus sericeus. Pharm. Bio. **43** (8), 726-731

Harborne JB, Williams CA (2000). Advances in flavonoid research since 1992. Phytochem. **55**, 481-504

Kelebek H, Selli S, Canbas A, Cabaroglu T (2009). HPLC determination of organic acids, sugars, phenolic compositions and antioxidant capacity of orange juice and orange wine made from a Turkish cv. Kozan. Microchem. J. **91**, 187–192

Kojs P, Włoch W, Rusin A (2004). Rearrangement of cells in storeyed cambium of *Lonchocarpus sericeus* (Poir.) DC connected with formation of interlocked grain in the xylem. Trees. **18**, 136-144

Lohachoompo V, Srzednicki G, Craske J (2004). The Change of Total Anthocyanins in Blueberries and Their Antioxidant Effect After Drying and Freezing. J. Biomed. Biotech. **5**, 248–252

Malešev D, Kunti V (2007). Investigation of metal–flavonoid chelates and the determination of flavonoids via metal–flavonoid complexing reactions. J. Serb. Chem. Soc. **72**(10): 921–939

Musa AM, Yaro AH, Abubakar MS (2006). Anticonvulsant activity of methanol extract of the stem bark of Lonchocarpus sericeus Poir (Papilionaceae). J. Trop. Biosci. **6**, 17-30

Okiei W, Ogunlesi M, Azeez L, Osunsanmi M, Obakachi V, Golda N (2009). The voltammetric and titrimetric determination of ascorbic acid levels in tropical fruit samples. Inter. J. Electrochem. Sci. (4), 276-287.

Provan GJ, Scobbie L, Chesson A (1994). Determination of phenolic acids in plant cell walls by microwave digestion. J. Sci. Food Agric. **64**, 63-65

Sarada SKS, Dipti P, Anju B, Pauline T, Kain AK, Sairam M, Sharma SK, Ilavazhagan G, Kumar D, Selvamurthy W (2002). Antioxidant effect of beta-carotene on hypoxia induced oxidative stress in male albino rats: J. Ethnopharma. **79**, 149–153

Shin SJ (2003). Vitamin E modulates radiation-induced oxidative damage in mice fed a high lipid diet. J. Biochem. Mol. Bio. **36** (2), 190-195.

Takagi S (1985). Determination of Green Leaf Caroteniods. Agric. Bio. Chem. **49**(4), 1211- 1213

Whitehead D, Dibb CH, Hartley RD (1983). Bound phenolic compounds in water extracts of soil, plant roots and leaf litter. Soil Bio. Biochem. **15**, 133-136