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Journal of Chemical and Pharmaceutical Research

J. Chem. Pharm. Res., 2011, 3(4): 538-541

ISSN No: 0975-7384
CODEN(USA): JCPRC5

Amino Acid Content and Proximate Analysis of the Ethanol Seed Extract of *Vigna unguiculata* Used in the Management of Sickle Cell Disease

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ABSTRACT

Natural herbal plants abound in tropical Africa which are rich sources of amino acids and proteins. Certain amino acids have been shown to play an important role in the management of sickle cell disease. This work evaluated the amino acid content and proximate analysis of the ethanol seed extract of Vigna unguiculata in view of proposing a rich source of amino acids and proteins which will help in the management of sickle cell disease. The amino acid content of the extract was determined using LKB amino acid analyzer based on the method developed by Ruhemann. The proximate analysis was carried out using standard biochemical methods. The amino acid analysis of the extract showed the presence of the following amino acids; aspartate (27.8%), threonine (3.3%), serine (2.6%), glutamine (43.5%), proline (17.6%), glycine (9.5%), alanine (18.7%), cysteine (3.6%), valine (8%), methionine (3.2%), isoleucine (5.3%), leucine (5.4%), tyrosine (0.5%), phenylalanine (5.5%), histidine (4.5%), lysine (0.5%), arginine (14.3%) and tryptophan (0.5%). The proximate analysis of the extract indicated appreciable content of protein (23.65%), moisture (12.85%), ash (3.4%), fats and oil (4.5%) and fiber (4.8%). From the result, Vigna unguiculata is a rich source of amino acid and protein thus will be beneficial in the management of sickle cell disease.

Keywords: Amino acids, Proteins, Sickle Cell, *Vigna unguiculata*.

INTRODUCTION

Sickle cell disease (SCD) caused by a single amino acid substitution at the sixth position of the beta-globin chain of sickle hemoglobin (HbSS) is an inherited hematological disorder

characterized by crescent-moon or sickle shaped human red blood cells (Balgir, 2007; Desai and Hiren, 2004; Uzoegwu, 1995). Sickling of red blood cells occur as a result of polymerization of deoxygenated HbSS molecules, so that they become stacked linearly (Wang, 2004). Clinical symptoms occur in homozygotes and develop at about 6 months old. There is a chronic hemolytic anemia and recurrent painful crises because of the sickled erythrocytes blocking small vessels (Solovey *et al,* 2001). This leads to tissue ischemia and infarction, mostly affecting the liver, spleen, lungs, brain and retina, leg ulceration and priapism are also evident. These crises may be precipitated by minor infections, severe cold, exercise, dehydration and pregnancy.

Natural herbal plants abound in tropical Africa which are rich sources of amino acid and protein. Because anti sickling effects of certain amino acids such as phenylalanine, lysine, arginine and others (Noguchi and Schechter, 1985; Ekeke and Shode, 1990), we were prompted to investigate the amino acid content and proximate analysis of *Vigna unguiculata* used in the management of sickle cell disease.

EXPERIMENTAL SECTION

Collection and preparation of plant materials:

The seeds of *Vigna unguiculata* were obtained from the herbal line, Ogbete main market Enugu State, Nigeria. They were authenticated by Mr. Njokuocha of the herbarium section, Department of Botany, University of Nigeria, Nsukka, Nigeria. The seeds were air dried under shade at room temperature and pulverized using an electrical grinding machine. The powdered material (500g) was passed through a 40-mesh sieve and then mascerated in 95% ethanol and filtered using a Whatman filter paper 125mm. the filtrate was concentrated to a solid matter using a rotary evaporator. The extracts were stored in the refridgerator at 2-8^{o}C.

Determination of amino acid constituents of the extract:

Amino acid contents of the extract was determined using LKB amino acid analyzer based on the method developed by Ruhemann (1911) as described below. The extract was hydrolyzed using 6N HCL at 110^{o}C for 24 hours and then stored frozen in pH 2.2 sodium citrate buffer until ready for analysis. Fifty microlitres of the hydrolysate was loaded into a sample capsule and placed into a refridgerated automatic injector which can take up to 50 samples at one time. The sample was automatically pumped onto an analytical column packed with sulphonated polystyrene cation exchanger resin. The amino acids were then eluted from the column by pumping buffers at an increasing pH, Na^{+} concentration and column temperature. Each amino acids were mixed with ninhydrin (2,2-dihydroxy-1,3-indandione) which reacts at 135^{o}C to form Ruhemann's purple which absorbs light in the photometer at 570nm. The photometer reads both wavelengths simultaneously and summates both signals for recording and integrating.

Proximate analysis:

Ash content, moisture content and fiber content were determined using the method outlined by Association of Official Analytical Chemists (AOAC, 1990). Determination of protein was done using the micro-kjedhal's method as described by Pearson (1976). Fats and oil content was done according to the method of Odo and Ishiwu (1999).

RESULTS

The amino acid content of the seed extract showed varying concentrations of the amino acids as shown in Table 1.

Table 1: Amino acid content of the seed extract of *Vigna unguiculata*

Amino acids	% Composition
Aspartate	27.8
Threonine	3.3
Serine	2.6
Glutamine	43.5
Proline	17.6
Glycine	9.5
Alanine	18.7
Cysteine	3.6
Valine	0.8
Methionine	3.2
Isoleucine	5.3
Leucine	5.4
Tyrosine	0.5
Phenylalanine	5.5
Histidine	4.5
Lysine	0.5
Arginine	14.3
Tryptophan	0.5

Table 2: Proximate analysis of the seed extract of *Vigna unguiculata*

Components	% Composition
Protein	23.65
Ash	3.4
Fiber	4.8
Fats and oil	4.5
Moisture	12.85

The result of proximate analysis of the seed extract showed it contains proteins, ash, fiber, fats and oil and moisture in varying amounts as represented on Table 2 above.

DISCUSSION

The anti sickling role of some amino acids had already been investigated and documented (Ekeke and Shode, 1990; Nwaoguikpe, 1993). The seed extract contains phenylalanine and other amino acids (table 1) which could explain in part many of the positive effects of the extract in management of sickle cell disease. Phenylalanine is the major anti sickling agent in *Cajanus cajan* (Ekeke and Shode, 1990). Apart from its effect in membrane stability, phenylalanine a model anti sickling agent is also known to stimulate the activation of membrane bound Na^+/K^+ and Ca^{2+} ATPase activities (Elekwa *et al,* 2005). The preponderance of anti sickling amino acids in the seed extract of *Vigna unguiculata* probably must have been responsible for its use in the management of sickle cell disease. Nutritionally, the high quantity of amino acids and crude proteins (23.65%) can be of profound benefit to the sicklier. One can rightly conclude that the

extract of *Vigna unguiculata* should be developed into an effective management therapy for sickle cell disease (SCD).

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