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Amino acids of seeds of some *acacia* taxa as taxonomic marker

Eiman Mohammed Ali Mustafa*[1], Hatil Hashim Alkamali[2], Ahlam Salih Eltahir[2], Magdah Abdelbasit Nory Salih[3]

[1,3]*Department of Biology, University College in Al-Khafji, Hafr El-Batin University, Saudi Arabia*

[2]*Department of Botany, Faculty of Science and Technology, Omdurman Islamic University, Omdurman, Sudan*

[3]*Department of Chemistry, University College in Al-khafji, Hafr El-Batin University, Saudi Arabia*

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Abstract

The aim of this study is to determine the composition of amino acids in eight *Acacia* seeds (*A cacia mellifera*, *A. nilotica* ssp*, adansonia*, *A. nilotica* ssp *nilotica*, *A. oerfota*, *A. polycantha* ssp. *campylacantha, A. senegal* var *senegal, A. seyal* var. *seyal* and *A. sieberiana* var *sieberiana*) of the Sudan and to add another marker in the taxonomy of these taxa. The study included a determination of amino acid composition using HPLC followed by pairing affinity between studied taxa based on distribution of free amino acids and fatty acids. The amino acid composition of seeds of the studied eight Acacia taxa indicated that glutamic acid is the most dominant in all *Acacia* taxa studied highest value appeared in Acacia seyal var. seyal (43.65mg/gm), and the lowest value in *Acacia polyacantha* ssp. *camplacantha* (32.62mg/gm). Whereas, methionine is the minor, in *Acacia mellifera* (1.25mg/gm), and the lowest value noted in *Acacia nilotica* ssp. *nilotica* (0.55 mg/gm). Highest degree of pairing affinity noted between *Acacia oerfota* and *Acacia siebriana* (89.91%) followed by *Acacia nilotica* ssp. *adansonia* and *Acacia nilotica* ssp. *nilotica* (71.37%) and between *nilotica* ssp. *nilotica* and *Acacia seyal var seyal* (70.91%), *Acacia nilotica* ssp. *adansonia* and *Acacia seyal* (70.54%) and between *Acacia nilotica* ssp. *nilotica* and *Acacia siebriana* (70.19%). Hence *Acacia orefota* and *Acacia siebriana* are the most closely related, also *Acacia nilotica adansonia* and *Acacia nilotica seyal.*

* **Corresponding Author:** Eiman Mohammed Ali Mustafa ✉ eimanlife7@gmail.com

Introduction

Acacia species predominate in Sudan and are of high importance because of the gum produced by some of them and for their medicinal and economic importance. The genus *Acacia*, belongs to the family *Fabaceae* and it is a large group of woody species comprising of shrubs (Elamin, 1972). The *Acacia* have been reported to exhibit significant potential to lower poverty in semi-arid regions of Africa (Adewusi, 2003).

Screening conducted by the Australian Tree Seed Centre in 1994 indicated that *Acacia colei* has good nutritional value and that known toxic and anti-nutritional factors were absent or at levels below those that would cause any concern (Harwood *et al*., 1999). The seeds of *A. colei* had been subjected to testing for nutritional potential and food safety from the early 1990's (Adewusi*, et al*., 2006; Adewusi, *et al*., 2003; Falade*, et al*., 2005; Falade, *et al*., 2008). Functional aspects of *A. victoriae* were investigated in some detail by a team from Charles Sturt University (Ee. *et al*., 2008, 2009 and 2011, Agboola, *et al*., 2012). Testing on *A. saligna* was conducted in 2012 by Youzbachi, *et al.* (2012) and by Ee and Yates (2013).

The amino acid balance in *Acacia* seed is similar to other legumes, such as lentils, and as such *Acacia* is an excellent complement to cereals, which are generally low in lysine. Adewusi, *et al.* (2006) tested Protein Efficiency Ratios and found the best complementarity was between *Acacia* seeds and the traditional cereal fonio (*Digitaria exilis*). Sorghum (*Sorghum bicolor*) was the next best complement, with pearl millet (*Pennisetum glaucum*) third. This was primarily due to the relatively low levels of methionine in *Acacia* seeds and compounded by the apparent interference of S-carboxyethyl cysteine (a non-protein amino acid) on methionine absorption (Falade, *et al*., 2012). This issue with methionine means that *Acacia* seeds are best eaten with a supplementary source of methionine. The aim of this study is to determine the composition of amino acids in *Acacia* seeds and to add another marker in the taxonomy of this genus.

Material and methods

Plant Materials

Acacia seeds of eight taxa (*A cacia mellifera*, *A. nilotica* ssp*, adansonia*, *A. nilotica ssp nilotica*, *A. oerfota*, *A. polycantha* ssp. *campylacantha, A. senegal* var *senegal, A. seyal* var *seyal* and *A. sieberiana* var *sieberiana*) were selected for this study. Seeds of the selected eight *Acacia* taxa were collected from various areas of Western Sudan. These seeds were identified by plant taxonomist of Soba Research Forests, Ministry of Science and Technology, Khartoum.(Ustaz Hasan AL-Bager). The identification of studied taxa was also done according to AL-Amin,1972. The voucher specimens are kept at Herbarium of Botany Department, Faculty of Science and Technology, Omdurman Islamic University.

Methods

Amino acids content

The amino acids composition of all studied of (8 taxa) *Acacia mellifera*, A. *nilotica* ssp*, adansonia*, A. *nilotica ssp nilotica*, A. *orefota*, *A. polycantha* ssp *campylacantha*, *A. senegal* var *senegal A. seyal var seyal*, and *A. sieberiana, var, seberiana* samples was determined according to the official methods (AOAC,1984). Using Sykam HPLC system (Model S7130). The system was equipped with a programmable auto injector.

The samples were prepared by 200 mg of each sample in hydrolysis tubes. Five milliliters of 6N-hydrochloric acid (6M) were added to each and tightly closed. The tubes incubated at 100°C for 24 hours. The hydrolysate of each sample was then filtered using 125mm filter paper. A 200 µl of the filtrates were evaporated at 140°C for about an hour. A diluted buffer was added to the dried samples and then the samples were ready for analysis. The HPLC system was calibrated with a standard amino acid kit solution and then the sample hydrolysate was injected into the HPLC analyzer system with an auto injector.

Pairing affinity values

The method of Pairing affinity between studied taxa of *Acacia* seeds based on distribution of free amino acids and fatty acids (or similarity Index) described

by Sokal and Sneath, (1963) was used to analyse the data of free amino acid composition and determine the pairing affinity between the eight taxa of *Acaica* species. The degree of pairing affinity (PA) between the two taxa was calculated according to the following formula:

$$\text{Pairing Affinity (PA)} = \frac{\text{Amino acids common to A and B}}{\text{Total amino acids in A and B}} \times 100$$

Where A and B are the compared taxa

Results and discussion

Amino acid composition of eight Acacia taxa

Table 1 shows the composition of amino acid of the studed *Acacia* taxa. Fig. 1-8 shows the HPLC chromatograms of each of the *Acacia* taxa studied. The most dominant of amino acid among all *Acacia* species studied was glutamine highest value noted in *Acacia seyal* var. *seyal* 43.65mg/gm, whereas, the lowest value existed in *Acacia polyacantha* ssp. *campylacantha* 32.62mg/gm. followed by arginine ranged from 21.25 to 12.08mg/gm for *Acacia polyacantha* ssp. *campylacantha* and *Acacia nilotica* ssp. *Adansonia*, respectively. While, the lowest values of amino acid in all *Acacia* species studied were noticed in methionine 0.55 to 1.25mg/gm for *Acacia nilotica* ssp. *nilotica and Acacia mellifera*, respectively. Histidine was ranged between 4.94 and 7.12mg/gm, *Acacia mellifera* got highest level from histidine and least level was observed by *Acacia sieberiana*. *Acacia senegal* provided high amount of isoleucine 13.31 mg/gm compared with other *Acacia* species studied. The lowest value of isoleucine was obtained by *Acacia adansonia* 6.85 mg/gm. The *Acacia senegal* gave highest values from leucine and lysine 19.54 and 14.85 mg/gm, respectively.

The least values obtained by *Acacia adansonia* 13.21 and 9.33mg/gm respectively. Phenylalanine showed highest value 11.60 mg/gm in *Acacia senegal*, whereas, the lowest value was observed by *Acacia adansonia*. *Acacia seyal var seyal* provided high level from threonine but the lowest value noticed in *Acacia adansonia*. From the current results, Table 1 showed that valine, alanine and glycine were found higher in *Acacia mellifera*. Tyrosine, serine and proline were appeared higher in *Acacia polycantha* spp *camplyacantha*. Aspartic acid was found high in *Acacia Senegal*, glutamic acid was noticed higher in *Acacia seyal var seyal*.

Table 1. Amino acid composition of eight *Acacia* taxa.

Amino Acid (mg/g)	*Acacia mellifera*	*Acacia nilotica* ssp. *adansonia*	*Acacia nilotica* ssp. *nilotica*	*Acacia oerfota*	*Acacia polyacantha* ssp. *campylacantha*	*Acacia senegal*	*Acacia seyal var. seyal*	*Acacia sieberiana* var. *sieberian*
Asp	20.61	12.27	14.83	17.58	18.5	23.27	18.02	16.95
Thr	6.17	4.35	4.55	4.66	5.2	6.51	6.52	5.33
Ser	12.24	7.01	5.07	6.15	12.51	8.79	6.85	4.97
Glu	36.88	39.5	33.8	37.49	32.62	39.85	43.65	36.13
Gly	11.37	8.55	8.63	10.97	10.09	11.13	9.88	8.71
Ala	11.17	7.48	7.64	7.67	10.72	11.02	10.07	8.54
Cys	3.64	8.5	6.82	5.75	1.9	4.75	7.02	7.38
Val	13.48	8.32	8.59	8.69	12.68	13.23	11.65	9.35
Met	1.25	0.64	0.55	0.67	0.98	1.22	1.15	0.72
Iso	12.97	6.85	6.88	7.1	10.72	13.31	9.75	7.78
Leu	19.3	13.21	13.57	13.64	17.9	19.54	17.34	15.91
Tyr	4.8	1.34	1.35	1.75	5.26	4.54	1.93	2.00
Phe	11.49	5.94	6.71	6.9	9.92	11.6	9.84	7.33
His	7.12	5.47	5.5	6.81	6.91	6.56	6.59	4.94
Lys	14.3	9.33	9.72	10.2	13.51	14.85	11.79	9.53
Arg	17.93	12.08	13.25	20.55	21.25	20.26	20.45	16.72
Pro	11.26	8.25	9.19	8.98	11.89	11.67	11.71	10.57

Asp = Aspartic acid, Thr = Threonine acid, Ser = Serine, Ala = Alanine, His =Histidine, Arg =Arginine, Pro = Proline Met=Methionine,

Lys = Lysine, Glu = Glutamic acid , Gly = Glycine acidCys = Cysteine , Val = Valine acidIso = Isoleucine, Leu = Leucine Phe = Phenylalanine, Tyr = Tyrosin.

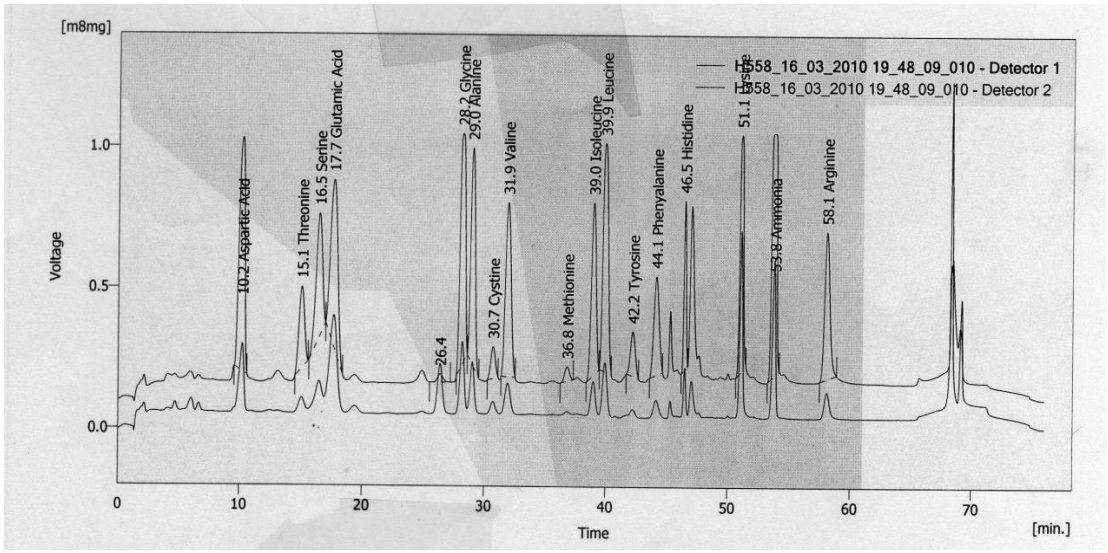

Fig. 1. HPLC chromatogram of *Acacia mellifera*.

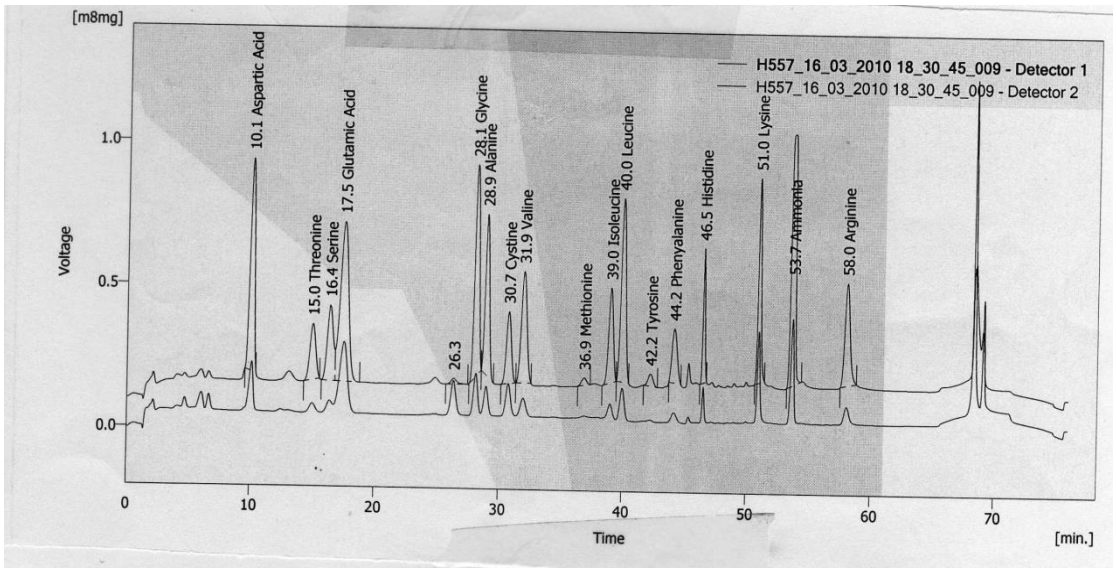

Fig. 2. HPLC chromatogram of *Acaia nilotica* ssp. *Adansonia*.

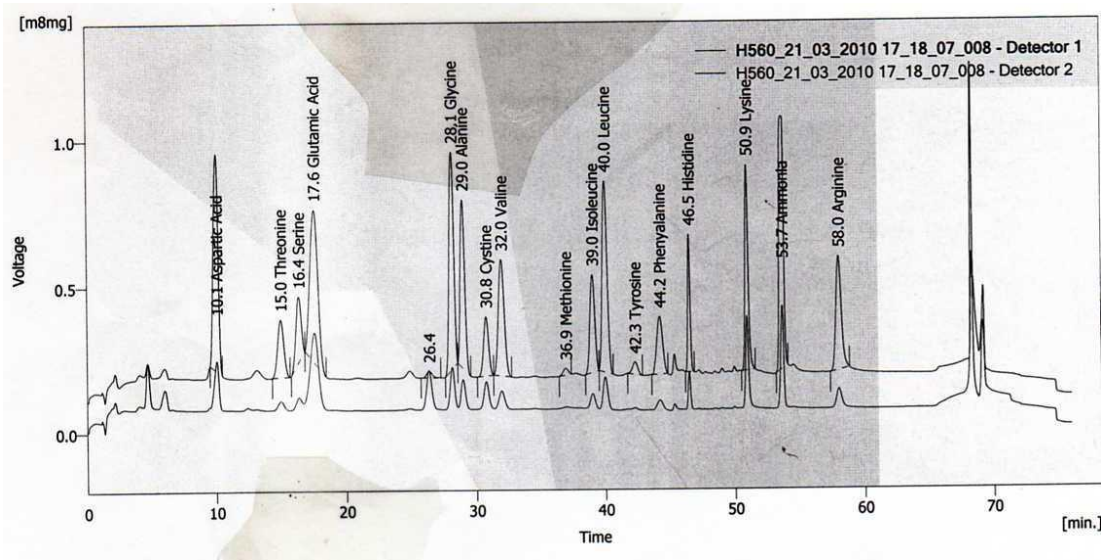

Fig. 3. HPLC chromatogram of *Acacia nilotica* ssp. *Nilotica*.

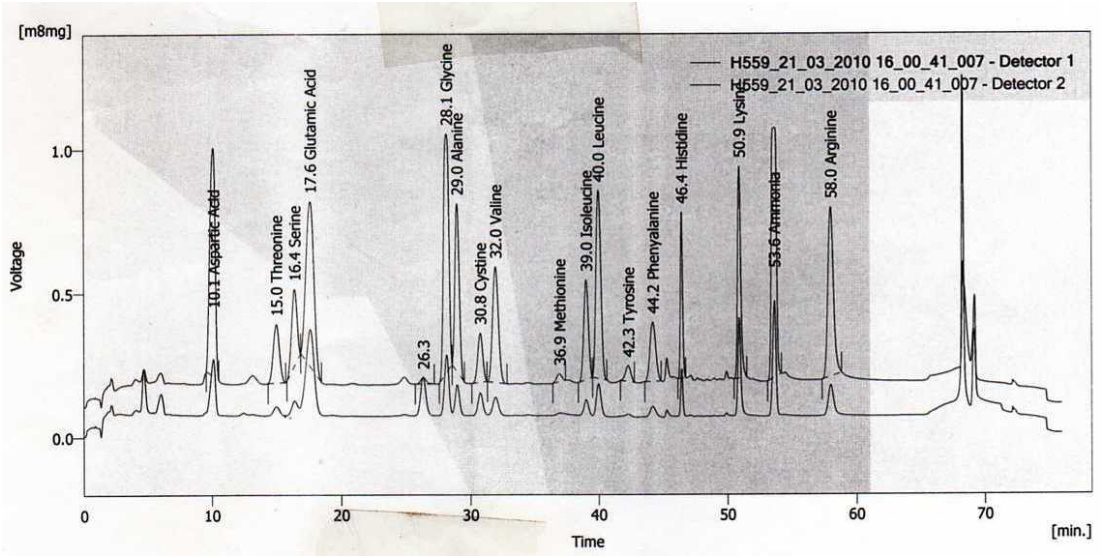

Fig. 4. HPLC chromatogram of *Acacia oerfota*.

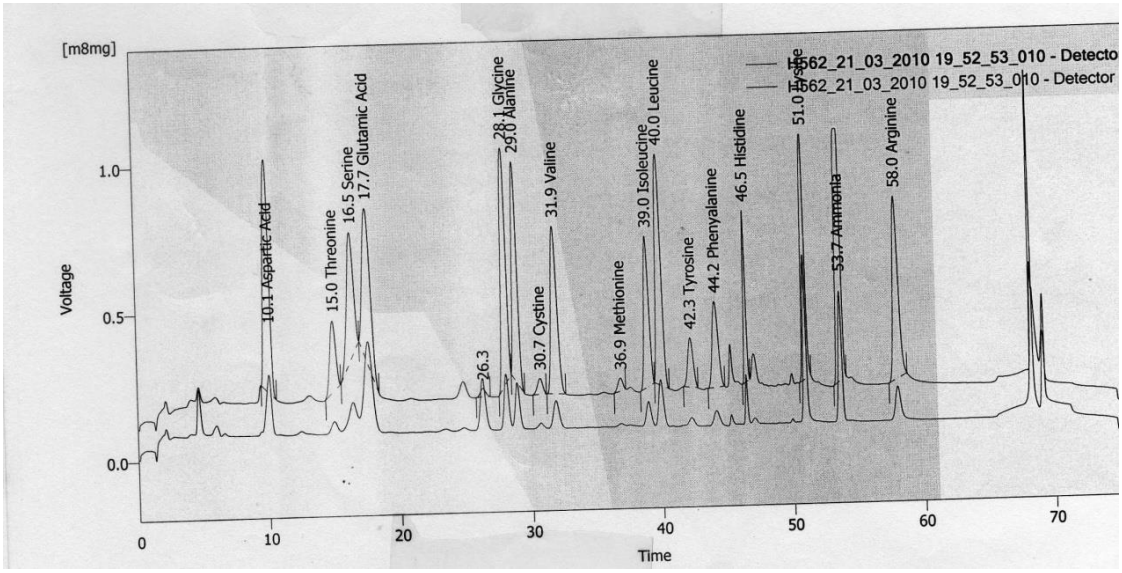

Fig. 5. HPLC chromatogram of *Acacia polyacantha* ssp. *Campylacantha*.

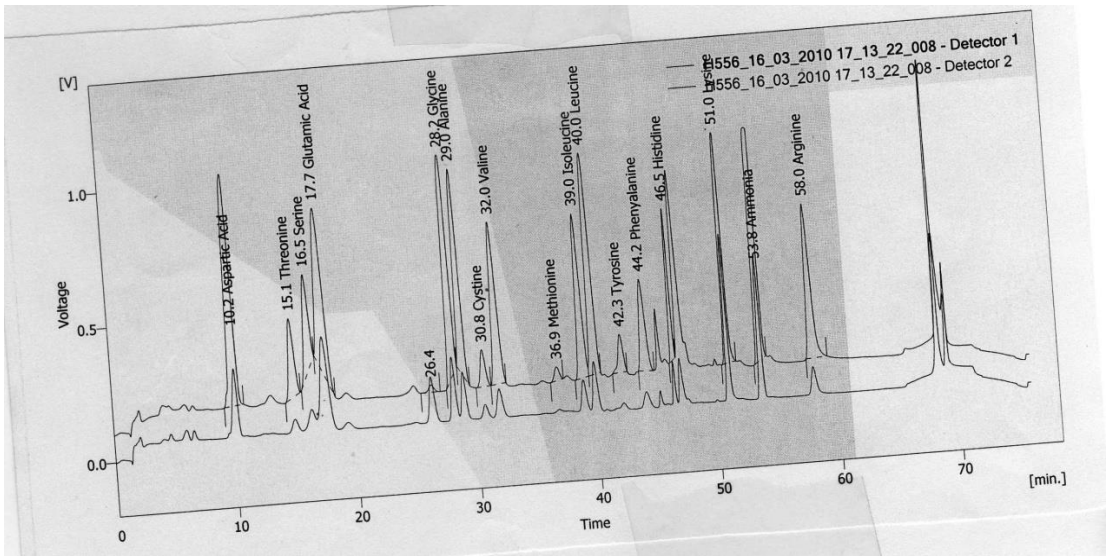

Fig. 6. HPLC chromatogram of *Acacia Senegal* var *Senegal*.

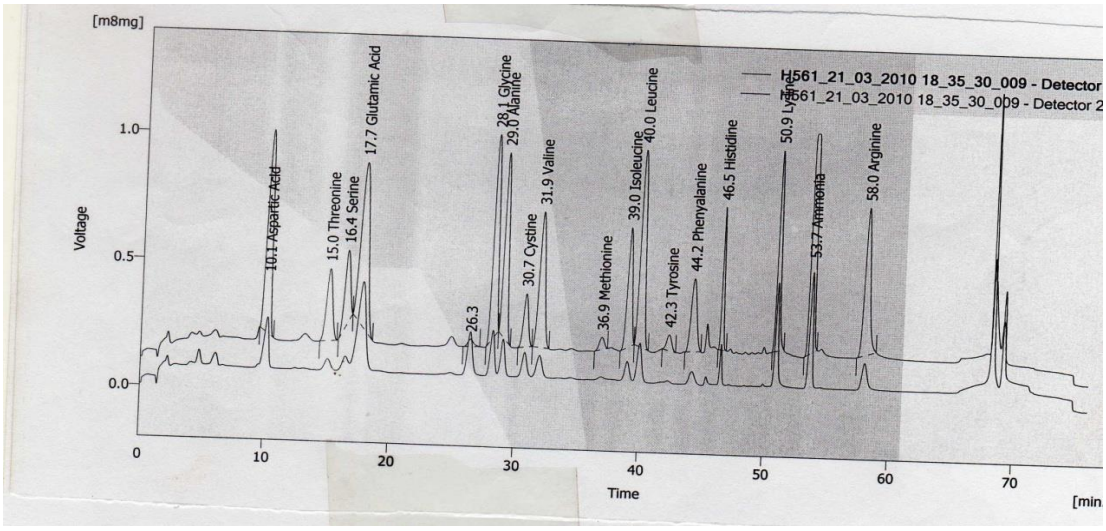

Fig. 7. HPLC chromatogram of *Acacia seyal* var. *seyal*.

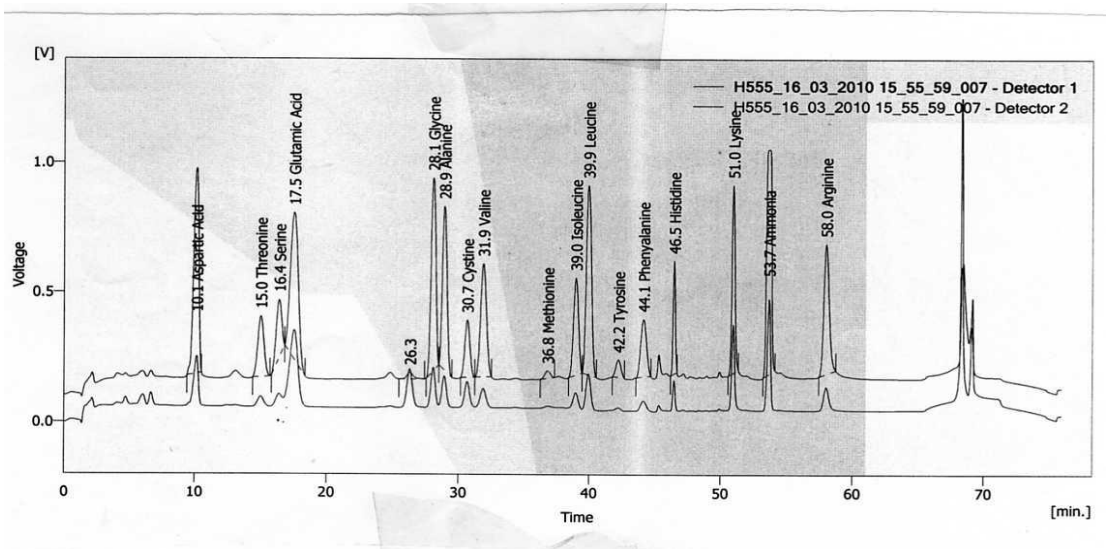

Fig. 8. HPLC chromatogram of *Acacia seiberiana*.

The total amino acids of the species studied reached 222.10 mg/gm of *Acacia senegal* seed. Therefore, it considered highest amount obtained, compared with others species. The total amount of amino acid differ from species to anther from *A. mellifera*, *A.seyal var seyal*, *A polycantha* spp *camplyacantha, A.orefota, A. sieberiana, A. adansonia* and the least amount obtained by *A. nilotica* spp. *nilotica* respectively.

The free amino acids of seeds of Acacia taxa as taxonomic marker

The free amino acids composition of the different *Acacia* taxa showed in Table 2. reveals a total of 17 amino acids, the most abundant amino acids in decreasing order were Glutamic acid, Aspartic acid, Leucine, Arginine, Valine and Serine. Whereas, the least abundant ones were Systeine, Tyrosine and specially Methionine.

The application of comparative free amino acid composition to taxonomic groups of different taxa and determination of the similarity index reveals the highest degree of pairing affinity between *Acacia oerfota* and *Acacia siebriana* (89.91%) followed by *Acacia nilotica ssp. adansonia* and *Acacia nilotica ssp. nilotica* (71.37%) and between *nilotica ssp. nilotica* and *Acacia seyal var seyal* (70.91%), *Acacia nilotica ssp. adansonia* and *Acacia seyal* (70.54%) and between *Acacia nilotica ssp. nilotica* and *Acacia siebriana* (70.19%). Hence *Acacia orefota* and *Acacia siebriana* are the most closely related, also *Acacia nilotica adansonia* and *Acacia nilotica seyal*.

Kinnari *et al.*, (2019) stated that *Acacia* seeds contained high amounts of essential amino acids (histidine, lysine, valine, isoleucine and leucine). There was variation in the sensory profile of the species.

Table 2. Results of Pairing Affinity between the eight taxa based on free amino acids of content

	A.n n	A.s	A.m	A.p	A.s	A.s	A.o	A.n a
A.nilotica ssp. *Nilotica*	100	70.19	68.98	69.27	69.95	70.91	68.69	71.37
A.sieberiana var sieberiana		100	64.28	65.60	59.36	67.22	89.91	67.02
A.mellifera			100	66.93	67.19	68.36	65.83	68.39
A.polyacanth spp *camplyacantha a*				100	68.18	69.25	66.89	69.43
A.senegal var *Senegal*					100	67.12	67.12	69.49
A.seyal var *seyal*						100	68.16	70.54
A.oerfota							100	68.15
A.nilotica ssp. *Adansonia*								100

A.n n = A.nilotica ssp. *nilotica*

A.s = A.sieberiana var sieberiana

A.m = A.mellifera

A.p = A.polyacantha spp *camplyacantha a*

A.s = A.senegal var *senegal*

A.s = *A.seyal* var *seyal*

A.o = A.oerfota

A.n a = *A.nilotica* ssp. *Adansonia*

Conclusion

The results of this study showed that the seeds of the studied *Acacia* taxa are potential of being exploited as a source of oil and protein and have good nutritional value and could be included in human diet for food formulations. The amino acid composition of studied *Acacia t*axa may provide useful information which may help clarify the taxonomic relationships of complex groups of plants like those belonging the *Acacia* species.

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