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Dosage of Some Chemical Substances in Two Plant Species: *Alysicarpus ovalifolius* (Sch. and Th.) and *Indigofera pilosa* (Poir)

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Abstract

The content of chemical compounds of two leguminous plants (*Alysicarpus ovalifolius* and *Indigofera pilosa*) was given through chromatographic methods (HPLC) and spectrocolorimetry. The principal isolated compounds are: *β*-Carotene in the sheets, the vitamin B in the roots and the vitamin C in the pods. Trace elements are not also insulated in the two plant species. The results show a content iodine (0.27 mg/100g of sample) particularly high in the stems of *Indigofera pilosa*, what could explain the use of the plant to fight against the thyroid dysfunctions. The ratio iron in mg/100g of sample 114.14 is rather important and would make *Indigofera pilosa* a palliative against anaemia. The two plants would be indicated in cases of treatment of hypocalcaemia.

Keywords

Leguminous Plants, Spectrocolorimetry, HPLC, Proteins, Vitamins, Trace Elements

1. Introduction

Human being needs a rich, varied and balanced food to enjoy a good health. The economic crisis created underprivileged zones installing the populations in an insufficiency of resources, even precariousness. Deficiencies in vitamins, iodine, iron, calcium and in other essential nutrients are noted in all the age brackets in particular in the children [1].

To rectify such situations, the introduction in the food of some plant species especially of the family of legu-

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minous plants, would be an opportunity, which challenges any development strategy should integrate and support. Most leguminous plants are excellent sources of folic acid, potassium, iron and magnesium. They also contain vitamins of the group B, zinc and copper [2]. Being of vegetable origin, the leguminous plants are deprived of cholesterol and constitute usually a very good source of fibers [3]. Then, we centered this study on the research of the nutritional value of two leguminous plants *Alysicarpus ovalifolius* and *Indigofera pilosa*. Among the fodder plants, these fabaceaes leguminous plants *Alysicarpus ovalifolius* (Sch. and Th.) and *Indigofera pilosa* (Poir) appear most important. They are plants with high added value spread in all the tropical areas [4]. The promotion is explained by their excellent fodder qualities (proteins, energy and digestibility), their adaptation in Sahelian medium and their exploitation to commercial purposes for the pasture fattening ovine practised by the populations [5].

The analysis of the principal components was carried out by well-proportioned use of spectrocolorimetry and HPLC after extraction with suitable solvents and reagents in order to justify the use of the two plants like food supplements to balance certain deficiencies.

2. Equipment and Methods

2.1. Material

2.1.1. Vegetable Material

Vegetable species consist of the various parts (sheets, stems, pods and roots) of *Alysicarpus ovalifolius* and *Indigofera pilosa*. The study carried out on the fresh plants and the dried plants in the shade and to the laboratory temperature.

2.1.2. Laboratory Material

It consists of:

- A chromatograph HPLC Jasco 880 PU equipped with a UV/visible detector and an integrator Spectra Physics 4270;
- A both portable pH meter-conductimeter NOR 824 microprocessor equipped with electrodes model 9453 Iodide Half-Concealment ORION;
- A spectrometer UV/Visible Novaspec II;
- A colorimeter with direct reading Novaspec II;
- A centrifugal machine Prolabo.

2.1.3. Chemical Products

For the extraction and the isolation of proteins, vitamins, calcium and trace elements the solvents and reagents used are generally analytical quality. These include among others methanolic potash, the ferric chloride, the potassium thiocyanate, the cadmium oxide, the ammonium oxalate; the ascorbic acid β-carotene is of sigma quality.

Concerning the chromatograpy, special solvents quality HPLC (hexane, acetonitrile) have been used for fractionation.

2.2. Methods

2.2.1. Extraction

One to 15 g of dry sample are crushed in a mortar then macerated in 100 to 150 ml of water distilled for the biogenic salts and proteins or 100 to 150 ml of ethanol. The extracts are filtered. For proteins, 10 drops of cold CH_3COOH are added and heated with a bain Marie to precipitate them and the residues are dried and weighed.

For the extraction of vitamin A or β-carotene, the samples are crushed with 10 ml distilled water, filtered then saponified.

2.2.2. Dosage of Proteins

Albumin is used as witness and a range of solutions of concentrations (200 ppm; 40 ppm; 28.6 ppm and 3.1 ppm) was prepared. The addition of 2 drops of $CuSO_4$ and 2 drops of NaOH to each solution gives a violet colouring characteristic of proteins. The reading with the colorimeter at the wavelength λ = 550 nm, established then the line of calibration (**Figure 1**).

A = F(C) with A being the absorbance, C: concentration in ppm.

From the line of calibration, we determined the protein concentration of the various parts of the studied plants.

2.3. Dosage of Carotenoids

One gramme of dry sample is crushed in a mortar. Then the powder is mixed with 10 ml distilled water. After filtration the aqueous phase was extracted with the mixture hexane/acetonitrile/ethanol (50/20/30V/V/V). The extract was saponified with caustic potash (KOH) methanolic (40%) in a flask of 5 ml closed and surrounded by aluminum foil to prevent the degradation of the carotenoids pigments sensitive to the light. After heating with a bain Marie at 56˚C during 20 mn, the contents are cooled then centrifuged during 5 mn at 2500 trs/mn.

The line of calibration (**Figure 2**) obtained with the range of solutions to the wavelength λ = 450 nm made possible to determine with the chromatographic analysis, the concentrations of β-carotene of the sheets, stems, pods and roots of both plants.

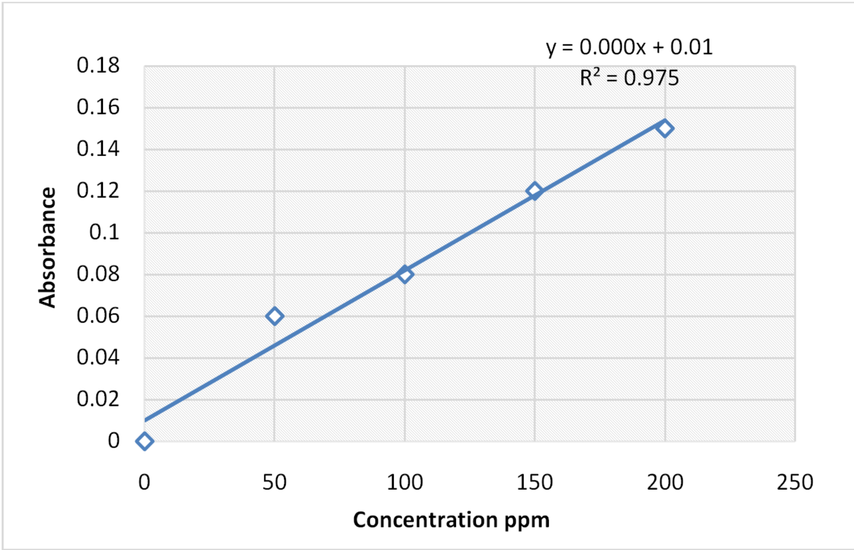

Figure 1. Right of calibration of proteins.

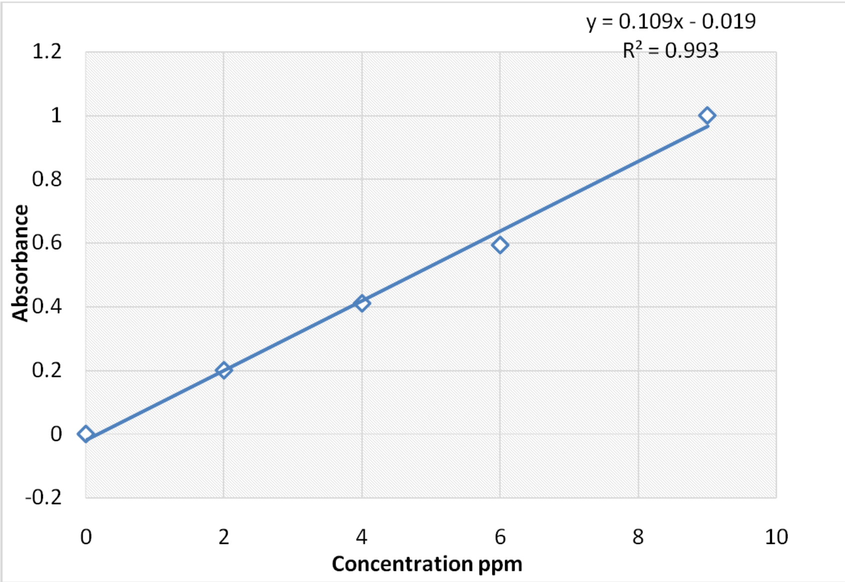

Figure 2. Right of calibration of β-carotene.

2.4. Dosage of the Vitamin B

It is carried out with the UV/visible spectrophotometer with 294 nm. The range of solutions having been used for the calibration is made up using the following concentrations: 5.01 g/L; 2.50 g/L; 0.83 g/L; 0.55 g/L and 0.50 g/L. On the basis of this range, we plotted the straight line of calibration (cf **Figure 3**) which enabled us to determine the various concentrations in vitamin B.

2.5. Dosage of the Vitamin C

The proportioning of the vitamin C carried out by chromatography HPLC with the wavelength λ = 254 nm. For the calibration A = F(c) (**Figure 4**), the range of concentrations is the following one: 100 ppm, 50 ppm, 20 ppm, 12.5 ppm, and 0.5 ppm of ascorbic acid in methanol.

2.6. Dosage of Iron

Iron in the presence of potassium thiocyanate gives a brown red complex characteristic of iron thiocyanate. The reading is made with λ = 520 nm the range of concentrations having been used for calibration (**Figure 5**) is: 20 ppm, 50 ppm, 125 ppm, 166.6 ppm, 500 ppm, 1000 ppm.

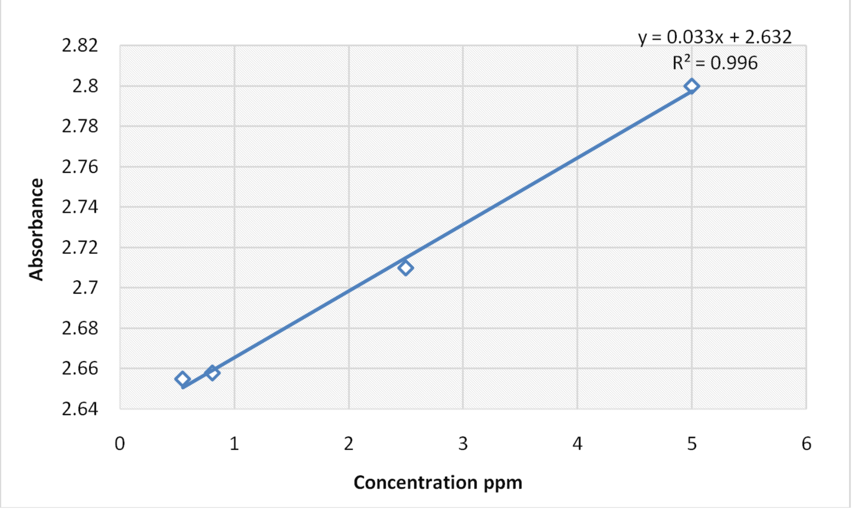

Figure 3. Right of calibration of the vitamin B.

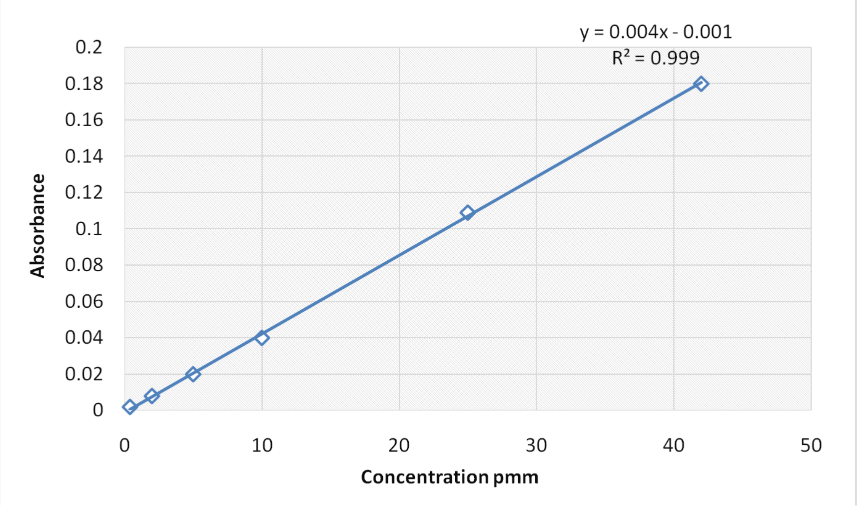

Figure 4. Right of calibration of the vitamin C.

2.7. Dosage of Iodine

The analysis is carried out with a both portable pH-metre conductimeter NOR 824 sensitive only to iodides.

One gramme of dry sample is crushed in a mortar then one 20 ml of distilled water is added to. The reading is carried out after filtration.

From the line of standard calibration (**Figure 6**) given by the manufacturer, the concentrations of the iodine of the various parts of the plants are given.

C = F(c) with C: Conductance in mV, c = concentration in iodide ppm.

2.8. Dosage of Calcium

The proportioning of calcium consists of making act 10 ml of ammonium oxalate on 100 ml of extract; it is formed a white precipitate of calcium oxalate.

$$C_2O_4\left(NH_4\right)_2 + Ca\left(OH\right)_2 \rightarrow CaC_2O_4\left(\text{precipitate}\right) + 2\left(NH_4OH\right)$$

After drying, the precipitate is weighed.

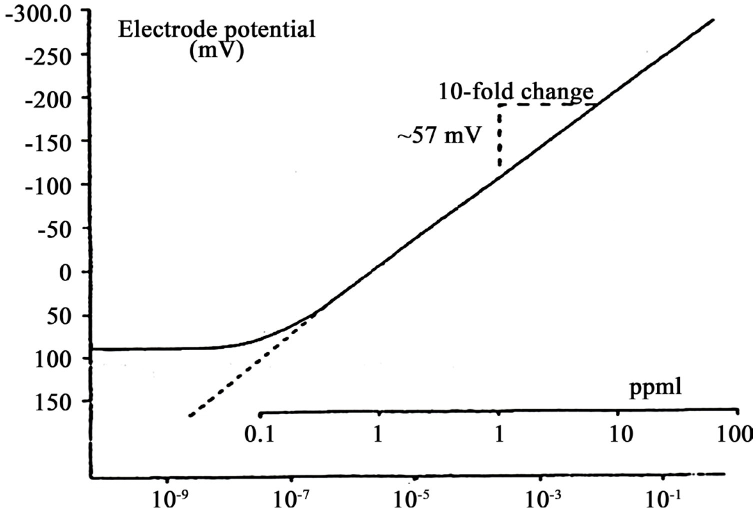

Figure 5. Right of calibration of the iodide [8].

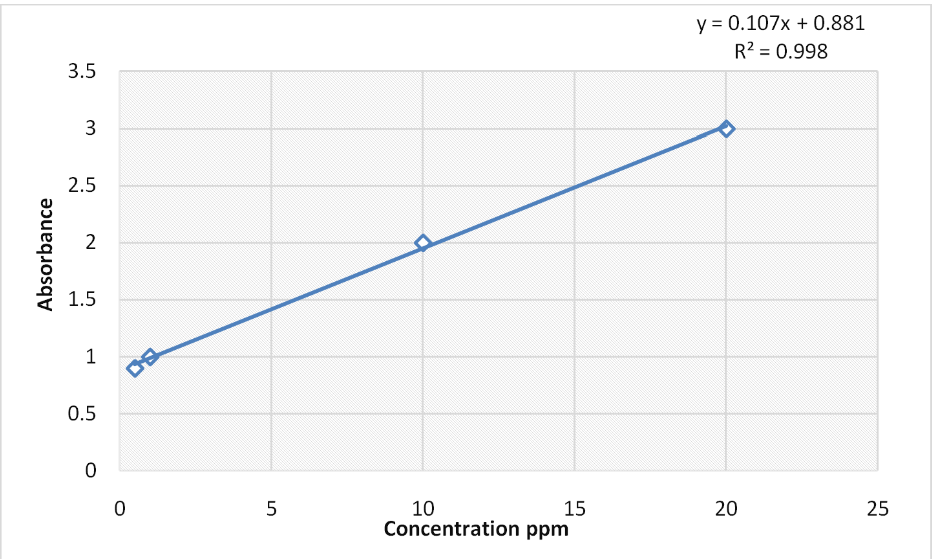

Figure 6. Right of calibration of iron.

3. Results and Discussion

3.1. Results

3.1.1. Dosage of Proteins

The content of proteins of the air parts of *Indigofera pilosa* varies between 18.2% to 24%, whereas at *Alysicarpus ovalifolius* it is of 17%. These results are not negligible since the daily requirements out of proteins at an individual are estimated between 12.5 g and 28.3 g for the children [6] [7].

3.1.2. Dosageof Carotenoids

The average *β*-carotene content forIndigofera pilosa is 0.628 mg/100g and 0.930 mg/100g for *Alysicarpus ovalifolius.*

3.1.3. Dosage of the Vitamin B

The plants *Indigofera pilosa* and *Alysicarpus ovalifolius* provide the respective average contents of vitamin B is 26.63% and 43.27%.

3.1.4. Dosage of the Vitamin C

The pods and the roots are richer in vitamin C than the other parts of the plants. Indeed the contents vary between 63 mg with 375 mg for *Alysicarpus ovalifolius* and 85 mg and 344 mg for *Indigofera pilosa*.

3.1.5. Dosage of Iodine

The results show that the pods and the roots of the two plants are very rich in iodine. Indeed the contents are of 0.39 mg and 0.15 mg/100g with 0.27 mg/100g respectively for *Indigofera pilosa* and *Alysicarpus ovalifolius*.

3.1.6. Dosage of Iron

The contents of iron are variable. The most important rates are noted respectively pods and roots with 44.8 mg/100g with 839.6 mg/100g for *Alysicarpus ovalifolius*.

The results of the various concentrations starting from the right-hand sides of calibrations are consigned in the following **Table 1**.

3.2. Discussion

The two plants gave after extraction and various analysis samples by HPLC and spectrocolorimetric method the results in various required components(iodine, iron, calcium, proteins, *β*-carotene, vitamins C and B).Indeed, we found contents of proteins from 18.20% to 24% in sheets and stems, bordering the daily needs which range between 12.50% and 28.30% for children [6] [7]. The average *β*-carotene contents are of 0.63 mg/100g for *Indigofera pilosa* and 0.92 mg/100g for *Alysicarpus ovalifolius* whereas the daily needs turn around 0.35 mg/kg for children and 0.7 mg/kg for adults [9]. The plants *Indigofera pilosa* and *Alysicarpus ovalifolius* provide the respective average contents of vitamin B of 26.63% and 43.27%. Knowing that the requirements in vitamins B recommended are 25 mg/kg, the two plants would be important sources vitamin B. In addition the requirements

Table 1. Summary of the average content of the various studied elements.

Plants	*Indigofera pilosa*					*Alysicarpus ovalifolius*			
Sample	Sheets	Stem	Pods	Roots	Average	Sheets	Stem	Pods	Average
Protein (g/100g)	18.20	19.30	24.00	ND	20.50	17.00	ND	ND	17.00
β-caroten (mg/100g)	1.62	0.80	0.05	0.04	0.63	2.49	0.09	0.17	0.92
Vitamin B (g/100g)	ND	ND	9.47	43.80	26.63	ND	21.17	65.38	43.27
Iodide(g/100g)	0.05	63.50	0.20	0.39	0.21	0.01	0.15	0.27	0.14
Vitamin C (mg/100g)	22.00	20.00	344	85.00	117.75	19.00	375	63.00	152.40
Iron (mg/100g)	5.00	15.00	44.80	391.76	114.14	8.50	98.25	839.50	315.40
Calcium (g/100g)	0.09	0.09	6.23	7.14	3.39	0.84	9.94	12.42	7.73

ND: Non Determined.

in vitamin C are in average 75 mg/kg for adult [9], and the plants provide 117.75 mg/100g for *Alysicarpus ovalifolius* and 152.40 mg/100g for *Indigofera pilosa*. The contents of biogenic salts are not in rest. Calcium accounts for in average 3.39%; iron gives 114.14 mg/100g and the iodine provide 0.21 mg/100g.Taking into account the important rather content of iron, *Indigofera pilosa* could be used to fight against anemias. The iodine contents especially high in the stems of *Indigofera pilosa* could be used to fight against the thyroid dysfunctions.

The well concentrated *β*-carotene in the sheets of the plants, the vitamin B in the roots and the vitamin C in the pods could make them a possible way to fight against the vitamin disorder generated by their deficiencies. We could also take into account these plants to balance some cases of hypocalcaemia.

4. Conclusions

All this diversity and this wealth in the composition do not allow for the moment to make a chemical classification of the plants. However, studies of characterization of some compounds such as anthracene and flavonic heterosides, tannins and saponosides would complete this investigation.

Taking into account the appreciable contents, of proteins, vitamins, the leguminous plants *Alysicarpus ovalifolius* and will *Indigofera pilosa* could be used as food supplements to balance certain deficiencies. However, the control of the possible toxicity of the plants would be a precondition.

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