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Article

CHEMOMETRICS STUDY OF GERMS PLANT FOR DETERMINING ANTICANCER ACTIVITY

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ABSTRACT

This project started from the premise that in nature, there are substances capable of preventing the occurrence of cancer, or, at least keeping it in its early stages, by using plant germs. Germs were obtained from Biovita germs machine, like: watercress, unshelled and genetically modified soybeans, hyssop, sage, fennel, amaranth, broccoli, radish, alfalfa, cumin, caraway, rye, wheat, buckwheat, and the next step was to extract the substances from these germs, and analyzed by LS/MS, for the identification of polyphenolic compounds, which are antioxidants, which play a very important role in the prevention of cancer, mainly alpha-linolenic acid, the main source of omega 3 fatty acids, essential for the human body. The process was carried out in several stages, namely: the germination period was 4-5 days, followed by maceration in hydroalcoholic solution with 99% ethyl alcohol, then the decoction and percolation in the system provided with separating funnel, filter and Erlenmayer glass. The antioxidant activity was determined using spectrophotometry (Tecan Sunrise™ – A Reliable Absorbance Reader), using as a calibration sample, ascorbic acid, the absorbance of which was

already known from the literature data, namely 600 nm. The presence of riboflavin, at an absorbance of 440 nm, allantoin, 517 nm, quercetin, at an absorbance of 385 nm, choline, at 570 nm, and thiamine at an absorbance of 520 nm, was identified. The calibration curve was obtained based on the values of the concentrations used and the absorbance obtained. The scavering was determined, on the basis of which the antioxidant activity was identified.

1. INTRODUCTION

Cancer is characterized by the rapid growth of abnormal cells and is often linked to oxidative stress. [1] Broccoli is loaded with compounds that are believed to protect against cancer. [2]

Observational studies suggest that the consumption of cruciferous vegetables, including broccoli, is linked to a reduced risk of many cancers, including lung, colorectal, breast, prostate, pancreatic, and gastric cancers [3]. A unique family of plant compounds called isothiocyanates sets cruciferous vegetables apart from other veggies. Studies suggest that isothiocyanates affect liver enzymes, reduce oxidative stress, decrease inflammation, stimulate your immune system, and combat the development and growth of cancer. [4] The main isothiocyanate in broccoli, sulforaphane, acts against the formation of cancer at the molecular level by reducing oxidative stress .[5] Sulforaphane occurs at 20–100 times higher amounts in young broccoli sprouts than in full-grown heads of this vegetable [6]. Though broccoli supplements are also available, they may not contribute an equivalent amount of isothiocyanates and thus may not give the same health benefits as eating whole, fresh broccoli [7].

Bile acids are formed in your liver, stored in your gallbladder, and released into your digestive system whenever you eat fat. [8] Afterward, the bile acids are reabsorbed into your bloodstream and used again. Substances in broccoli bind with bile acids in your gut, increasing their excretion and preventing them from being reused. [9]

This results in the synthesis of new bile acids from cholesterol, reducing total levels of this marker in your body. [10]

Two of the main carotenoids in broccoli, lutein and zeaxanthin, are associated with a decreased risk of age-related eye disorders.[11]Vitamin A deficiency may cause night blindness, which can be reversed with improved vitamin A status. [12]

Broccoli contains beta carotene, which your body converts into vitamin A.

This vegetable may thus boost eyesight in individuals with a low vitamin A intake. [13]

Broccoli is considered a goitrogen, which means that high amounts may harm the thyroid gland in sensitive individuals. Cooking this vegetable on high heat can reduce these effects [14]

Individuals taking the blood thinner warfarin should consult with their healthcare practitioner before increasing their broccoli intake because its high vitamin K1 content may interact with this medication. [15]

All plants and animals produce squalene as a biochemical intermediate, including humans. It occurs in high concentrations in the stomach oil of birds in the order Procellariiformes.[16] Squalene is a natural organic compound originally obtained for commercial purposes primarily from shark liver oil (hence its name, as *Squalus* is a genus of sharks), although plant sources (primarily vegetable oils) are now used as well, including amaranth seed, rice bran, wheat germ, and olives. Yeast cells have been genetically engineered to produce commercially useful quantities of "synthetic" squalane. [17]

Squalene is a hydrocarbon and a triterpene, and is a precursor for synthesis of all plant and animal sterols, including cholesterol and steroid hormones in the human body.[18]

Phosphaturic mesenchymal tumor (PMT) [19] is a rare distinctive mesenchymal neoplasm with heterogeneous but recognizable histologic appearances. It frequently elicits a clinical paraneoplastic syndrome consisting of hypophosphatemic hyperphosphaturic osteomalacia due to increased secretion of FGF23 [20]. The patients typically present with gradual muscular weakness, bone pain, and pathologic fractures. The diagnosis is commonly delayed for years due to the non-specific nature of these symptoms, lack of clinical suspicion, failure to include serum phosphorus levels in routine blood chemistry testing, and difficulty in identifying the responsible tumor. Additionally, these tumors are often missed histologically because of their rarity and morphologic overlap with other mesenchymal neoplasms. [21] Complete excision of the tumor is crucial as it typically resulted in the resolution of the osteomalacia, clinical symptoms, and laboratory abnormalities. Observational studies suggest that the consumption of cruciferous vegetables, including broccoli, is linked to a reduced risk of many cancers, including lung, colorectal, breast, prostate, pancreatic, and gastric cancers [3]. A unique family of plant compounds called isothiocyanates sets cruciferous vegetables apart from other veggies.Studies suggest that isothiocyanates affect liver enzymes, reduce oxidative stress, decrease inflammation, stimulate your immune system, and combat the development and growth of cancer.

2. METHOD

2.1. THEORETICAL MODEL

This method was developed in Oncogen laboratory, for determining antioxidant activity by the bases of the absorbance values, obtained by the UV-VIS analysis. It was used the ethanol extract for *in vitro* antioxidant activities and quantitative determination of bio-active compounds,and was made after next steps:

1. The germination of the seeds / Germination
2. Physical Purity
3. The composition of botanical
4. Humidity
5. Molecular weight
6. Viability
7. The germination energy
8. The control surfaces taken

2.2. Experimental Method/Model

This method is based by the Standard Protocol, from that have used:

2.2.1. Chemicals and basic tools:

2.2.1.1.Ethanol 99,0%

2.2.1.2.Na2SO4 , anhidrous
2.2.1.3.Aluminium oxide with granulation for cromatography
2.2.1.4.DSS
2.2.1.5.NaOH sol.
2.2.1.6.HCl sol.
2.2.1.7.ddH2O
2.2.1.8.DMSO
2.2.1.9.Weighing material
2.2.1.10.Filter material
2.2.1.11.Pasteur pipettes

2.3. Equipment

2.3.1. Digital balance
2.3.2. pHmeter
2.3.3. Sonicator
2.3.4. Vortex
2.3.5. Heating source
2.3.6. Graduated 10ml cylinder
2.3.7. Bath water
2.3.8. Liquid column of cromatography
2.3.9. A solvent in which the sample is very soluble (i.e.: carbon tetrachloride, deuterated methanol, DMSO,or ethanol etc.). *It is prudent to try this out first with ordinary methanol, DMSO etc. to have an idea about the relative volumes required before using the deuterated solvents).*

The pH can also be adjusted up or down with NaOH or HCL to aid dissolution.
Add about 3ml of the sonicate, vortex or shake lightly to dissolve sample (avoid frothing).

Glassware
- Molding conical plug
- Pipetts graduated of glass by different capacities
- glass filter
- 50ml cylindrical
-Coloumns of glass chromatography with a length of about 100 mm, an inner diameter of about 10 mm, cock provided at the inner side and having the lower end tapered to a diameter of mm.
-Capsuls porcelain, 50 ml.
-Germinater BIOVITA G1
-Analytical balance
-Stirrer
-Sonicator or Shaker

2.4. Measurement Materials

-site mesh size 2 mm
-Map medium porosity filter
- Porcelain pestle
-Thermometer
-Glass wool

3. APPLICATION

In the first step it was checks the Material Safety Data Sheet for the compounds. From the literature (THE MERCK INDEX),it was check the standard protocol of this method in vitro. Next it was check the qualitative and quantitative characterization of the seeds, that were puts on the BIOVITA germinator.

The obtain germs and the steps of the process , was showed in the next figures:

FIGUREs 1-9. Types of germs plant from different type of beans,like: Schinduf;Fennel; Sage; Hyssop; Kresse ; Soy; Chia.

It was check the temperature,that can also be adjusted to aid the rapid dissolution of sparingly soluble solutes.
After 4-5 days of the germination,it was continuos with the decoction and percolation process of the obtained germs.
The next step it was to pursued the measurement of the concentration of the desired hydrocarbons compounds absorbed by the plant. Adjust the pH back to 7.0 (6.8 – 7.2) and make total volume to 4.0 ml in the graduated tube, by identification the negative effects. There will be pursued an comparison of the efficiency of desired eight types of plants used, tested, namely (kresse, hyssop, sage,soybean,fennel,chia, amaranthus,schinduf).

3.1. Results

The antioxidant activity was determinated by the absorbance values.The values of the measurement values of the molar concentration (mg/mL) ,were showed in the next table.

Table1. The measurement values of the molar concentration of the kresse germs; hyssop germs; sage germs; soy germs; amaranthus germs; chia germs; fennel germs and schinduf germs. The absorbance obtained values are showed in this table.

Type of germs plant of beans	Molar Concentration (mg/mL)								Absorbance (nm)			
									Name of			
									Reference	Antioxidant substance present in germs		
Kresse	0.1179	0.05	0.1332	0.0567	0.0396	0.0464	0.1431	0.0518	**0.1406**	**0.0449**	**Ascorbic acid 518**	**Quercetine**	**385**
Hyssop	0.049	0.051	0.0565	0.0464	0.0506	0.0458	0.0464	0.0461	**0.0461**	**0.0486**		**Hysopine**	**280**
Sage	0.047	0.0463	0.0451	0.047	0.0473	0.0505	0.0431	0.0472	**0.0485**	**0.048**		**Hystamine**	**506**
Soy	0.0452	0.9608	0.0499	0.7696	0.0495	0.3669	0.0456	0.4933	**0.046**	**0.5299**		**Flavin**	**440**
Amaranthus	0.2442	0.2102	0.1971	0.1925	0.285	0.1416	0.2051	0.2194	**0.2396**	**0.2536**		**Choline**	**570**
Chia	0.2485	0.3316	0.3289	0.3685	0.3139	0.3192	0.3484	0.2498	**0.2918**	**0.2519**		**Cyannine**	**694**
Fennel	0.2971	0.3109	0.2432	0.3466	0.2284	0.2669	0.2597	0.2835	**0.2379**	**0.2556**		**Tanin**	**760**
Schinduf	0.1068	0.1159	0.1115	0.1114	0.1046	0.1202	0.0925	0.0964	**0.0734**	**0.0932**		**Quercetine**	**385**

3.2. Discussion

The scavering was calculated by the plot of the concentration values, by the obtained absorbance values (nm). The next figures are:

1. The plot of the creson(kresse) concentration, besides of the absorbance values (Figure 10);
2. The plot of the hyssop concentration, besides of the absorbance values (Figure 11);
3. The plot of the sage concentration, besides of the absorbance values (Figure 12).
4. The plot of the soy concentration/ by absorbance values by the absorbance values (Figure 13).

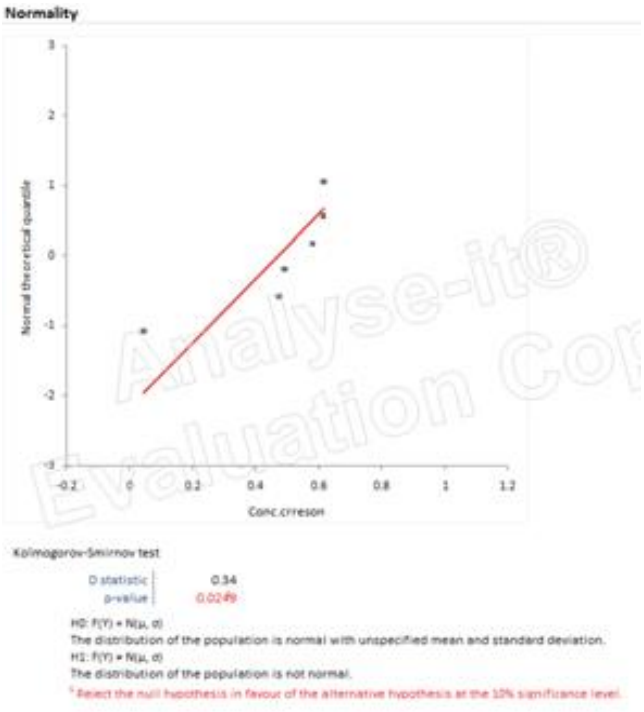

Figure 10.The plot of the c_{creson}(kresse) concentration

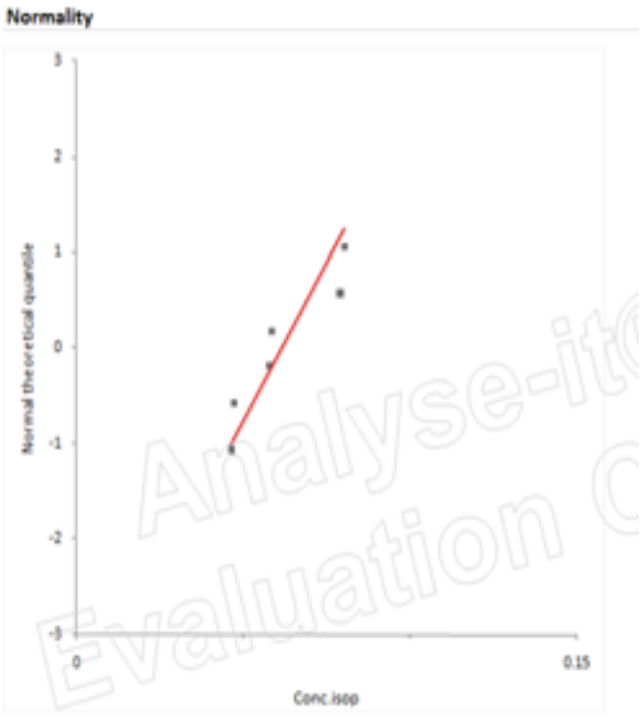

Figure11.The plot of the $c_{\text{hyssop(mg/mL)/Abso}}$

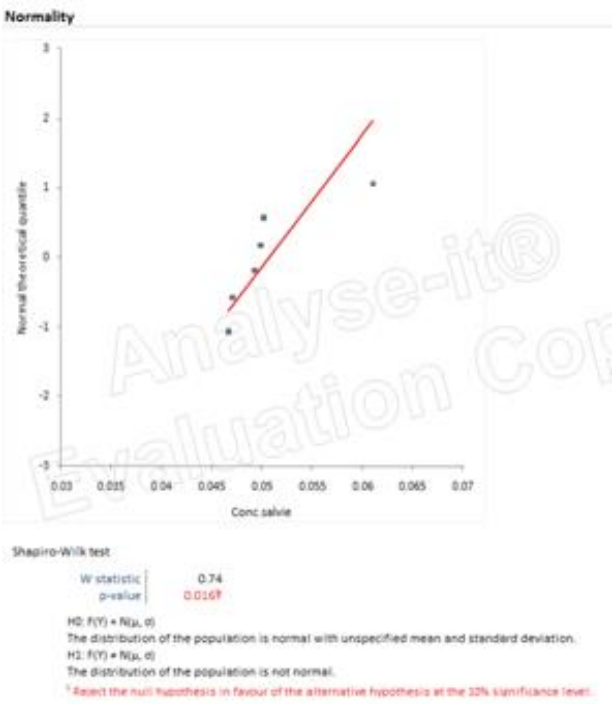

Figure12. The plot of the c_{sage} (sage)concentration/absorbance values

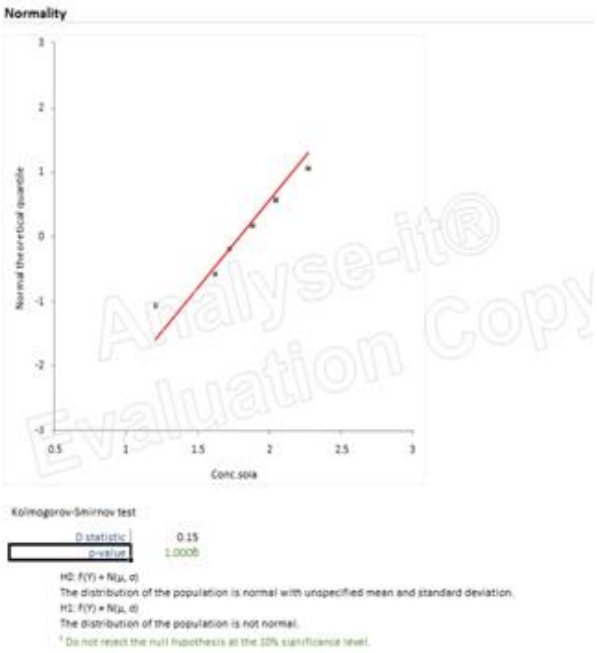

Figure 13. The plot of the $c_{soy\ concentration/\ by\ absorbance}$ values

4. CONCLUSION

In this paper, we are used a series of hydroalchools extracts, with different concentrations, by 15ml, 25ml, 50ml si 75ml, with 99.0% ethanol. The germs were obtained by different plants like: broccoli, kresse, Hyssop, Sage, Soy, Amaranthus, Chia, Fennel and Schinduf. In this UV-VIS analyse,for identify the absorbances, we are obtain : Quercetine , (A= 385 nm); Hyssopina, (A= 280 nm), Hystamine (A=506 nm), Phlavine (A=440nm), Choline (A=570nm), Cyannine (A=694nm), Tannin (A=760nm).

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