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ResearchPaper

Phytochemical Study and Antioxydant Activity of Extracts of Shoots of 3 Varieties of Soy Glycine Max Growing In the Central Highlands of Madagascar

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ABSTRACT:*The current research concerns essentially the study of antioxidant molecules of natural origin. This study falls into this context and consists in making at first a phytochemical screening of extracts of* ***shoots of 3 varieties of soy*** *Glycine maxgrown in the Highlands of Madagascar Secondly, we estimated the antioxidant activity of these extracts The phytochemical study allowed to highlight the existence of saponins, polyphenols and flavonoids in aqueous extracts and unsaturated steroids in chloroform extracts.The method used to measure the antioxidant activity was the free radical scavenging by using DPPH•(2,2-diphenyl-1-picrylhydrazyl).Scavenging capacity of DPPH free radical is very interesting with a respective IC50 = 239 µg / ml for* ***F10,*** *74 µg / ml for* ***Malady*** *and 33 µg / ml for* ***OC11;*** *these values remain greater than the capacity of DPPH radical-scavenging ascorbic acid whose IC50 =4,575 µg /ml. The results obtained showed that the extract****OC11*** *demonstrate a significant antioxidant activity*

Key words*: antioxidant activity, phytochemical screening, soybean shoots ,malagasy varieties, aqueous and chloroformic extracts, Central Highlands of Madagascar*

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I. INTRODUCTION

Soy is a plant whose seed is the basis of a diet that is beneficial to human health. Some authors have reported the importance of lecithin, lipid, mineral and other minor soybean content, which the pharmaceutical industry has used for many uses. (Kim et al, 2006).In particular, Schryver's work, 2002, and Galan, 2011 , noted the soy isoflavone richness , and that their content varies according to soybean varieties and compartmentalization of the seed, i-e the cotyledons are poor in these compounds, while its concentration is higher in the seeds . Rasolohery, in 2007, studied genotypic and environmental variation factors potentially involved in isoflavone synthesis in cotyledons and soybean. All these reasons pushed us to make the phytochemical screening of the extracts of germs of the 3 varieties of soybeans growing on the Highlands of Madagascar, **F10, Malady, OC11,** to confirm the presence of phytonutrients in the studied plants.

The study of phytotherapeutic properties as an antioxidant is still attracting renewed interest from many researchers, particularly for plants used in traditional pharmacopoeia. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population rely on traditional medicine for their primary health care needs (Jon C Tilburt et coll, 2008) These plants represent a new source of active compounds such as phenolic (Mohammedi, 2005). Free radicals and oxygen species have been associated with cardiovascular and inflammatory diseases, and even intervene in cancer and aging. Efforts to compensate for the damage caused by these species are increasingly recognized as a basis for new therapeutic approaches, and in the field of preventive medicine, antioxidants are regaining interest (Isren et al., 2001).).

The development of new antioxidants with a good antioxidant capacityis essential to fight against the phenomena of oxidation. For this reason,the investigation of plants containing new antioxidant substances

remains a topical issue, considering that these plants may contain hundreds or even thousands of secondary metabolites.

Recently, interest in natural antioxidants, in relation to their therapeutic properties, has increased compared to previous years. In the various specialties, scientific research has been developed to extract, identify and quantify these compounds from several natural substances including medicinal plants and food products (Cai YZ, Sun M, Corke H (2003);(Congo M., 2012)(Khady B, et al. (2009).

The share of unexplored plants in both chemistry and biology is still immense. This offers the hope of discovering treatments for still devastating diseases and offering inexpensive therapeutic alternatives with fewer side effects. Current studies on secondary metabolites obviously focus on exploring their pharmacological activities (AbdelwahedAand al. (2007). Flora MALGACHE is full of several species of plants still little or not studied, but with real pharmacological properties (Rasoanaivo P,2000). The total and perfect control of the various properties of these plants, which involves the determination of all the physicochemical groups capable of generating one or more pharmacological effects, is today a goal that occupies a first-order order (Bulletin de l'Organisationmondiale de la Santé, 2008).That is why we are interested in carrying out a phytochemical study of extracts of germs of 3 Glycine max soybean varieties growing on the Madagascar Highlands. The present work aims to study the antioxidant activity of the methanolic extract of these extracts by the technique of DPPH (2,2-diphenyl-1-picrylhydrazyl).

Phytochemical determination:

Powdered plant samples (5 g) were extracted with a mixture of methanol and water (150 ml) in the volume ratio 4:1 using Soxhlet for 12 h. The extract with 75 ml (3×25 ml) chloroform in a separating funnel. The chloroform layer was separated and evaporated to dryness on a water bath maintained

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II. MATERIALS AND METHODOLOGY

2.1Materials

2.1.1- Plant material

Three varieties of soybeans are used for the realization of this study. These are the varieties FT10, OC11, FASTO which have been kindly provided by Fifamanor. It should be noted that these seeds have about the same morphological characteristics.

2.1.2-Reactive chemicals

Methanolic solution of FeCl3 10%,Gelatin solution 1%,Sodium chloride 1%),FeCl3 aqueous solution (10%),Glacial acetic acid,ConcentratedHCl,Concentrated sulfuric acid,Isoamylalcohol,Aceticanhydride,Ammonia solution 25%,Reagents from MAYER, WAGNER and DRAGENDORFF, Methanol solution of DPPH (8%),methanol,Ascorbic acid (vitamin C)

2.2 Methodology

2.2.1- Preparation of extracts

The present study is carried out with extracts of soybean shoot obtained by the following phyto-chemical extraction technique:

0.100 g of seed powder of each variety of soya are macerated in 5 ml of 80% methanol for 2 hours and after centrifugation, the supernatant is transferred to other previously coded tubes allowing the identification of the variety, the extracts are evaporated, the yield is calculated, the extracts are stored in a refrigerator at 4 ° C.

To find the different chemical families, 4 types of extracts are prepared from the seed powders of each variety and the dry evaporation residue of the extract to be studied:

Aqueous extract: the residue is stirred in distilled water, the suspension is boiled for 30 min.Chloroformic extract: the residue is suspended in chloroform, the mixture is filtered after stirring.Hydro-alcoholic extract: the residue is stirred in 80% ethanol for at least 2 hours.Acid extract: the residue is macerated for 30 min in 3 ml of 2N HCl, the solution then filtered.

2.2.2-Phyto-chemical screening

We have characterized the different chemical groups by referring to the techniques described in the work ofBékro Y. A.and al., 2007, Badiaga M.,2011.The phyto-chemical screening is carried out in order to search for the following active elements:

The detection of saponins is based on their ability to give foamy aqueous solutions (foam index)N'GuessanK, et coll., 2009.Tannins and polyphenols: The following tests are carried out on the aqueous extract: Ferric chloride test - Gelatin test 1% - Saline gelatin test:(Duncan el al. 1999)
Desoxyoses: KELLER-KILIANI test
Iridoids: The aqueous extract is treated with concentrated HCl. After heating in boiling water bath for 30 min, the turn of the blue stain shows the presence of iridoid.
Flavonoids: WILSTATER test (Fong et al., 1974)The presence of flavonoids is characterized by the turning of the color of the upper phase: from orange to red for flavones and from red to purple for flavonols, to purplish red for flavonones.
Leucoanthocyanans: The search for leucoanthocyanin is performed by the BATE-SMITH Test.
Steroids and Triterpenes: LIEBERMANN-BURCHARD Test (Fong et al., 1974)
Unsaturated sterols: SALKOWSKI test
Anthraquinones: BORNTRAGER test

Alkaloids :pH partitioning for alkaloids was performed according to Brimer el al. (1989). Dragendorff's reagent (Wagner et a/. 1984) was added to one part of the prepared extracts, and Mayer's reagent (Wagner el a/. 1984) to the other. Observations were made for the development of a red-orange precipitate on the addition of Dragendorff's reagent, and a white precipitate on the addition of Mayer's reagent.

2.2.3- Qualitative identification of antioxidant activity

For the evaluation of the antioxidant activity of the shoots of 3 varieties of soy Glycine max; we used aqueous methanolic extracts .10 mg / ml of each extract was deposited on a silica plate (silica gel 60 F254 on aluminum foil) and this was dried and developed in the appropriate solvent system DCM / Acetate (20/80). The revelation is made using a methanolic solution of DPPH.

2.2.4- DPPH free radical scavenging test.

DPPH (2,2-Diphenyl-1-picrylhydrazyl) is a free radical, stable or acceptorof intense violet color . This radical loses its native color when it binds with antioxidant substances, which transfer electrons or protons to it. The reduced form of DPPH confers a yellow color on the solution (Brand Williams1995). The shift towards this coloration and the intensity of the discoloration results from the nature, concentration and potency of the present active ingredients .The radical scavenging activity of the DPPH radical was measured according to the protocol described by Athamena et al., 2010: 5 µl of each methanolic solution of extracts at different concentrations are added to 195 µl of the methanolic solution of DPPH (8%). The mixture is vigorously stirred, then the tubes are incubated at room temperature and in the dark for 30 minutes. The maximum absorption wavelength has been previously determined. All readings are taken at 517 nm (Marksen K. and al,2007).The negative control is composed of 1 ml of the methanol solution of DPPH and 1 ml of methanol. The positive control is represented by a solution of a standard antioxidant: ascorbic acid whose absorbance was measured under the same conditions as the samples and for each concentration

III. RESULTS

3.1-The result of the phyto-chemical screening is shown in Table 1.

We have characterized the presence of saponins, polyphenols and flavonoids in aqueous extracts and unsaturated steroids in chloroform extracts

3.2- Qualitative test

The antioxidant activity of the extracts was observed on the plate by the appearance of yellow spots on the purple background (**Fig 1**)

3.3-DPPH assay

The inhibition percentages (%) of the DPPH radical ● are calculated from the following formula:
I% = [1- (Abs Sample-Abs Negative Control)] X 100
I%: percentage of anti-radical activity
Abs Sample: Sample Absorbance
Abs Negative control: Absorbance of the negative control. (Meddour, 2013).

The curves shown in **Figures 2, 3, 4,5** show the variation of the percentage of inhibition as a function of the concentrations of our extracts. We also determined graphically the concentration corresponding to 50% inhibition (IC50). The IC50 is inversely proportional to the antioxidant capacity of a compound, because it expresses the amount of antioxidant required to decrease the free radical concentration by 50%. The smaller the IC50 value, the greater the antioxidant activity of a compound (Parejo and al, 2003)The IC50 values of the different methanolic extracts are shown in **Table 2**.The methanolic extracts of the 3 varieties of soybean shoots make the free radical stable (2.2 diphenyl-1-picrylhydrazyl) yellow-colored diphenyl-picrylhydrazine with an

IC50 ranging from 33µg / ml to 239 µg / ml showing a very important activity for the **OC1**1 variety that brings stability to DPPH with an **IC50 of 33µg / ml**. From these results it is proved that ascorbic acid remains the most effective antioxidant with an IC50 of 4.575µg / ml compared to the methanolic extracts of the studied soya beans. According to Table 2, the **OC11** variety has a high antioxidant activity because its IC50 is twice lower than that of **Malady** and 7 times lower than that of **FT10**.

IV. DISCUSSION AND CONCLUSION

The phytochemical screening carried out using characterization reactions reveals the richness of our plant in secondary metabolites in the studied extracts ie polyphenols and flavonoids. The **Salkowski test** reveals the presence of unsaturated sterols in the chloroformic extracts of the 3 varieties.According to the results obtained in this study, we can say that this analysis will find an important application in the pharmaceutical industry as well as a potential utility in the food industry.

Many methods are currently used to evaluate antioxidant activity. The DPPH radical has been widely used for studying the antiradical activity of various plant extracts. The chemical 2,2-diphenyl-1-picrylhydrazyl was one of the first free radicals used to study the structure-antioxidant relationship of phenolic compounds [9].The results of measuring the percentage of inhibition of the DPPH radical as a function of the concentration of the compounds tested show that the percentage of inhibition of the free radical increases with the increase in the concentration for either ascorbic acid or for the methanolic extracts of the shoots.of soy. It is observed that the percentage of inhibition of ascorbic acid is greater than that of the extracts tested for all concentrations, that is to say that ascorbic acid remains the most effective antioxidant with an **IC 50 of 4,575µg. / ml** relative to the methanolic extracts of soybean shoots.

Similar results are reported from studies of corn and oregano, respectively, ethanolic extracts are very effective at trapping DPPH radicals due to their high content of phenolic acids and flavonoid glycosides (Ksouri et al., 2007). A study by Kang et al.(2003) suggested that the polar molecules present in the plant extracts contribute to the increase of the antiradical activity. It is thus obvious that the strong activity of the crude extracts is attributed to its richness to the phenolic compounds, which possess the strongest content of polyphenols, flavonoids.

In general, the activities of our extracts are "good", this suggests that this part of our plant is rich in phenolic components that are responsible for antioxidant activity according to many studies [22].The antioxidant activity tested by the use of the DPPH radical is positive for each extract and of different intensity, **OC11** () has an antioxidant activity more intense than **Fasto** () and **FT10** () because the higher the value of IC50 is small, the greater the antioxidant activity of a compound is great.

Antioxidants contribute significantly to the prevention of diseases, also in the pharmaceutical industry, the development of new synthetic methodologies and the preparation of molecules for therapeutic use are a major objective and a permanent concern for many researchers. In this context, we plan in the future to make the phytochemical study and evaluation of the antioxidant power of soybean extracts of other varieties harvested in other regions of Madagascar. Variation in antioxidant activity could be due to the amount and / or nature of the antioxidant substances present in the extracts

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Figures

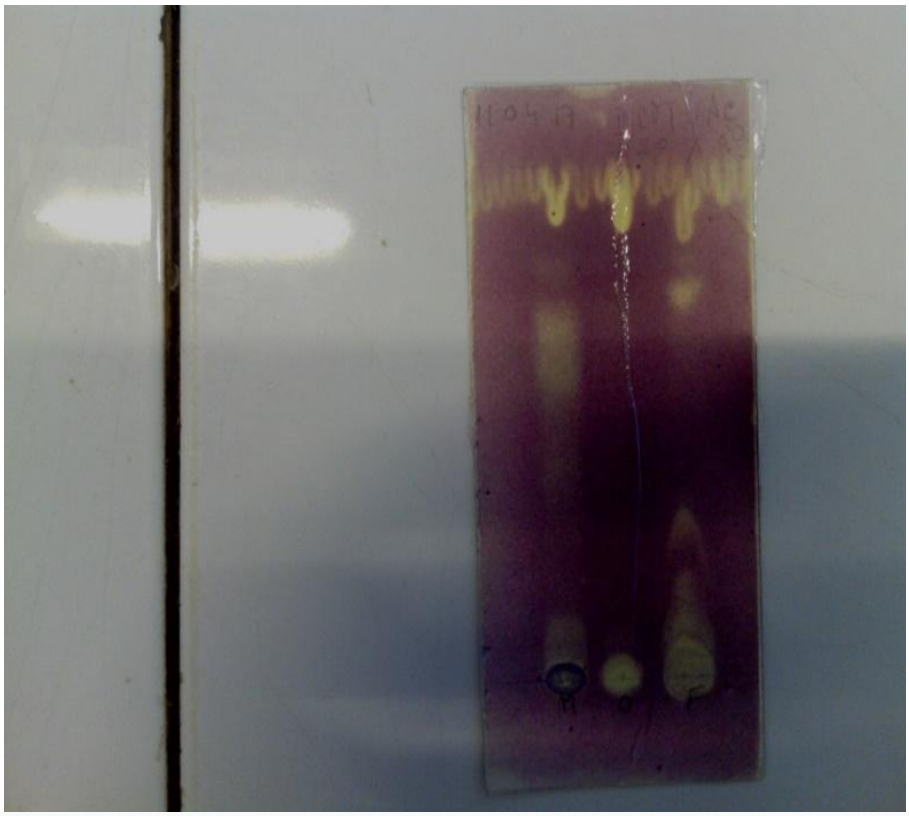
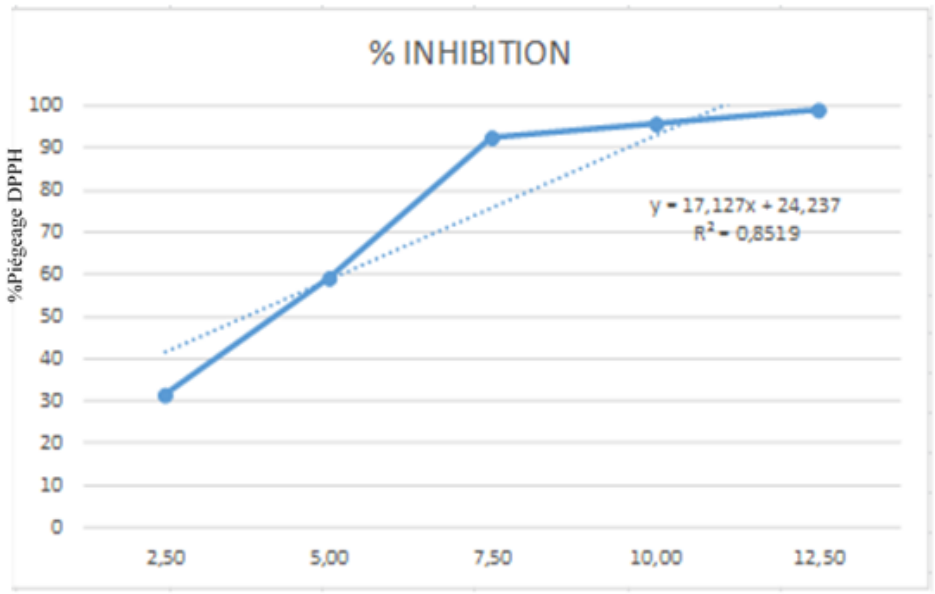


Figure 1: Result of the qualitative test of the antioxidant activity of the 3 extracts

Concentration en Vit C et % inhibition											
		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
	µg/ml	0	0,5	1	1,5	2	2,5	5	7,5	10	12,5	25
	% I	0,00	4,37	13,23	18,01	24,65	31,64	59,15	92,60	95,69	99,01	101,63

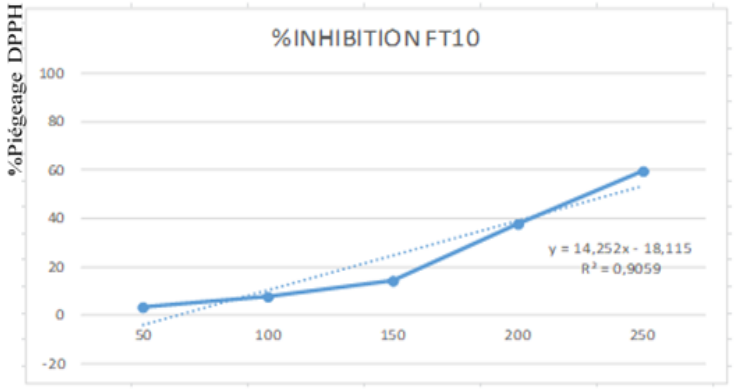


Concentration µg/ml

Fig 2:Anti-free radical activity of Vit C

IC50=4,575µg/ml

Concentration en FT10 et % inhibition											
		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
	µg/ml	0	10	20	30	40	50	100	150	200	250	500
	% I	0,00	2,64	2,18	1,45	2,90	3,56	7,65	14,50	37,71	59,79	65,72



Concentration µg/ml

Fig3:Anti-free radical activity of extracts of Soy sprouts variety F10

IC50=239mg/ml

Concentration en M et % inhibition											
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
µg/ml	0	5	10	15	20	25	50	75	100	125	250
% I	0,00	0,14	-1,63	10,82	13,57	27,79	38,41	51,10	65,55	69,32	70,91

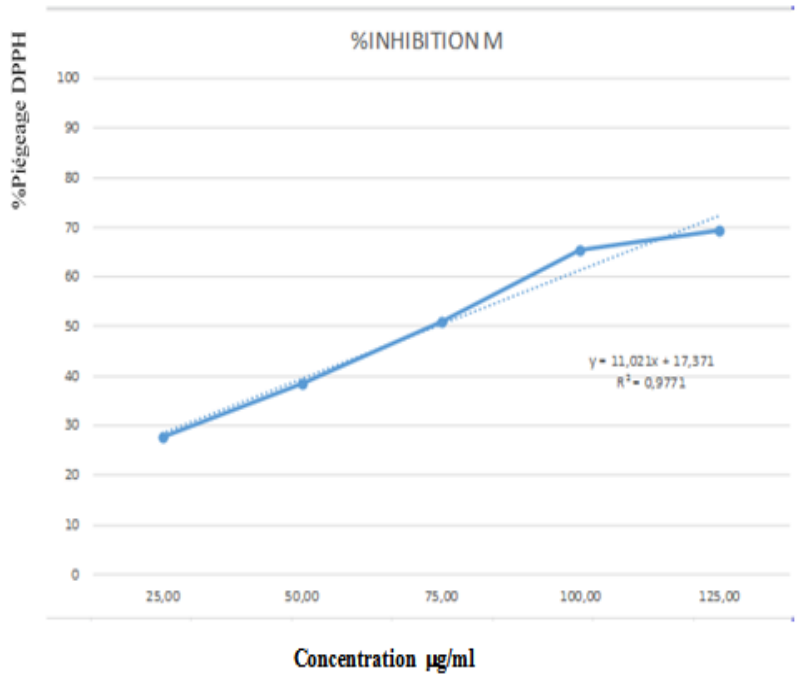


Fig4:Anti-free radical activity of extracts of Soy sprouts variety Malady (M)

Concentration en OC11 et % inhibition											
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
µg/ml	0	5	10	15	20	25	50	75	100	125	250
% I	0,00	3,25	3,78	10,58	19,21	44,39	58,87	73,17	80,38	93,14	95,69

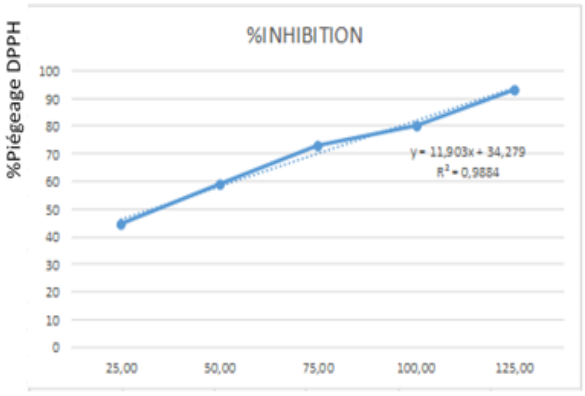


Fig5:Anti-free radical activity of extracts of Soy sprouts variety OC11
IC50=33µg/ml

Table

Table 1: Phytochemical Screening Results

Famille chimique	Réaction	FASTO	OC11	FT10
Alcaloïdes (Cordell,Bruneton)	MAYER	-	-	-
	WAGNER	-	-	-
	DRAGENDORFF	-	-	-
Désoxyoses	Test de KELLER-KILIANI	-	-	-
Tanins	HEMINGWAY et KARCHESY	-	-	-
Polyphénols		+	+	+
Flavonoïdes	Test de WILSTATER	+	+	+
Leucoanthocyanes	Test de BATE-SMITH	+	+	+
Anthraquinones	Test de BORNTRÄGER	-	-	-
Iridoïdes		-	-	-
Saponines		+	+	+
Stéroïdes et triterpènes	LIEBERMANN-BURCHARD	-	-	-
Stérols insaturés	SALKOWSKI	+	+	+

+ = positive reaction; - = negative reaction

Table 2: IC50 values of extracts of the soybean varieties studied: F10, Malady and OC11

Variété	F10	Malady (M)	OC11
IC50 (mg /ml)	239	74	33

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