Quest Journals
Journal of Research in Pharmaceutical Science
Volume 5 ~ Issue 1 (2019) pp:08-13
ISSN(Online) : 2347-2995
www.questjournals.org

Research Paper

Antimicrobial potential of Germs extractsof 3 soybean(Glycine max) varieties growing on the Central Highlands of Madagascar

Florence Randriamamonjy(1), Herisetra Lalaina Tsirinirindravo(2), Laurence Ralamboranto(3)

Laboratory of Applied Biochemistry to Medical Sciences, Fundamental and Applied Biochemistry Departement,Faculty of Sciences, University of Antananarivo. Antananarivo 101,
Laboratory of Applied Biochemistry to Medical Sciences, Fundamental and Applied Biochemistry Departement,Faculty of Sciences, University of Antananarivo. Antananarivo 101, Madagascar ,
Laboratory of Applied Biochemistry to Medical Sciences, Fundamental and Applied Biochemistry Departement,Faculty of Sciences, University of Antananarivo. Antananarivo 101, Madagascar,
Corresponding Author ; Laurence Ralamboranto

ABSTRACT:*Natural products have been the main source of drugs used in traditional Malagasy pharmacopoeia . In addition, many modern drug molecules also come from natural products . Among the constituents of bean sprouts, isoflavones have been shown to have beneficial properties for human health, especially the antimicrobial properties of polyphenols and flavonoids have been mentioned in the literature. Also in our current work, we have tried to explore the antimicrobial potential of ethanolic extract of shoots of the three varieties of Glycinemax**: FT10, OC11 and Fasto or Malady**. The results obtained showed that they exert a variable antimicrobial activity with respect to all the strains tested: Thus, Staphylococcus aureus, Shigella dysenteriae, Salmonellatyphi, Escherichia coli are sensitive to the OC11 extract; Shigella boydii, Pseudomonas aeruginosa and Listeriamonocytogeneis are sensitive to the **FT10** extract, Gardnerella vaginalis and Salmonella typhi are susceptible to **Malady or Fasto** extract. In addition, the minimum inhibitory concentration (MIC) in a liquid medium with the extract **OC11** is respectively: 2.3 mg / ml for Staphylococcus aureus, of: 0.9 mg / ml for Vibrio parahaemolyticus:1.6 mg / ml with Salmonella typhi and 1.7mg / ml for Klebsiella and finally 2.3mg / ml for Escherichia coli .The minimum bactericidal concentration in solid medium with the same extract varies from 3.4mg / ml for Salmonella typhi to 5.1mg / ml with Staphylococcus aureus and Escherichia coli. The OC11 extract is bactericidal with respect to the tested microorganisms because the CMB / MIC ratio varies between 2.12 and 2.2.*

KEY WORDS: *antibacterial activity, soybean shoots, malagasy varieties, aqueous extract,, isoflavones.*

R*eceived 02 June, 2019;* ***A****ccepted 18 June, 2019*
*Published with open access at****www.questjournals.org***

I. INTRODUCTION

For over two millennia, soybeans have been cultivated in the Far East and their cultivation has since developed in Western countries since the 19th century. The majority of intensive soybean crops remain in the United States, Brazil, Argentina and China (FAOSTAT). The pharmaceutical industries have also taken advantage of the many potential uses of soy, thanks to its high levels of lecithins, lipids, minerals and other minor compounds potentially beneficial to health. (Guillaume 1947)(Barrett ,2002) (Schryver,2002).

Soy is rich in isoflavones (Berger and al.2008). They are made by plants in response to environmental stresses such as infections or lack of nutrients (Howitz and Sinclair, 2008).Among these compounds, isoflavones are particularly noted, present in low concentration. The content of the latter varies according to the varieties of soya and the compartmentalisation of the seed, that is to say the cotyledons are poor in isoflavones, whereas its concentration is higher in the germs. (Kim,2007),(Boscredon,2001)(Rasolohery,2007)

This study aims to provide some answers concerning some biological activities of extracts of three varieties of soybeans growing on the Central Highlands of Madagascar.

Numerous studies have described the antibacterial properties of plant polyphenols, among others the flavonoids to which the class of isoflavones belong. (Djeussi,2013), reported that plant extracts and many other flavonoid-rich phytochemicals possessed antimicrobial activity.

On the other hand, thanks to their structure characterized by the presence of phenolic group, and other chemical functions, flavonoids are considered very good antimicrobial agents (Harborne and Williams, 2000).

It is known that soy is used in traditional medicine for the treatment and prevention of several diseases. Indeed, this product is considered an alternative in the treatment of many infections. Thus, it is essential to accurately determine the germs that are sensitive or not to the isoflavones contained in the extracts.

It should be noted that in Madagascar the promotion of the soy sector contributes to food security and thus fight against malnutrition and poverty by improving the incomes of the actors.

II. MATERIALS AND METHODOLOGY

2.1Materials

2.1.1 Description of the study material: soySoy is known under the scientific name of Glycine max.

-Systematic position

The botanical classification of Glycine max after L. Merr., 1917.

The plant belongs to:

Kingdom:Plantae,Subkingdom:Tracheobionta ,Division: Magnoliophyta,,Class: Magnoliopsida, Subclass:Rosidae,Order:Fabales, Family: Fabaceae, Subfamily: Faboideae, Tribe:Phaseoleae, Subtribe: Glycininae, Genus:wistaria

- Soybean varieties in Madagascar

The genus Glycine comprises about forty species. Soybean itself includes about 4000 varieties. By natural crosses, hybridizations and selections, cultivated varieties have taken a variety of shapes and colors.

For the moment, preliminary results from trials conducted in various regions suggest that the most interesting varieties according to their vegetative cycle are:

• For the Highlands: DAVIS (115 days)

• For Lake Alaotra: DAVIS (115 days) short size (46 cm), ACADAN (115 days) tall (90cm)

• For the province of Diégo: HOOD (96 days), BOURKE (96 days) and can be varieties GEDULD (97 days), HILL (83 days), WILLONI (83 days)

Soybeans are grown mainly in the regions of Vakinankaratra, Itasy and Imerina Central. With wheat, it is mainly produced on family farms. The average size of family farms is 0.9 ha and the average number of active family members working there is 6 with a probable use of paid external labor when needed.

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 Chemicals

Solvents and reagents

Several reagents have been used to highlight the different characteristics of soy isoflavones:

- Glacial acetic acid,Hydrochloric acid,Sulfuric acid, Iso amyl alcohol,Ammonia, Acetic anhydride
- Benzene,Ferric chloride, Chloroform,Dulbecco's Modified Eagle Medium (DMEM),Ethanol, Methanol.
- PBS(phosphate buffer solution)

2.1.3 Microorganism strains

Antimicrobial test is carried out on fifteen bacterial strains: Staphylococcus aureus, Streptococcus faecalis ATCC 19433 Vibrion cholerae ATCC15748, Vibrion parahaemolyticus, Shigella dysenteriae, Shigella boydii, Salmonella typhi, Klebsiella, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa ATCC9027, Rhodococcus sp, Gardnerella vaginalis , Listeria monocytogenes, Neisseria gonorrhea.

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 each in 5 ml of 80% methanol for 2 hours and then centrifuged, the supernatant is transferred to other previously coded tubes allowing the identification of the variety, the extracts are evaporated, the yield is calculated. They are stored in a refrigerator at 4 ° C.

In this study, the ethanolic phase extract of the shoots of the three soybean varieties are used to evaluate the antibacterial activity .

2.2.2 Antibacterial assay

It is known that soy is used in traditional medicine for the treatment and prevention of several diseases. Indeed, this product is considered an alternative in the treatment of many infections(World Health Organization (WHO)2002.)(Rasoanaivo,2000). Thus, it is essential to precisely determine the germs that are sensitive or not to the isoflavones contained in the extracts(Marmonier,1990)

2.2.2.1 Antibiogram test(Courvalin and al, 2012),(Sabine,1995)

The sensitivity of bacterial strains to anti-bacteriological agents is assessed according to the antibiogram technique, using the diffusion method:

All microorganisms were stocked in appropriate conditions and regenerated twice before using. A disc diffusion method was used for the antibacterial assay (Hayes and Markovic, 2002). Sterile nutrient agar plates were prepared for bacterial strains and inoculated by a spread plate method under aseptic conditions. The inoculum used is 10^6 CFU / ml. Filter paper discs of 5 mm diameter (Whatman No. 1 filter paper) were prepared and sterilized. The sterile impregnated discs with plant extracts were placed on the agar (MUELLER-HINTON medium) surface with flamed forceps and gently pressed down to ensure complete contact of the disc with the agar surface. . Each test is repeated three times.Two controls were performed: a negative control with sterile distilled water and an antibiotic disc as a positive control .The antibacterial activity of each extract was expressed in terms of the mean of diameter of zone of inhibition (in mm) produced by the respective extract after 24h incubation at 37 ° C. ± 1 °C.

2.2.2.2 MIC and MBC determination(Courvalin , 1990.)(Denis and al, 2011)

The MIC or minimal inhibitory concentration is the lowest concentration for which there is inhibition of bacterial growth. It is determined by the solid-state disk method and the liquid dilution method:

Solid state disc method: Geometrically progressive concentrations of the extract to be studied are prepared, the autoclaved discs are impregnated with 20 µl of solution to be tested, and then applied to the Mueller Hinton medium uniformly inoculated with 1 ml of inoculum. . The Petri dishes thus prepared are then incubated

at 37 ° C. for 24 hours. The MIC corresponds to the lowest concentration of extract giving a positive result (inhibition halo diameter between 7 to 8 mm).

Dilution method in liquid medium: seven concentrations in geometric progression of the test extract are prepared then 1ml of each solution is poured into a test tube containing 9 ml of nutrient broth previously inoculated with the microorganism to be tested. Non inoculated culture medium (10 ml) serves as a control; These tubes are then incubated at 37 degrees C for 24 hours. The reading of the results is done macroscopically by comparison with the control tube. The growth of the germ results in the appearance of a disorder of the culture broth. On the other hand, in the case of inhibition of growth, the contents of the tube remain clear. MIC is the lowest extract concentration for clear tubes

The minimum concentration or bactericidal concentration (MBC) is the lowest concentration for which no bacterium survives. The contents of each of the clear tubes used for the determination of the MIC are inoculated on agar medium . After incubation at 37 ° C. for 24 h, the colonies developed are counted.
. The lowest concentration of extract causing 0 survivors corresponds to CMB.

The extract is bactericidal for MBC/MIC ≤ 4 and bacteriostatic as these ratios are >4 (Djeussi and al., 2013)

III. RESULTS

3.1 Extraction efficiency

variety	Yield (%)
FASTO	12,6
FT10	14
OC11	12,7

3 .2Antimicrobial activity(Antibiogram)

The **OC11** extract (inhibition zone 7-13 mm) found out to be the most effective than the other two varieties **Malady** and **F10** (inhibition zone 6-8 mm) (Table 1). The most sensitive germs whith **OC11** extract were Staphylococcus aureus , Salmonnella typhi and Escherichia coli. Antibiotics used like references in this study (gentamycin) were more efficient than the extracts**.**

3.3 MIC, MBC

MIC, MBC, and MBC/MIC ratio values are presented in Table number2. For **OC11** extract ,the MBC/MIC report varies from 2,12 to 2,2. According to Marmonier (1990), when the report of MBC / MIC of an

antimicrobial substance is lower or equal for four (≤ 4) the substance has a bactericidal effect. On the contrary, if the MBC/ MIC report is higher than four (> 4), the effect is bactériostatic. Ethanolic extract **OC11** has a bactericidal effect against Staphylococcus aureus , Salmonnella typhi and Escherichia coli. (CMB/CMI 2,12 – 2,2)

IV. DISCUSSION AND CONCLUSION

The extracts of the shoots of the three varieties of glycine max: **FT10, OC11** and **Fasto or Malady** exert a variable antimicrobial activity with respect to all the strains tested. The figure1 illustrate the antibacterial activity of different extracts against Staphylococcus aureus

The antibacterial activity of the extracts on the 15 bacterial strains is verified by the measurements of inhibitory halos of the imbibed extract disks on the cultures of the seeds. Of the 15 bacterial strains tested, 8 strains were susceptible: Staphylococcus aureus, Shigella dysenteriae, Shigella boydii, Salmonella typhi, Escherichia coli, Pseudomonas aeruginosa, Gardnerella vaginalis and Listeria monocytogenes

This sensitivity depends on the varieties tested:

Staphylococcus aureus, Shigella dysenteriae, Salmonella typhi, Escherichia coli are susceptible to **OC11** extract.

Therefore the study supplies scientific basis of its traditional application. Any investigations and more pursues of its activity against a wider range of bacteria, identification and purification of its chemical constituents, and toxicological investigations about extracts must be carried out with the aim of developing new drugs intended for human consumption

The results obtained show that the OC11 ethanolic extract has a significant bactericidal activity against the bacterial species tested.

There is an urgent need for alternative antibiotics to fight infections caused by bacteria. Therefore, we can rely on drugs being used by traditional practitioners.

ACKNOWLEDGEMENT

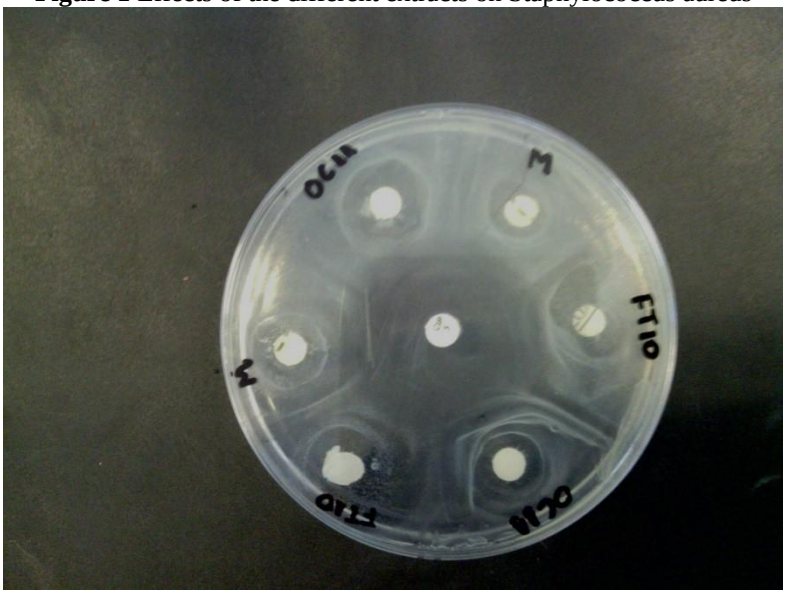
The authors are grateful :
-toLaboratory of University Athenee Saint Joseph Antsirabe
-toFIFAMANOR laboratory for supplying soybean varieties

BIBLIOGRAPHIE

[1]. -BARRETT, J. R. Soy and children's health: a formula for trouble. Environ Health Perspect, 110**,**A294-A296.2002
[2]. -BERGER, M.; RASOLOHERY, C. A.; CAZALIS, R.; DAYDÉ, J. Isoflavone accumulation kinetics in soybean seed cotyledons and hypocotyls: Distinct. pathways and genetic controls. Crop Sci,48**,**700-708.2008
[3]. -BOSCREDON, A., Variabilité de la teneur et de la composition en isoflavones de la graine de soja (Glycine max L. Merr): effet des facteurs génotypiques et environnementaux. Mémoire d'ingénieur,laboratoire d'agrophysiologie, Ecole d'Ingénieur purpan, Toulouse**.,** pp 83.2001
[4]. -COURVALIN P, LECLERCQ R., Antibiogramme. Paris : ESKA ; 2012.
[5]. -COURVALIN P., Bactéricidie : aspects théoriques et thérapeutiques. Paris : Maloine ; 1990.
[6]. -DENIS F., PLOY MC., MARTIN C.,BINGEN E., R. QUENTIN R., Bactériologie Médicale Techniques usuelles. Paris : Elsevier/Masson; 2011.
[7]. -DJEUSSI DE, NOUMEDEM JAK, SEUKEP JA, FANKAM AG, VOUNKENG IK, TANKEO SB, NKUETE AHL, KUETEV., Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram bacteria. BMC Complement. Altern. Med. 13(164):1-8.,2013
[8]. -GUILLAUME A., L'utilisation du soja dans l'alimentation et dans l'industrie,dans : Journal d'agriculture traditionnelle et de botanique appliquée, pp. 191-197,1947
[9]. -HARAGUCHI H., TANIMOTO K., TAMURA Y., MIZUTANIK.,and KINOSHITO T. (1998). Mode of antibacterial action of retrochalcones from Glycyrrhizainflata. Phytochemistry. 48:125-129
- HAYES AJ, MARKOVICK B (2002) Toxicity of australian essential oil Backhousia citriodora (Lemon myrtle). Antimicrobial activity and in vitro cytotoxicity. Food Chem Toxicol.40: 535-543;
[10]. -KIM, J. A. et CHUNG, I. M.,Change in isoflavone concentration of soybean (Glycine max L.) seeds at different growth stages. J Sci Food Agric, 87**,** 496-503,2007.
[11]. MARMONIER, A.,Introduction aux techniques d'étude des antibiotiques in Bactériologie Médicale, technique usuelles,3ème édition, Doin, Paris, 227-236p,1990.
[12]. -MICHAEL J. PELCZAR,Jr ;E.C.S. Chan ;NOEL R. Kneg, Microbiology. 5th edition, ED. Mc Graw-Hill. 918p, 1986.
[13]. -RaASOANAIVO P., Une banque de données sur les plantes médicinales de Madagascar, Info-Essences. 15 : 5-6p, 2000.
[14]. -RASOLOHERY C. A ., 2007 : Etude des variations de la teneur en isoflavones et de leur composition dans le germe et le cotylédon de la graine de soja [Glycine max (L.) Merrill]
- SABINE D.R.,Antibiotiques et antibiogrammes. Decarré-Vigot, Monreal. 322p, 1995.
[15]. -SCHRYVER T, Increasing health benefits using soy germ. Cereal Foods world 47.185-188,2002
[16]. -World Health Organization (WHO): Traditional medicine. Growing needs and potential, WHO policy Perspectives on Medicines, pp.1ñ6, WHO, Geneva 2002

Figures

Figure 1 Effects of the different extracts on Staphylococcus aureus



Tableaux

Table 1: In vitro antibacterial activity (diameter halo /mm) of aqueousextracts Malady ,OC11, FT10 on pathogenic bacteria

	MALADY 6.38	**OC11 6.45**	**FT10 6.72**
Staphylococcus aureus	**6**	**12,5**	**6**
Streptococcus faecalis ATCC 19433	**6**	**6**	**6,5**
Vibrio cholerae ATCC 15748	**6**	**7**	**6**
Vibrio parahaemolyticus	**6.5**	**-**	**6**
Shigella dysenteriae	**6**	**9**	**6**
Shigella boydii	**6**	**6**	**8**
Salmonella typhi (aliments)	**8**	**13**	**6**
Klebsiella	**-**	**6**	**6**
Escherichia coli	**6**	**11**	**6**
Bacillus cereus (aliments)	**7**	**7**	**6**
Pseudomonas aeruginosa ATCC 9027	**6**	**6**	**9**
Rhodococcus sp.	**7**	**6**	**6**
Gardnerella vaginalis	**8**	**6**	**6**
Listeria monocytogenes	**6**	**6**	**8**
Neisseria gonorrhoe	**7**	**6**	**6**

0 = No activity, 0-10 = moderately sensitive, 10-20 =sensitive, 20 and above =very sensitive

Table 2: **MIC and MBC values (mg/mL) of OC11 extract on bacteria**

Bacteria	MIC (mg/ml)	MBC (mg/ml)	MBC/MIC
Staphylococcus aureus	2,3	5,1	2,2
Salmonella typhi	1,6	3,4	2,12
Escherichia coli	2,3	5,1	2,2

MIC: **minimal inhibitory concentration**
MBC: **minimum concentration or bactericidal concentration**

Laurence Ralamboranto" Antimicrobial potential of Germs extractsof 3 soybean(Glycine max) varieties growing on the Central Highlands of Madagascar" Quest Journals Journal of Research in Pharmaceutical Science, vol. 05, no. 01, 2019, pp. 08-13