British Journal of Pharmaceutical Research
15(1): 1-6, 2017; Article no.BJPR.31695
ISSN: 2231-2919, NLM ID: 101631759

SCIENCEDOMAIN *international*
www.sciencedomain.org

Bersama abyssinica Fresen. (Melianthaceae): Antifungal Activity on Aflatoxin B_1-producing Mold

Kouadio Bene[1*], Adou Koffi Mathieu Kra[2], Kouadio Fernique Konan[3] and Guédé Noël Zirihi[1]

[1]Laboratory of Botany, UFR of Biosciences, University Felix Houphouët-Boigny, Abidjan, 22 BP 582 Abidjan 22, Côte d'Ivoire.
[2]Laboratory of Pharmacodynamy Biochemistry, UFR of Biosciences, University Felix Houphouët-Boigny, Abidjan, 22 BP 582 Abidjan 22, Côte d'Ivoire.
[3]Laboratory of Bacteriology-virology Antibiotics, Natural Substances and Monitoring Resistances of Microorganisms in Anti-Infectives (ASSURMI), Institut Pasteur of Côte d'Ivoire, 01 BP 490, Abidjan 01, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration between all authors. Author KB conducted ethnobotanical investigations, extractions, evaluation of antifungal activities and wrote the manuscript. Author AKMK supervised the evaluation of antifungal activities. Author KFK supervised the extractions and made an improvement to the manuscript. Author GNZ proposed the study protocol, supervised all the work. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2017/31695
Editor(s):
(1) R. Deveswaran, M.S. Ramaiah College of Pharmacy, Bangalore, India.
Reviewers:
(1) Shimaa Ragab Hamed, National Research Centre, Egypt.
(2) Fatih Kalyoncu, Manisa Celal Bayar University, Turkey.
(3) Raul Ricardo Gamba, Universidad Nacional de La Plata, Argentina.
(4) Shohei Sakuda, The University of Tokyo, Japan.
(5) Mohamed M. Deabes, National Research Centre, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/18084

Original Research Article

Received 20th January 2017
Accepted 2nd March 2017
Published 7th March 2017

ABSTRACT

Aspergillus flavus is the main aflatoxin-producing mold (only group B). In view of the extent of the pathologies caused by these toxins, we have begun a series of research on medicinal plants with the aim of finding new bioactive substances capable of neutralizing the action of these mycotoxins and endowing a safety guaranteeing their taking in The therapeutic treatments of affections related to the production of aflatoxins in general but aflatoxin B_1 in particular. An ethnobotanical survey

**Corresponding author: E-mail: kouadio777@gmail.com;*

conducted in the Department of Transua (East of Cote d'Ivoire) revealed *Bersama abyssinica*, medicinal species highly recommended in the treatment of mycoses. After extraction of the active ingredients, the Sabouraud double tube dilution method was used to evaluate the antifungal activities of two extracts of this plant. The aqueous total (TAE) and ethanolic extracts 70% (EE70%) of tested leaves possess an antifungal activity. EE70% is the most active (MIC = 0.781mg/ml and IC_{50} = 0.08 mg/ml). These results justify the traditional use of *Bersama abyssinica* in the treatment of mycotic diseases in the Department of Transua. *Bersama abyssinica* is an alternative in the bio-fight against *Aspergillus flavus* producing aflatoxin B_1.

Keywords: Bersama abyssinica; Brong; antifungal activity; Aspergillus flavus; aflatoxin B_1.

1. INTRODUCTION

Aflatoxins are natural toxins [1], toxic to humans and animals [2]. Aflatoxins represent major challenges for global food security systems, health, nutrition and economies, as they are produced both during agricultural production, harvesting, transport, storage and food processing [3]. Aflatoxin B1 (AFB1), the major representative of the aflatoxin group [4], is the most frequent and the most toxic. It is considered one of the most potent natural genotoxic carcinogens. Its target organ is the liver. *Aspergillus flavus* is the main Aflatoxin-producing species (only in Group B) [5].

Aflatoxins are molecules of low molecular weight. The AFB1 ($C_{17}H_{12}O_6$) has a molar mass of 312 g/mol, it is fluorescent of blue color under UV light. *Aspergillus flavus* is a species of ascomycete fungus. This mold is very cosmopolitan (soil, decaying organic matter, oil seeds and cereals). It is particularly abundant on peanuts and its derivatives, it is also found in tropical regions.

Aspergillus flavus is a toxic and pathogenic mold for humans. It is responsible for aflatoxicoses including otomycoses (pulmonary aspergillosis) and mycotic keratitis. Aflatoxin can cause serious liver problems and cancers (lung, liver, spleen, stomach, colon and kidneys). It is also an entomopathogenic species. Mold producing aflatoxin affects grains and other food crops, including corn and groundnut. Toxins could be transported along the food chain. It causes health problems in livestock with contaminated food and in humans with dangerous concentrations of aflatoxins in staple foods. Poisoning occurs mainly through the consumption of contaminated plant and animal products, but also by inhalation and dermal absorption [6]. The most severe case of aflatoxin poisoning in the world occurred in Kenya in 2004. According to reports from local medical institutions, 317 people were hospitalized and 125 people died. This intoxication was attributed to the consumption of indigenous maize stored in a humid environment [7,8].

In view of the threats caused by *Aspergillus flavus* in agriculture on the one hand and the multiple and severe affections linked to the production of aflatoxins, particularly of type B_1, which can cause death, it is necessary to look for new bioactive molecules allowing To effectively combat these toxins and the associated pathologies. It is with this concern that we undertook an ethnobotanical study of medicinal plants in the District of Zanzan (Côte d 'Ivoire). The survey carried out specifically in the Department of Transua revealed various uses of *Bersama abyssinica* in traditional medicine including the treatment of mycoses.

2. MATERIALS AND METHODS

2.1 Material

2.1.1 Plant material

The plant material consists of the leaves of *Bersama abyssinica* harvested during the ethnobotanical survey.

2.1.2 Fungal strain

The strain tested is *Aspergillus flavus*. It comes from the mycology laboratory of Institut Pasteur of Côte d'Ivoire (IPCI). Mold was collected from a patient in the Infectious Diseases Department of the University Hospital of Treichville (Abidjan, Côte d'Ivoire). It was taken on 31 December 2014. It was isolated and grown on Sabouraud agar. Its powdery or fluffy appearance requires some precautions during handling.

The characteristics of this strain are given in Table 1.

Table 1. Characteristics of the fungal strain

Strain	Code	Organic products	ATFQ Sensitivity	Type	Services
Aspergillus flavus	14-669	Pus of ear	AMB-(R) / NYS-(R) / MCZ-(S) / KET-(S) / ITZ-(S) / CAS-(S) / VRZ-(I)	Mold	SMI-CHU Treichville

AMB (Amphotericin B), NYS (Nystatin), MCZ (Miconazole), KET (Ketoconazole), ITZ (Itraconazole), CAS (Caspofungin)/ VRZ (Voriconazole)/, PSZ(Posaconazole)
R: resistant, S: Sensitive

2.2 Methods

2.2.1 Preparation of plant extracts

The harvested leaves of *Bersama abyssinica* were rinsed with water and dried out of the sun. These dried medicinal plant organs were then reduced to fine powder using an IKA-MAG RTC electric mill. The extraction of the active ingredients was carried out according to the method of Zirihi et al. coupled with the exhaustion method [9].

One hundred grams (100g) of medicinal plant powder is homogenized in one (1) liter of distilled water in a Blender (Mixer) brand of Life's Superb (LS-317) for three times three minutes at room temperature. The homogenate obtained is filtered successively on a square of white cloth, on hydrophilic cotton and then on Wattman paper. With the aid of an oven set at 50℃., the extraction solvent is eliminated. The dry evaporate is recovered in the form of powder and constitutes the total aqueous extract (TAE).

Ten grams (10g) of the TAE are dissolved in 200 ml of an ethanol-water solution (70/30) and then homogenized in a Blender. After decantation in a separating funnel, a liquid phase with a solid residue which precipitates is obtained, since it is insoluble in the alcohol-water mixture 70-30. The supernatant is collected, filtered on cotton to remove any residue and dried in an oven (50℃). The powder obtained constitutes the 70% ethanolic extract (EE70%).

2.2.2 Antifungal activity

2.2.2.1 Preparation of culture media

Incorporation of plant extracts into Sabouraud agar was carried out using the dual tube dilution method [10]. All extracts were tested separately. Each series contains 14 test tubes including 12 test tubes (containing plant extract) and 2 control tubes (one without plant extract serves as a control for growth control of the germ, the other without germ and without extract serves as a control Control of sterility of the culture medium).

For the twelve test tubes, the concentrations of the extracts varied from 50 to 0.024 mg/ml according to a geometrical sequence of ½.

The 14 tubes of each series were sterilized in an autoclave (PBI STEMATIC III) at 121℃ for 15 minutes and then tilted with small pellet at room temperature in order to allow the cooling and the solidification of the agar [11].

2.2.2.2 Evaluation of antifungal activity

The inoculum is prepared from 48-hour germ cultures on sloping agar. These germs are removed with a platinum loop and then homogenized in 10 ml of sterilized distilled water. There is thus obtained a suspension 10^0 of seeds from which the suspension 10^{-1} is prepared by dilution to the tenth by transferring 1ml of the suspension 10^0 into 9 ml of sterile distilled water to have a final volume of 10ml.

The culture of the germs on the previously prepared media was done by seeding in tight transverse streaks until 1000 yeasts of the *Aspergillus flavus* strain equivalent to 10µl of the suspension 10^{-1} containing 10^5 germs/ml [12]. The cultures thus produced were incubated at room temperature for 72 hours.

Colonies are counted between 36 to 48 hours before the end of the experiment. Colonies of fungal germs were counted by direct counting using a Geiger-type colony counter pen. Growth in the 12 experimental tubes was evaluated as percent survival, calculated relative to 100% survival in the growth control control tube [13]. The method of calculating the percentage of survival was done according to the following formula:

$$S = (n/N) \times 100$$

(S = survival of *Aspergillus flavus* in percent, N = number of colonies in the control tube, n = number of colonies in the experimental tube).

2.2.2.3 Required antifungal parameters

Data processing allowed the following antifungal parameters to be determined [13]:

- **MIC** (Minimum Inhibitory Concentration): is the concentration of extract in the tube for which there is no visible growth to the naked eye.
- **IC_{50}** (Concentration for 50% Inhibition): this is the concentration that gives 50% inhibition. It is determined graphically from the plot of sensitivity curve of each extract on *Aspergillus flavus*.
- **The sensitivity curve:** it represents the evolution of the sensitivity of *Aspergillus flavus* as a function of the variations in the concentration of the extract.

2.2.2.4 Method of analyzing the results of antifungal activities

The comparison of the activities of the extracts is the method used and the performances of the extracts are compared on the basis of criteria such as:

- **The values of the antifungal parameters** (IC_{50}, and the appearance of the activity curves). An extract is all the more active as its IC_{50} value is low. Thus, an extract A is considered more active than another extract B if and only if the value of the IC_{50} of A is lower than that of B. As for the sensitivity curve, its general appearance (decreasing, regular or irregular) And the relative value of its slope (strong, medium or low) provide information on the antifungal activity potential of the extract considered. The most active extract is the one whose activity curve has the strongest slope [9,13] ; Or the one whose sensitivity curve is closest to the ordinate axis.
- **The effectiveness reports**, they make it possible to determine the number of times that a given extract is *n* times more effective than another. For a given antifungal parameter, this ratio is calculated by dividing the highest value by the lowest value.

For example:

$$\frac{IC_{50A}}{IC_{50B}} = k$$

Then this means that the extract (B) having the lowest IC_{50} value is "k times more active" than the extract (A) having the highest IC_{50} value.

3. RESULTS

After 72 hours of incubation, a gradual decrease in the number of colonies of *Aspergillus flavus* as the concentrations of the TAE and EE70% extracts were increased in the test tubes compared with the control tube.

The sensitivity curves of the two extracts show a decreasing pattern with steep slopes (Fig. 1). The slope of EE70% is stronger and closer to the ordinate axis than that of TAE. The two curves intersect the abscissa axis at different concentrations (respectively 195 and 98 µg/ml for TAE and EE70%), which represent the MIC values. This illustrates the dose-dependent sensitivity of *Aspergillus flavus* to the various extracts tested.

The antifungal parameters (MIC and IC_{50}) of the extracts are reported in Table 2.

Table 2. Values of the antifungal parameters of the two extracts of *Bersama abyssinica* at 72 hours of incubation

***Bersama abyssinica* extracts**	**Antifungal parameters**	
	MIC (µg/ml)	**IC_{50} (µg/ml)**
TAE	195	92
EE70%	98	57

4. DISCUSSION

The results show that *Aspergillus flavus* is sensitive to Total Aqueous Extracts (TAE) and ethanolic 70% (EE70%) according to a dose-response relationship. The values of the inhibitory concentrations obtained attest that the extracts have enhanced antifungal activities with better activity for the 70% hydroethanol extract (MIC = 98µg/ml). The sensitivity tests of the two extracts (aqueous and ethnaolic) of the leaves of Bersama abyssinica revealed an antifungal activity of these extracts.

This study shows that the partitioning of TAE by the solvent ethanol / water (70% and 30% respectively) is a method that allows to better concentrate the active ingredients. Their effects are thus revealed.

TAE being a *totum*, would contain a set of molecules of small, medium and large sizes. Indeed, water is a solvent that extracts a large group of chemical compounds. Thus, after the ethanol / water partition, the EE70% therefore

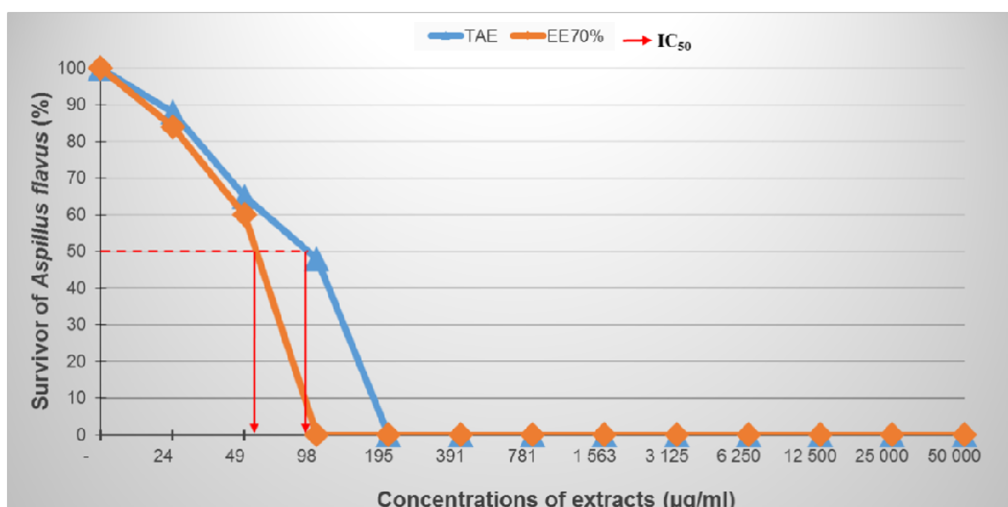

Fig. 1. Sensitivity curves of *Aspergillus flavus* with *Bersama abyssinica* extracts

concentrates many active ingredients of low and medium sizes (terpenoids, phenols, polyphenols, quinones etc.) which can better express their antifungal potential, hence l Activity.

The effectiveness report shows that:

> $IC_{50(TAE)}$ / $IC_{50\ (EE70\%)}$ = 92/57 = 1.6; This means that EE70% of *Aspergillus flavus* is 1.6 times more active than TAE.

The studies conducted on phytochemical composition of the leaves, stem bark and root bark of *Bersama abyssinica* revealed the presence of 24 chemical compounds in the leaves of which 3,7,11,15-Tetramethyl-2-hexadecen-ol (Terpene Alcohol) ; 7,8-Epoxylanostan-11-ol,3-acetoxy2 (Alcoholic compound); Furancarboxaldehyde,5-(hydroxymethyl) (Aldehyde) they possessed antimicrobial and antiinflammatory activities [14,15]. These authors also showed a fungicide, the 1,2,3-Benzenetriol (Pyrogallol). This would justify the antifungal activity found on *Aspergillus flavus* producing aflatoxin B_1 in our study.

Thermal treatments (sterilisation, pasteurisation, freezing) or drying (dehydration, lyophilisation), with the exception of roasting, have little effect on aflatoxins. Even roasting groundnuts only brings about a reduction of 50 to 80% of the initial level of aflatoxins. During processes for oil extraction, aflatoxins are mostly found in the resulting cattle cake. Detoxification processes for cattle cake with ammonia associated with formaldehyde can eliminate up to 95% of the initial level of AFB1 [5].

These chemical processes are not without consequence for the human organism. These results suggest that Bersama abyssinica is an alternative in the biological control of aflatoxin B1-producing *Aspergillus flavus*.

The discovery that Ivorian *Bersama abyssinica* extracts contains secondary metabolites with antifungal activity against *Aspergillus flavus* producing aflatoxin B1 is a novel finding.

5. CONCLUSION

This work made it possible to demonstrate the antifungal properties of the aqueous and ethanolic extracts 70% of the leaves of *Bersama abyssinica* on the *in vitro* growth of *Aspergillus flavus* producing aflatoxin B_1. The ethanolic 70% extract shows the best activity. These results could justify certain traditional uses of the plant in the treatment of pathologies such as mycoses. They demonstrate that *Bersama abyssinica* could be used to treat infectious diseases. However, this work must continue to isolate the active compounds responsible for the observed activities and to know the mode of action of these bioactive substances on the aflatoxin B_1-producing mold.

By this study, aqueous and ethanolic extracts of leaves of *Bersama abyssinica* are revealed as potential candidates in the alternative of biological control against *Aspergillus flavus* producing aflatoxin B_1, a struggle which until now is only purely chemical.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. CODEX. CODEX general standards for contaminants and toxins in food and feed. Available:http://www.codexalimentarius.org/input/download/standards/17/CXS_193e. (Accessed 23 August 2015, 2013)
2. Van Egmond HP, Ronald CS, Marco AJ. Regulations relating to mycotoxins in food. Perspectives in a global and European context. Analytical and Bioanalytical Chemistry. 2007;389:147-157.
3. Murphy PA. Hendrich S, Bryant C.M. Food mycotoxins: An update. Journal of Food Science. 2006;71(5):51-65.
4. Afssa. Évaluation des risques liés à la présence de mycotoxines dans les chaînes alimentaires humaine et animale. Agence française de sécurité sanitaire des aliments, France. 2009;308. French.
5. Anses. *Aspergillus flavus* et autres moisissures productrices d'aflatoxines. Agence nationale de sécurité sanitaire, France. 2012;3. French.
6. Wagacha JM, Muthomi JW. Mycotoxin problem in Africa: Current status, implications to food safety and health and possible management strategies. International Journal of Food Microbiology. 2008;124:1-12.
7. Centers for Disease Control and Prevention (CDC). Outbreak of aflatoxin poisoning - Eastern and Central provinces. Kenya. 2004;53(34):790-793.
8. Lewis L, Onsongo M, Njapau H, Schurz-Rogers H, Luber G, Kieszak S, et al. The Kenya aflatoxicosis investigation group. Aflatoxin contamination of commercial maize products during an outbreak of acute aflatoxicosis in Eastern and Central Kenya. Environmental Health Perspectives. 2005;113:1763-1767.
9. Zirihi GN, Kra AKM, Guédé-Guina F. Évaluation de l'activité antifongique de *Microglossa pyrifolia* (Lamarck) O. Kuntze (Asteraceae) « PYMI » sur la croissance in vitro de Candida albicans, Revue de Médecine et de Pharmacopées Africaines. 2003;17:11-18.
10. Coulibaly K. Études botanique, pharmacologique et explorations phytochimiques des extraits de Terminalia ivorensis et Terminalia superba, deux espèces ligneuses commerciales, médicinales antimicrobiennes de la forêt de Mopri, Tiassalé (Sud de la Côte d'Ivoire). Thèse de Doctorat Unique de botanique, spécialité ethnobotanique, Université de Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire. 2012;200. French.
11. Kporou KE, Kra AKM, Ouattara S, Guédé-Guina F, Djaman AJ. Évaluation de l'activité antifongique de Mitracarpus scaber sur Candida tropicalis. J. Sci. Pharm. Biol. 2009;10(1):13-20. French.
12. Holt JR. Laboratory test of antifungal drugs. J. of Clinical Pathology. 1975;1(18): 767-774.
13. Kra AKM. Recherche bioguidée de composés antifongiques à partir de plantes médicinales de Côte d'Ivoire. Thèse de Doctorat d'État, Pharmacologie des substances naturelles, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire. 2016;242. French.
14. Zekeya N, Chacha M, Shahada F, Kidukuli A. Analysis of phytochemical composition of *Bersama abyssinica* by gas chromatography - mass spectrometry. Journal of Pharmacognosy and Phytochemistry. 2014;3(4):246-252.
15. Lather A, Gupta V, Tyagi V, Kumar V, Garg S. Phytochemistry and pharmacological activities of Bersama engleriana Guerke – An overview. International Research Journal of Pharmacy. 2010;1(1): 89-94.

© 2017 Bene et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18084