International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2736-1756 Vol. 18 (3), pp. 001-010, March, 2024. https://internationalscholarsjournals.org/journal/ajmr/table-of-contents © International Scholars Journals

Full Length Research Paper

Chemical composition and antifungal activities of hydroethanolic extracts of cocoa (*Theobroma cacao* L.) pod waste, Ivory Coast

AKAFOU Ethien Felix[1*], GOLY Kouassi Roselin Cyrille[2], ATOBLA Koua[3], SORO Yaya[1] and ADIE Adjehi[4]

[1]Laboratoire des Procédés Industriels, de Synthèse et des Energies Nouvelles (LAPISEN), Institut National Polytechnique Félix HOUPHOUET-BOIGNY de Yamoussoukro, BP 1093 Yamoussoukro, Côte d'Ivoire
[2]Department of Science and Technology, UFR of Medical Sciences, Alassane Ouattara University, Bouaké, 01 BPV 18 Bouaké 01, Côte d'Ivoire
[3]Department of Biosciences, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire
[4]Laboratory of Biotechnology and Food Microbiology, Department of Food Science and Technology, Nangui Abrogoua University, 02 BP 801, Abidjan 02, Côte d'Ivoire.

Received 23 December 2023; Accepted12 February, 2024 and Published 6 April 2024

Abstract

Cocoa cultivation generates large quantities of cocoa pod shells, the management of which is a concern for producing countries. This study aims to valorize cocoa pod waste by determining the chemical composition and antifungal activities of their hydroethanolic extracts. Secondary metabolites were extracted from the dried powder of plant material by maceration in hydroalcoholic solvent. Their families were determined by phytochemical screening and certain contents determined by dosage. Antifungal activities were evaluated in solid and liquid media. Extraction yields were 14.10 ± 0.08 and 11.92 ± 1.81% respectively for green and yellow pod shells. Phytochemical screening revealed the presence of all the families sought in the yellow and green pod shells. Total polyphenol and flavonoid contents of yellow pods are higher than those of green pods with values of 35.50 ± 0.50 mg EAG/g and 65.33 ± 0.33 mg EQ/g of dry extract, respectively. However, condensed and total tannins are more abundant in green pods with contents of 16.40 ± 0.02 and 23.98 ± 0.02 mg EAT/g, respectively. Antifungal tests revealed that the four fungal strains tested were more sensitive to hydroethanol extract of yellow pod husks. Results obtained show that cocoa pod waste can be used in pharmaceutical field.

Key words: Cocoa pod waste, hydro-ethanolic extracts, secondary metabolites, antifungal activity.

INTRODUCTION

Plants are universally recognized as an essential component of biodiversity and a vital resource for the planet (Lesley Bremness, 2022). According to Soro et al., 2012, they are an important source of bioactive molecules and play an important role in the health of populations worldwide, particularly in Africa.

Theobroma cacao is a plant which is prized for its beans that are used in chocolate-production. According to (Vásquez et al., 2019) beans represent 20-30% of the dry fruit of cocoa tree. Côte d'Ivoire is the world's leading cocoa-producing country. The production of the 2019-2020 season, estimated at 2.18 million tons of cocoa beans (ICCO P, 2021), is accompanied by around 15 million tons of pod shells (waste) (GBN, 2021).

*Corresponding author:ethien.akafou20@inphb.ci

After harvesting and hulling, the residue (pod shell) is released back into the environment. The degradation of cocoa pod shells encourages the production of spores and the spread of pathogens (Meraz-Pérez et al., 2021), including *Phytophthora spp*, which is responsible for a significant loss (up to 30%) of world cocoa production (El-Sayed & Ali, 2020). Cocoa pod shells constitute an important renewable resource, used in several fields such as chemical, petrochemical, biodiesel (Ofori-Boateng and Lee, 2013), pharmaceutical (Campos-Vega et al, 2018), food and biomedical fields (Barrios-Rodríguez et al, 2022). They are rich in secondary metabolites (Ofori-Boateng et Lee, 2013; Mansur et al., 2014; Barrios-Rodríguez et al., 2022) known for their antifungal, antibacterial and antioxidant effects (Kassi et al., 2014). Despite the potential of this biomass, we found only one study in literature of its antifungal activities carried out on *Fusarium oxysporum* (Rachmawaty et al, 2018).
The aim of this work is to valorize cocoa waste (cocoa pod shells) by evaluating the antifungal activity of hydroethanol extracts against four fungal strains. The choice of *Candida albicans* is justified by the fact that it mainly infects people whose immune systems are weakened, and it is one of the major causes of opportunistic mycosis which ranks fourth in deaths due to infectious diseases with a rate of mortality of 45.1% (OMS, 2018). The Aspergillus genus, for its part, is responsible for the production of aflatoxins in smoked and dried fish and constitutes a real public health problem for consumers (Abdoullahi et al., 2018).

MATERIALS AND METHODS

Substances tested

Hydro-ethanolic extracts derived from cocoa pod shell grindings were tested against four fungal strains. Cocoa pods were harvested in March 2021 at Logbakro (6°44'115" North and 5°12'091" West) in Yamoussoukro in the central part of Côte d'Ivoire. Very early in the morning, before sunrise, pods were separated from stalk using a pair of scissors, placed in a dark bag and immediately transported to the laboratory of Institut National Polytechnique Félix HOUPHOUËT-BOIGNY (INP-HB) in Yamoussoukro (Côte d'Ivoire), where they were debulked. In addition to hydroethanol extracts of cocoa pod shells, two reference antifungal molecules were also tested. These were ketoconazole (Strides Pharma Science Limited, India) and ltraconazole (HOVID Bhd, Malaysia).

Extraction of secondary metabolites

After hulling, fresh pod shells were crushed and dried away from the sun for 14 days (Figure 1), then ground using an IKA MF 10 basic electric grinder with a 0.5-mm sieve.

Secondary metabolites were extracted following the method used by Kouamé et al. (2021), using a hydro-ethanol solvent consisting of 70% ethanol and 30% distilled water. The dried cocoa pod husk powder (Figure 2) was macerated in the ethanol-water mixture using a magnetic stirrer for 24 hours at a ratio of 1g / 10 ml (mass of powder per volume of macerating solvent). The resulting macerate was filtered twice on absorbent cotton and once on 3 mm Wattman filter paper. The filtrate was then evaporated in an oven at 50°C.
The yield of the extractions was determined by the ratio of the mass of the plant extract obtained by that of the crushed material.

$$R_1\ (\%) = \frac{M_1}{M_0} \times 100$$

R_1 : Extract yield
M_1 : Mass of plant extract (in g)
M_0 : Mass of plant material (in g)

Phytochemical screening and determination of total polyphenols, total flavonoids and condensed tannins

The phytochemical screening, based on the method used by Coulibaly et al. (2020), aims at identifying the presence of several families of secondary metabolites in cocoa pods. The families sought are sterols, polyterpenes, alkaloids, polyphenols, flavonoids, anthocynes, gallic tannins, catechic tannins and quinones.
The content of secondary metabolites was determined using various dosage methods. Total polyphenols were determined using the method described by Wood et al. (2002). Total flavonoids were determined using the method described by Kouamé et al. (2021) and condensed tannins using the method described by Julkunen-Titto (1985). The total polyphenol content of the hydroalcoholic extract was determined from calibration curve Y = 1.129 X + 0.0544 with R^2 = 0.9967 plotted using gallic acid as standard. Flavonoids were determined from calibration curve Y= 0.6455X - 0.0079 with R^2 = 0.9983 plotted using quercetin as standard. Condensed tannin content in hydroethanol extract of dried cocoa pod grinds was determined from calibration curve Y = 0.1165X + 0.00003 with R^2 = 0.9996 plotted using stock solution of tannic acid as standard (Kouamé et al., 2021). Assay tests were carried out in triplicate for each sample.

Antifungal activity

Antifungal tests were carried out against three mycelial strains of Aspergillus genus and a Candida strain (*Candida albicans*). Fungal strains were plated on Sabouraud chloramphenicol agar and incubated at 25 ± 2°C.

Fig. 1: Crushed cocoa pod shells.

Fig. 2: Dried cocoa pod husk powder

Inhibition of mycelial growth by hydroethanolic extracts of cocoa pods

Mycelial growth inhibition test consists in making a central puncture of 5 mm diameter explants taken from a 72 hours old culture. The strains tested were *AspergillusNiger, Aspergillus flavus* and *Aspergillus cabonarius.*

Prepared Sabouraud agar (according to the manufacturer's instructions) is distributed in 6 test tubes as follows: 29.4 mL in tube T1 and 15 mL in five other tubes (T2 to T6). Volume of tube T1 increases to 30 mL by adding 0.6 mL of a mixture consisting of 300 mg extract in 1 mL DMSO/sterile distilled water (1/13 : V/V) (Ismail et al., 2008). From this agar with 2% of extract concentration (6 mg/mL), dilutions in geometric progression of reason 2 are performed, giving a final concentration range of 0.18, 0.37, 0.75, 1.5; 3 and 6 mg/mL in tubes T6 to T1, respectively. Two control tubes (growth control (GC) and sterility control (SC)) containing 15 mL Sabouraud agar are also prepared.After homogenization, the content of each tube is transferred to a Petri dish of 90 mm diametermarked beforehand. Once agar has solidified, explant of 5 mm is removed using a platinum loop and placed in the center of agar. Mycelial growth is determined every 24 hours, by determining the average of two perpendicular diameters passing through the middle of the Petri dish (Goly et al., 2015).

Determination of Minimum Inhibitory Concentration (MIC) and Fungicidal Concentration (FMC)

Fungal inoculum is prepared from 72 hours young cultures at 25°C on chloramphenicol Sabouraud medium (bio-rad) (France). An aliquot is taken using a calibrated loop (diameter = 2 mm) and homogenized in 10 mL sterile distilled water. This suspension yields a solution with a loading of 10^6 cells/mL, obtained by diluting the suspension to obtain an optical density between 0.18 and 0.20, at 623 nm (Zerzouret Hamimed, 2022).

Concentration range is prepared as mentioned previously. Two control tubes (growth and sterility controls) are prepared. However, extract/agar mixture is left at room temperature (25°C ± 2) in inclined tubes until the agar solidifies. Fungal inoculum is then streaked onto the surface of sloping agar. After 72 hours of incubation at 25°C ± 2, MIC is determined by comparing experimental tubes with growth control. Concentration of experimental tube in which there is no visible fungal growth corresponds to MIC. MFC is determined from tubes in which no growth is observed. Subculture is performed on fresh agar. The surface of agar is scraped with a platinum loop and streaked onto the surface of sloping new agar in tubes numbered according to initial concentrations. After 3 days of incubation, FMC is determined. This is the lowest concentration at which fungal growth does not resume (Guede-Guina et al., 1997).

Analysis of results

Statistical analysis was carried out using GraphPad Prism 8 software (Dotmatics, Boston, Massachusetts). Comparisons of the means of the various parameters were made using the analysis of variance (ANOVA) test. Analyses were performed with a margin of error of 5% (α=0.05). The presence of a different letter on the values indicates that they are statistically different and the presence of the same letter on the values indicates that they are statistically identical.

RESULTS AND DISCUSSION

Yield of hydroalcoholic extractions

Hydroalcoholic extraction from green pod crush gave higher yield (14.10 ± 0.08%) than that from yellow pod crush (11.92 ± 1.81%) (Table1). Comparison of the two yields by analysis of variances showed a significant

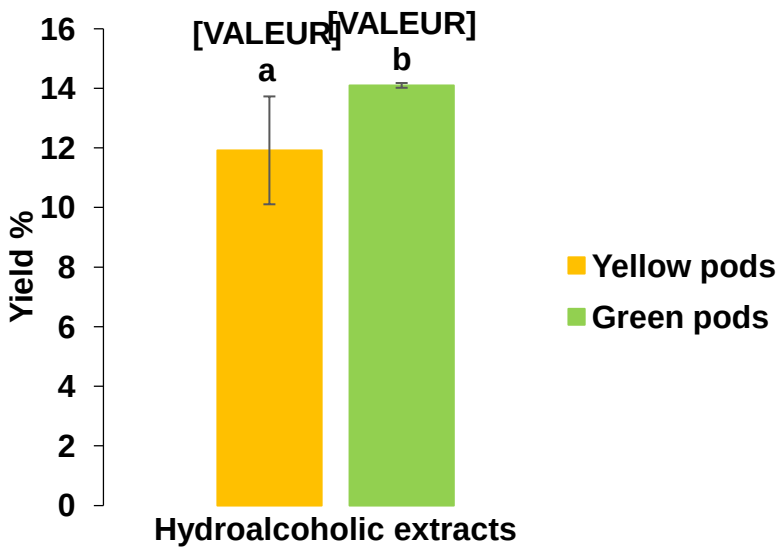


Fig. 3: Yield of hydroethanol extracts from cocoa pod shells.

Table 1. Chemical composition of hydroethanol extracts from cocoa pod shells.

Secondary metabolites ought	Hydro-ethanolic extracts of cocoa pods	
	Yellow pod	Green pod
Alkaloids	+	+
Polyphenols	+	+
Flavonoids	+	+
Anthocyanins	+	+
Saponosides	+	+
Catechic tannins	+	+
Gallic tannins	+	+
Quinones	+	+
Polyterpenes and sterols	+	+

+: Present

difference between them with a *p-value <0.0001* (α=0.05).

The lower extraction yield could be explained by the stress undergone at ripening stage. According to Isah (2019), the type and concentration of secondary metabolites produced by a plant are determined by species, genotype, physiology, developmental stage and environmental factors during growth. This suggests physiological adaptive responses employed by various taxonomic groups of plants to cope with stress and defensive stimuli (Ncube et Van Staden, 2015).

Chemical composition and secondary metabolite content of hydroethanolic extracts from cocoa pod shells

All secondary metabolites families sought are present in cocoa pods (yellow and green) (table 1).

Statistical analysis shows a significant difference between the total polyphenol, total flavonoid, condensed tannin and total tannin content of the yellow cocoa pod shell with a *p-value <0.0001* (α=0.05).

Yellow pods are richer in total polyphenols (35.50 ± 0.50 mg EAG/g dry extract) than green pods (25.02 ± 0.01 mg EAG/g dry extract) (figure 4). However, green pods are richer in total and condensed tannins than yellow pods. Total tannin content almost halves in yellow pod. It drops from 23.98 ± 0.02 to 12.23 ± 0.35 mg EAT/g dry extract. Condensed tannins are at least twice as concentrated in green pod (16.40 ± 0.02 mg EAT/g dry extract) than in yellow pod (6.65 ± 0.15 mg EAT/g dry extract). Apparently, pod ripening does not influence total flavonoid content. However, statistical analysis shows a difference in total flavonoid content between yellow cocoa pod shells (65.33 ± 0.33 mg EQ/g dry extract) and

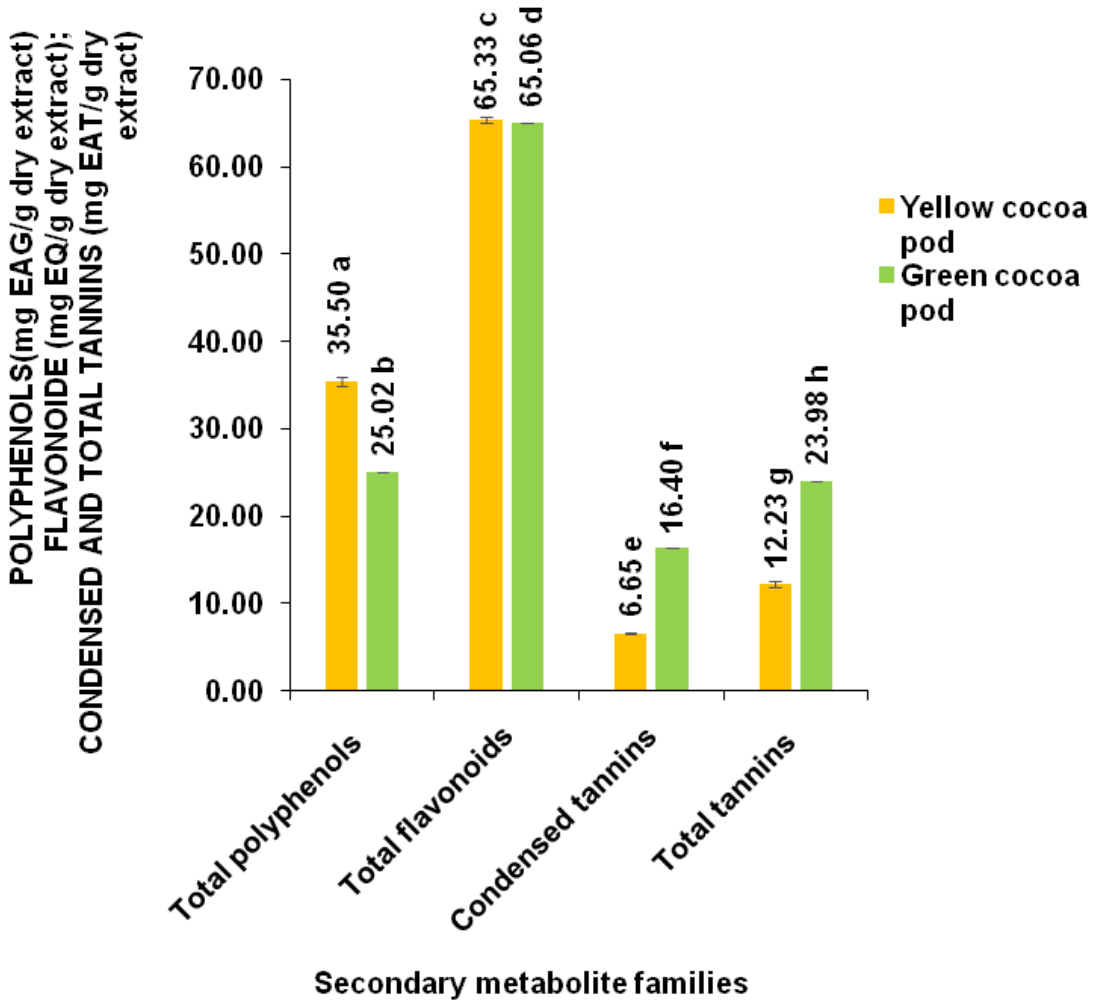


Fig. 4. Polyphenol, flavonoid, condensed tannin and total contents of hydroalcoholic extracts from yellow and green cocoa pods.

green cocoa pod shells (65.06 ± 0.04 mg EQ/g dry extract) (*p-value <0.0001,*α=0.05). This difference means that the shell of yellow cocoa pods is richer in total flavonoids than that of green pods.

Although they have not worked on cocoa, Mohammedi (2020) and Shuai et al. (2021) have already observed an increase in total polyphenol levels during fruit ripening. This increase in total polyphenol content from unripe to semi-ripe fruit could be explained by accumulation of polyphenols (Scarano et al., 2022). Decrease in tannin content was also observed by Ivancic et al. (2022) in olive pulp as opposed to skin. Reduction in tannin content could be explained by changes in phenolic compounds between ripening stages and explains why fruit astringency due to tannins decreases (Alarcão-E-Silva et al., 2001; Mohammedi, 2020). Astringency depends on the molecular structure of tannins, which determines their cross-linking with proteins and glycoproteins, so that they give an astringent taste when dissolved in saliva. During ripening, the molecular weight of tannins increases due to polymerization, resulting in tannin insolubility and a lack of astringency (Maduwanthi Marapana, 2019).

Inhibition of mycelial growth

All three Aspergillus strains (*Aspergillus Niger, Aspergillus flavus* and *Aspergillus cabonarius*) are more sensitive to the hydroethanol extract derived from crushed yellow cocoa pods (figure 5). This sensitivity is dose-dependent. *Aspergillus flavus* was the most sensitive. With a concentration of 1.5 mg/mL, hydroethanol extract of yellow pod crush inhibits mycelial growth of *Aspergillus flavus* for the first four day. From the fifth day onwards, mycelial growth resumes. Twice this concentration (3 mg/mL) completely inhibits mycelial growth of the strain for five days (figure 6E), while the same extract from green pod crush has no effect on mycelial growth of *Aspergillus flavus* (figure 6F).

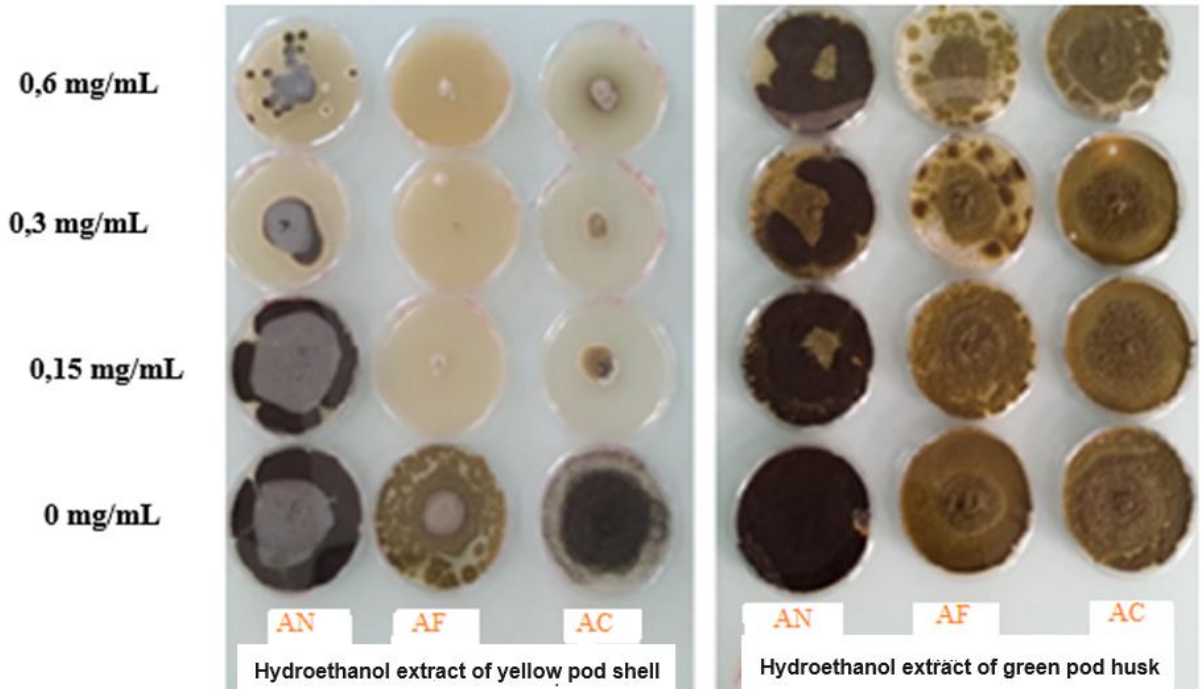
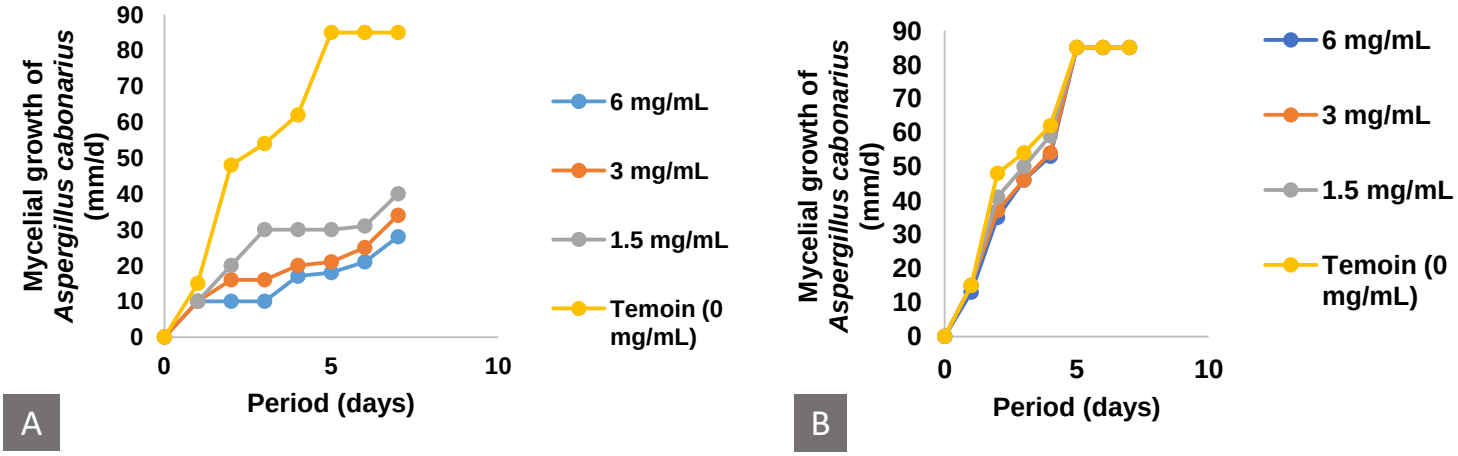
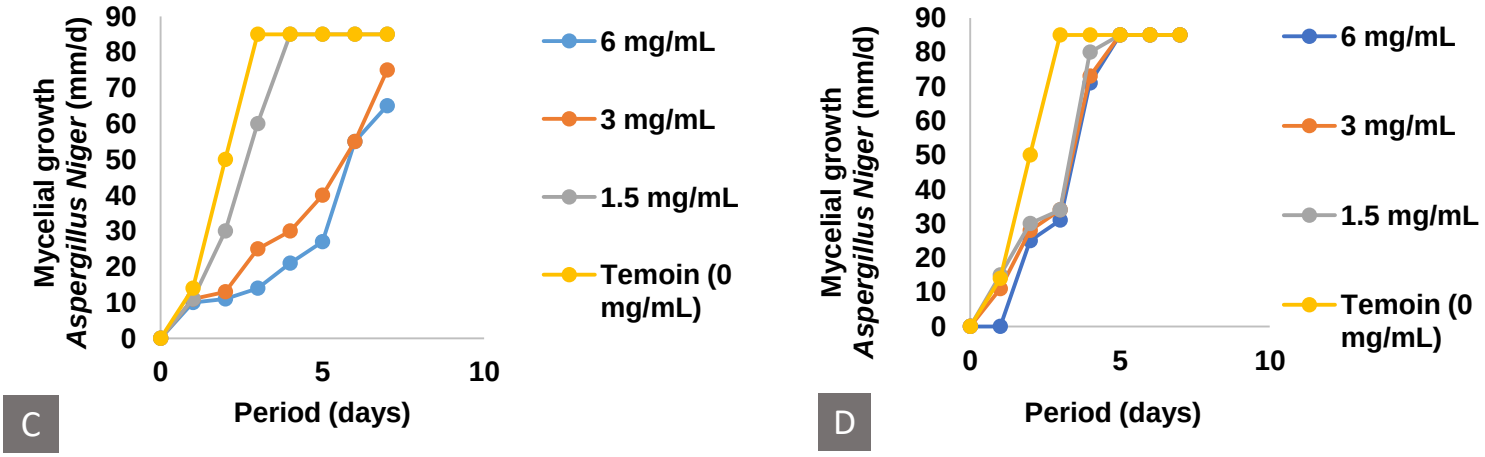


Fig. 5: Mycelial growth of *Aspergillus Niger* (AN), *Aspergillus flavus* (AF) and *Aspergillus cabonarius* (AC) in the presence of different concentrations of *hydroalcoholic extracts of cocoa pods* (yellow and green).

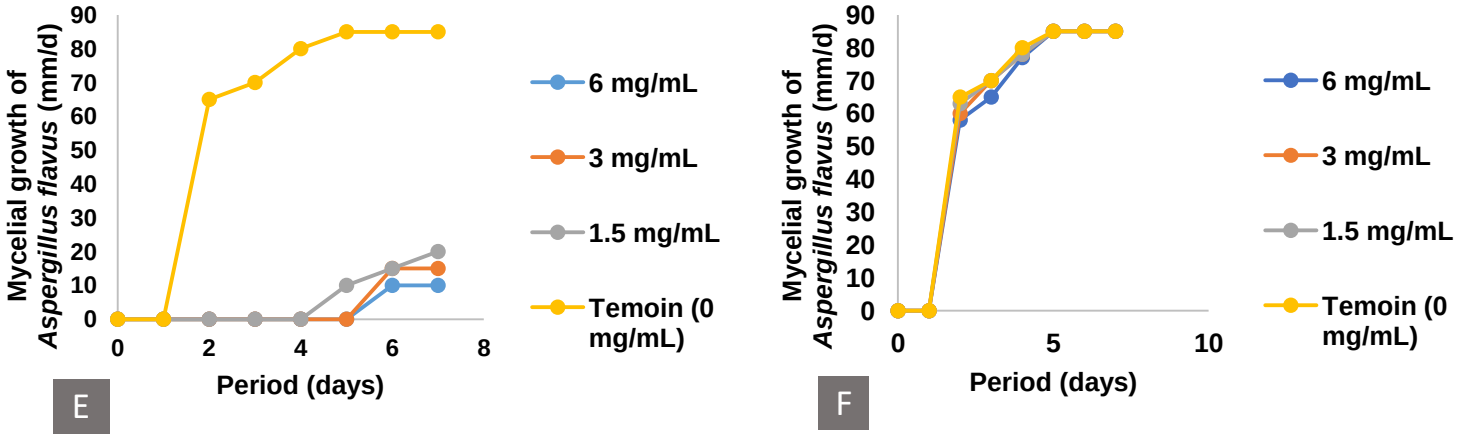


Evolution of mycelial growth of *Aspergillus carbonarius* in the presence of hydroethanol extract of yellow (A) and green (B) cocoa pods.

Compared with *Aspergillus flavus* and *Aspergillus carbonarius*, *Aspergillus niger* is resistant to hydroethanol extract of cocoa pod crush. Like the control, mycelial growth begins on the first day, even at 6 mg/mL (Figures 6A, B, C and D). However, with 6 mg/mL of hydro-ethanolic extract of green pod crush, mycelial growth of *Aspergillus niger* begins only 24 hours after the test started (figure 6D). Hydro-ethanol extract from yellow pods is far more effective than that from green pods. This could be explained by its high polyphenol content, compounds with antimicrobial properties (Jurd et al., 1971; Wang et al., 2021).



Evolution of mycelial growth of *Aspergillus niger* in the presence of hydroethanol extract of yellow (C) and green (D) cocoa pods.



Evolution of mycelial growth of *Aspergillus flavus* in the presence of hydroethanol extract of yellow (E) and green (F) cocoa pods.

Antifungal parameters of substances tested

For all strains tested, hydro-ethanolic extract from yellow pod crush was more effective than that from green pod crush (Table 2). However, all antifungal parameter values determined were higher than those of the two reference molecules tested (Itraconazole and Ketoconazole). In some cases, the results obtained with hydro-ethanol extracts of cocoa pods are comparable to those obtained with synthetic drugs, which are pure molecules.

In fact, hydro-ethanolic extract of yellow cocoa pod grinds, with MICs and MFCs of 1.5 and 3 mg/mL respectively for *Aspergillus cabonarius* and *Aspergillus flavus* strains, is more effective than that obtained from green cocoa pods. With a minimum fungicidal concentration equal to 18.75 mg/mL, *Candida albicans* strain appears to be more resistant than strains tested. Among Aspergillus strains tested, *Aspergillus niger* was most resistant.

Antifungal activity of these extracts is due to their chemical composition. Pronounced antifungal activity of hydro-ethanolic extract of yellow pod crush is justified by its high content in total polyphenols and total flavonoids known for their antibacterial and antifungal activities(Wang et al., 2021; Ould Kaddour, 2019).

Table 2: Antifungal parameters of tested substances.

ANTIFUNGAL ACTIVITY								
Substances tested	*Aspergillus cabonarius*		*Aspergillus Niger*		*Aspergillus flavus*		*Candida albicans*	
	CMI (mg/mL)	CMF (mg/mL)	CMI (mg/mL)	CMF (mg/mL)	CMI (mg/mL)	CMF (mg/mL)	CMI (mg/mL)	CMF (mg/mL)
ETHCV	3	6	6	> 18,75	6	> 18,75	9,38	>18,75
ETHCJ	1,5	3	3	6	1,5	3	3	18,75
Ket	0,55	2,22	0,14	2,22	0,14	2,22	**ND**	**ND**
Itr	0,14	2,22	0,07	2,22	0,07	1,11	**ND**	**ND**

ETHCV = Hydro-ethanolic extract from green pod shell; ETHCJ = Hydro-ethanolic extract from yellow pod shell; Itr = Itraconazole and ket = Ketoconazole; ND = Not Determined.

The influence of phenolic compounds from cocoa pod shells on the antifungal activity carried out on *F.oxysporum* has also been reported (Rachmawaty et al., 2018). Indeed, according to Kumalasari and Sulistyani (2011), this family of secondary metabolites can damage cell membranes or denature proteins and constrict the cell wall of cells that can lyse fungal cell walls, thus leading to inhibition of cell growth or cell death. Other families of compounds such as saponins, present in cocoa pod shells, could also contribute to antifungal activity.

Our results show that the hydroethanolic extract of cocoa pod shells has antifungal activities on the fungal germs tested. However, this extract from cocoa pod shells collected in Indonesia did not show any antifungal activity on the growth of *F. oxysporum* fungi contrary to acetone-water extract (Rachmawaty et al., 2018). These results show the influence of solvent and harvest area of plant material on activities of extracts as already reported in literature (Boutabia et al., 2016).

CONCLUSION

Our work was devoted to studying the antifungal activity of hydro-ethanolic extracts from cocoa pod waste. Hydro-ethanolic extracts of cocoa pod shells (yellow and green) contain all the families of secondary metabolites sough and have antifungal activity on all fungal strains tested. However, extract from yellow pods, richer in total polyphenols and flavonoids, was more effective against all the strains tested. Mould strains are more sensitive.This waste can therefore be used as a source of antifungals in the agricultural and pharmaceutical industries.The structural determination of secondary metabolites of this waste is in progress.

ACKNOWLEDGEMENT

The team of researchers in charge of this article would like to thank the Fonds pour la Science, la Technologie et l'Innovation (FONSTI) for funding this project, the Institut National Polytechnique Félix HOUPHOUËT-BOIGNY (INP-HB) of Yamoussoukro, Côte d'Ivoire, and the Ecole Doctorale Polytechnique (EDP) of this institution.

REFERENCES

Abdoullahi H.O., Tapsoba F., Guira F., Zongo C., Abakar L.I., Tidjani A. & Savadogo A. (2018). Technologies, qualité et importance socioéconomique du poisson séché en Afrique. *Synthèse: Revue des Sciences et de la Technologie*, 37: 49–63.

Alarcão-E-Silva M.L.C.M.M., Leitão A.E.B., Azinheira H.G. & Leitão M.C.A. (2001). The Arbutus Berry: Studies on its Color and Chemical Characteristics at Two Mature Stages. *Journal of Food Composition and Analysis*, 14(1): 27–35.

Barrios-Rodríguez Y.F., Salas-Calderón K.T., Orozco-Blanco D.A., Gentile P. & Girón-Hernández J. (2022). Cocoa Pod Husk: A High-Pectin Source with Applications in the Food and Biomedical Fields. *Chem. Bio. Eng. Reviews*, 9(5): 462–474.

Boutabia L., Telailia S., Bouguetof I., Guenadil F. & Chefrour A. (2016). Composition chimique et activité antibactérienne des huiles essentielles de Rosmarinus officinalis L. de la région de Hammamet (Tébessa-Algérie). *Bulletin de la Société Royale des Sciences de Liège*, 85: 174–189.

Campos-Vega R., Nieto-Figueroa K.H. & Oomah B.D. (2018). Cocoa (Theobroma cacao L.) pod husk: Renewable source of bioactive compounds. *Trends in Food Science &Technology*, 81: 172–184.

Coulibaly Y.O. (2020). La noix de cajou en Côte d'Ivoire : la filière cajou, un succès en trompe-l'oeil : le sénateur Jean-Baptiste Pany répond à vingt-neuf questions fondamentales, dans une interview réalisée par Yao Olivier Coulibaly. *La noix de cajou en Côte d'Ivoire*,: 1–207.

El-Sayed A.S.A. & Ali G.S. (2020). Aspergillus flavipes is a novel efficient biocontrol agent of Phytophthora parasitica. *Biological Control*, 140: 104072.

GBN (2021). Partnership Ready Côte d'Ivoire: Gestion et recyclage des déchets organiques.

Goly C., Soro Y., Kassi B., Dadié A., Soro S. & Dje M. (2015). Antifungal activities of the essential oil extracted from the tea of savanna (Lippia multiflora) in Côte d'Ivoire. *International Journal of Biological and Chemical Sciences*, 9(1): 24–34.

Guede-Guina F., Kra A.M., Vangah-Manda M., Bonga G.M. & De Souza C. (1997). Inhibition par MISCA-F 2 de la croissance de Aspergillus fumigatus; Candida albicans et Cryptococcus neoformans, 3 germes fongiques opportunistes du SIDA. *J. Afr. Biomed*, 2: 11–16.

ICCO P (2021). Organización Internacional de Cacao.

Isah T. (2019). Stress and defense responses in plant secondary metabolites production. *Biological Research*, 52:

Ismail H., Lemriss S., Ben Aoun Z., Mhadhebi L., Dellai A., Kacem Y., Boiron P. & Bouraoui A. (2008). Antifungal activity of aqueous and methanolic extracts from the Mediterranean sea cucumber, Holothuriapolii. *Journal de Mycologie Médicale*, 18(1): 23–26.

Ivancic T., Jakopic J., Veberic R., Vesel V. & Hudina M. (2022). Effect of Ripening on the Phenolic and Sugar Contents in the Meso- and Epicarp of Olive Fruits (Olea europaea L.) Cultivar 'Leccino'. *Agriculture*, 12(9): 1347.

Julkunen-Titto R. (1985). Phenolic constituents in the levels of northern willows: methods for precursors of clarified apple juice sediment. J. Food Sci., 33: 254–257.

Jurd L., Stevens K.L., King A.D. & Mihara K. (1971). Antimicrobial Properties of Natural Phenols and Related Compounds II: Cinnamylated Phenols and Their Hydrogenation Products. *Journal of Pharmaceutical Sciences*, 60(11): 1753–1755.

Kassi A.B., Soro Y., Fante B., Golly K.J., Sorho S., Toure A.S. & Coustard J.-M. (2014). Isolation and identification of bioactive compounds from kernel seed cake of the mango (Mangifera indica Lam). *International Journal of Biological and Chemical Sciences*, 8(4): 1885–1895.

Kouamé T.K., Siaka S., Kassi A.B.B. & Soro Y. (2021). Détermination des teneurs en polyphénols totaux, flavonoïdes totaux et tanins de jeunes feuilles non encore ouvertes de Piliostigma thonningii (Caesalpiniaceae). *International Journal of Biological and Chemical Sciences*, 15(1): 97–105.

Kumalasari E. & Sulistyani N. (2011). Aktivitas antifungi ekstrak etanol batang binahong (Anredera cordifolia (Tenore) Steen.) terhadap Candida albicans sertaskriningFitokimiaJurnalIlmiahKefarmasian 1. 2 59-60.

Lesley Bremness (2022). Plantes aromatiques et médicinales.

Maduwanthi S.D.T. & Marapana R. (2019). Induced ripening agents and their effect on fruit quality of banana. *International journal of food science*, e2520179.

Mansur D., Tago T., Masuda T. & Abimanyu H. (2014). Conversion of cacao pod husks by pyrolysis and catalytic reaction to produce useful chemicals. *Biomass and Bioenergy*, 66: 275–285.

Meraz-Pérez I.M., Carvalho M.R., Sena K.F., Soares Y.J.B., Estrela Junior A.S., Lopes U.V., dos Santos Filho L.P., Araújo S.A., Soares V.L.F., Pirovani C.P. & Gramacho K.P. (2021). The Moniliophthora perniciosa – Cacao pod pathosystem: Structural and activated defense strategies against disease establishment. *Physiological and Molecular Plant Pathology*, 115: 101656.

Mohammedi Z. (2020). Étude de l'évolution de la capacité anti-radicalaire du fruit de l'Arbutus unedo L. à différents stades de maturation. *Bulletin de la Société Royale des Sciences de Liège*, 89: 130-146.

Organisation mondiale de la Santé & Organization W.H. (2018). Results of the 2017 global WHO survey on mycetoma–Résultats de l'enquête mondiale de l'OMS surle mycétome, 2017. *Weekly Epidemiological Record= Relevé épidémiologique hebdomadaire*, 93(33): 423–428.

Ncube B. & Van Staden J. (2015). Tilting Plant Metabolism for Improved Metabolite Biosynthesis and Enhanced Human Benefit. *Molecules*, 20(7): 12698–12731.

Ofori-Boateng C. & Lee K.T. (2013). The potential of using cocoa pod husks as green solid base catalysts for the transesterification of soybean oil into biodiesel: Effects of biodiesel on engine performance. *Chemical Engineering Journal*, 220: 395–401.

Ould Kaddour A.S. (2019). Etude de l'effet antifongique des extraitspolyphénoliques de l'Atriplexhalimus L., sur la croissance de certains champignons dermatophytes.Thèse de Doctorat, Université Abdelhamid Ibn Badis de Mostaganem, Algérie, 149 pages.

Rachmawaty, Mu'nisa A., Hasri, Pagarra H., Hartati & Maulana Z. (2018). Active Compounds Extraction of Cocoa Pod Husk (*Thebroma Cacao* l.) and Potential as Fungicides. *Journal of Physics: Conference Series*, 1028: 012013.

Scarano A., Gerardi C., Sommella E., Campiglia P., Chieppa M., Butelli E. & Santino A. (2022). Engineering the polyphenolic biosynthetic pathway stimulates metabolic and molecular changes during fruit ripening in "Bronze" tomato. *Horticulture Research*, 9: uhac097.

Shuai L., Liu H., Liao L., Lai T., Lai Z., Du X., Duan Z., Wu Z. & Luo T. (2021). Widely targeted metabolic analysis revealed the changed pigmentation and bioactive compounds in the ripening Berchemia floribunda (Wall.) Brongn. fruit. *Food Science & Nutrition*, 9(3): 1375–1387.

Soro Y., Kassi A.B.B., Bamba F., Siaka S., Toure S.A. & Coustard J.M. (2012). Flavonoids and gallic acid from

leaves of *Santaloides afzelii* (Connaraceae). *Rasāyan Journal of Chemistry*, 5(3): 332–337.

Vásquez Z.S., de Carvalho Neto D.P., Pereira G.V., Vandenberghe L.P., de Oliveira P.Z., Tiburcio P.B., Rogez H.L., Neto A.G. &Soccol C.R. (2019). Biotechnological approaches for cocoa waste management: A review. *Waste Management*, 90: 72–83.

Wang D., Cui L., Ren H., Wang Y., Long D. & Niu Y. (2021). Anti-fungal activity and preliminary active components separation from ethanol extracts in Saffron (Crocus sativus L.) lateral buds. *Industrial Crops and Products*, 173: 114081.

Wood J.E., Senthilmohan S.T. & Peskin A.V. (2002). Antioxidant activity of procyanidin-containing plant extracts at different pHs. *Food Chemistry*, 77(2): 155–161.

Zerzour I. & Hamimed S. (2022). Tests phytochimiques de l'extrait hydroalcoolique des racines d'Anacyclus pyrethrum L.