

ETHNOPHARMACOLOGY AND CANDIDA-INHIBITORY EFFECTS OF XYLOPIA AETHIOPICA (ANNONACEAE) FRUIT EXTRACTS: SYNERGISTIC ENHANCEMENT WITH MICONAZOLE

Tchaa KOZOLINA¹, Poyodi KOLA², Diana OBISTIOIU³, Doris FLOARES³, Anca HULEA³, Aboudoulatif DIALLO⁴, Batomayéna BAKOMA⁵, Ciprian STROIA³, Ilinca IMBREA^{3*}

¹Laboratory of Pharmaceutical Sciences, Faculty of Health Sciences, Doctoral school ED732-STIS, University of Lomé, 01 BP: 1515, Lomé, Togo

²Laboratory of Applied Agronomic and Biological Sciences (LaSABA), Faculty of Sciences and Techniques, University of Kara, BP: 404, Kara, Togo

³University of Life Sciences "King Michael I" from Timisoara, Calea Aradului 119, 300645 Timisoara, Romania

⁴Department of Pharmacy, Laboratory of Pharmacology-Toxicology, Faculty of Health Sciences, University of Lomé, BP: 1515, Lomé, Togo

⁵Department of Pharmacy, Laboratory of Pharmaceutical Sciences, Faculty of Health Sciences, University of Lomé, BP: 1515, Lomé, Togo

ABSTRACT: An ethnopharmacological survey was conducted involving 203 participants in the Central Region of Togo. We assessed the anti-*Candida* activity and the ability of hydroalcoholic extracts and essential oils, obtained through hydrodistillation and steam distillation, to enhance the effectiveness of miconazole. This evaluation was performed using the microdilution method and optical density measurements against *Candida albicans* and *Candida parapsilosis*. The survey identified 30 disorders, with gynecological and obstetrical issues being the most prevalent at 90.14%. The essential oils achieved mycelial inhibition percentages exceeding 70% at a concentration of 8 mg/mL, indicating a clear dose-dependent effect. Among the hydroalcoholic extracts, the hydromethanolic extract (1:2) exhibited the most promising antifungal activity against the tested *Candida* strains. Furthermore, essential oils obtained through hydrodistillation exhibited a synergistic effect when combined with miconazole. *Xylopiya aethiopic*a essential oils are more potent than solvent extracts and could serve as promising natural adjuvants for treating fungal infections.

Keywords: boosted, *Candida*, ethnomedicinal, microdilution, Togo, *Xylopiya aethiopic*a.

INTRODUCTION

The use of medicinal plants as remedies for various diseases, attributed to their bioactive compounds, has been documented since antiquity. Although modern medicine long questioned their biological potential, current research focuses on identifying specific natural bioactive compounds from medicinal plants that, on the one hand, exert a beneficial effect on the body's energy and vitality and, on the other hand, provide an alternative to conventionally synthesized bioactive compounds used in disease treatment (Obistioiu *et al.*, 2023; Balasoiu *et al.*, 2024; Degi *et al.*, 2022; Han *et al.*, 2022; Beicu *et al.*, 2021). This is the case, for instance, for *Xylopiya aethiopic*a, commonly known as Ethiopian pepper, black pepper, or Guinea pepper, which is widely distributed across different regions of the world, particularly in Africa, and specifically in West and Central Africa. This spice holds significant cultural importance in various West African regions due to its medicinal virtues. In Nigeria, *Xylopiya aethiopic*a is used as an ingredient in the preparation of decoctions for women postpartum; this aromatic plant is also recognized for its role in relieving and reducing postpartum contractions (Owokotomo *et al.*, 2021). *Xylopiya aethiopic*a may also be used in the treatment of infectious diseases, as various parts of the plant (fruits, pods, seeds, leaves, stem, bark, and roots) have demonstrated activity against different pathogenic microorganisms and are thus used as an alternative by

populations (Houehouha *et al.*, 2020). In Togo, 80% of the population relies on medicinal plants for healthcare (Agody *et al.*, 2019).

Research in phytochemistry and phytopharmacology therefore represents an alternative for developing healthcare in developing countries (Jiofack *et al.*, 2010). Many studies have been conducted in this regard on *Xylopiya aethiopic*a. Examples of relevant studies include the evaluation of antimicrobial activity (Fleischer *et al.*, 2008), the toxicological effects of hydroethanolic extract (Assih *et al.*, 2022), anti-anaphylactic and anti-inflammatory actions (David *et al.*, 2013), potential improvements in hepatic damage (Folorunso *et al.*, 2013), and the treatment and relief of incurable wounds and tumors (Kola *et al.*, 2020). Moreover, antimicrobial substances are defined as agents used to destroy microorganisms or inhibit their growth, including antibiotics and other antibacterial and antifungal agents (Erhirnie *et al.*, 2014).

However, due to growing consumer concern regarding products containing such synthetically derived additives, the search for natural additives, particularly of botanical origin, has notably increased in recent years. Consequently, the valorization of these natural products possessing antifungal activity is both necessary and valuable (Ali *et al.*, 2016). Few studies have investigated the ethnopharmacological profile and comparatively assessed the anti-candidal activity of hydroalcoholic and essential oil extracts of *Xylopiya*

*Correspondence: Ilinca Imbrea, University of Life Sciences "King Michael I" from Timisoara, Calea Aradului 119, 300645 Timișoara, Romania., email: ilinca_imbrea@usvt.ro

aethiopica. In the context of growing interest in complementary medicine, a comprehensive evaluation is therefore warranted to explore not only the intrinsic antifungal potential of these extracts but also their possible synergistic effects when combined with conventional antifungal agents. Accordingly, this study aims to contribute to the valorization of this aromatic plant from the Togolese flora.

MATERIAL AND METHODS

Ethnopharmacological survey design

This ethnopharmacological study is primarily descriptive. It was based on sampling techniques established in quantitative ethnobotany, with slight modifications (Houéhanou *et al.*, 2016). To better

understand issues related to ethnomedicinal knowledge and the utilization forms of *Xylopi*a *aethiopica* fruits, discussions were held with 3 leaders of Village Development Committees (CVD) in each surveyed commune, totaling 24 CVDs across the 8 villages (Fig. 2). The surveyed population thus comprised three main groups: 114 traditional practitioners (herbalists), 66 spice vendors, and 23 household heads for a total of 203 individuals (Table 1). The ethnopharmacological survey was carried out from February to March 2024, covering 11 localities across 8 of the 14 villages in Togo's Central Region. Data were collected from each type of participant through structured interviews based on a questionnaire.

Table 1.

Distribution of respondents by status and locality

Central Region of Togo				
Municipality	Locality	Herbalists (114)	Retailers (66)	Principals of houses (23)
Tchaodjo 1	Sokodé	9	6	4
Tchaodjo 2	Lama Tessi	10	5	1
Tchamba 2	Bago	9	4	1
Sotouboua 1	Sotouboua	11	8	2
	Tabindè	9	3	2
Sotouboua 2	Fazao	11	5	1
	Adjengré	12	7	2
Sotouboua 3	Tchébébé	13	9	4
	Kaza	11	3	2
Blitta 1	Pagala	10	10	2
Blitta 3	Tchifama	9	6	2

Plant Material

The fruits of *Xylopi*a *aethiopica* were harvested in December 2024 in Djokoto, a township within the Kloto 2 commune, located in the Plateaus Region of Togo.

This township is situated 120 km northwest of Lomé. The fruits were dried and stored at room temperature. The coordinates for the cultivation and harvesting site are: Latitude: 7.008135°; Longitude: 0.62979°; Accuracy: 1.8 m.

A voucher specimen was identified at the Laboratory of Biology and Plant Ecology at the University of Lomé, under the reference number TOGO15895. To obtain a powder from the dried *Xylopi*a *aethiopica* pods, approximately 2.5 kg of the material was pulverized at the Center for Research and Training on Medicinal Plants (CERFOPLAM) of the University of Lomé.



Fig. 1. Dried fruits of *Xylopi*a *aethiopica*.

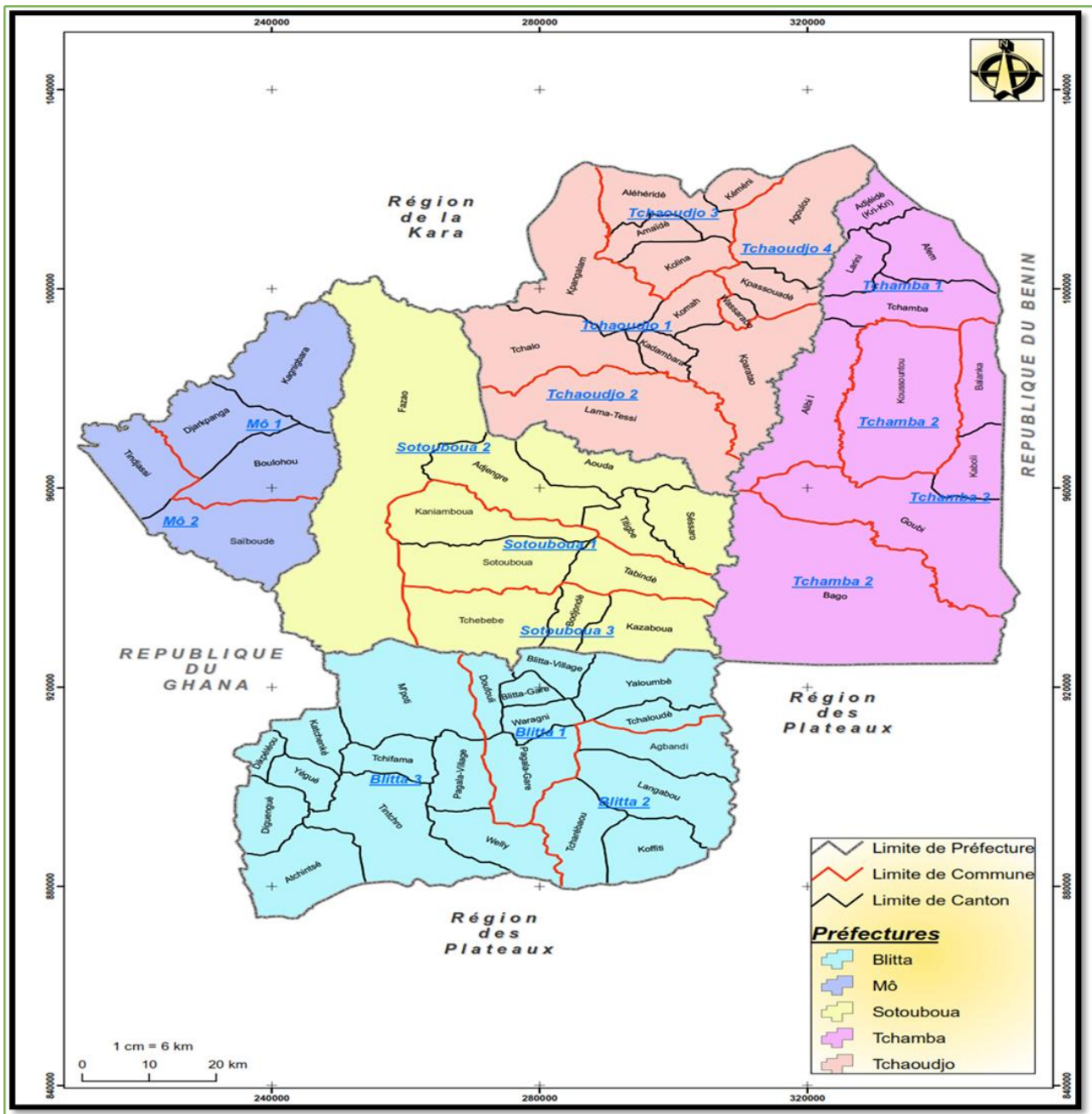


Fig. 2. Geographic map of the Central Region of Togo. Source: (MATDCC, 2025).
MATDCC = Ministry of Territorial Administration, Decentralization and Customary Chieftaincy

Biological Material

Cultures of *C. albicans* (ATCC 10231) and *C. parapsilosis* (ATCC 22019) strains were obtained from the Microbiology Laboratory of the Interdisciplinary Research Platform at the 'King Michael I' University of Life Sciences in Timișoara, Romania.

Chemicals

Ethanol (96%), methanol (96%), NaCl solution (0.5 µg/mL) (CHIMREACTIV S.R.L, Bucharest, Romania), Miconazole at 0.5 µg/mL (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany).

Extraction Materials and Equipment

Beakers, plastic containers, graduated cylinders, Erlenmeyer flasks, pipettes, Clevenger-type apparatus (Witeg, Germany), cotton, filter paper, Soxhlet extractor, precision balance (Kern & Sohn, D-72336 Balingen, Germany), UV-VIS spectrophotometer (Specord 205; Analytik Jena AG, Jena, Germany), ELISA microplate reader (BIORAD PR 1100, Hercules, CA, USA), 96-well microdilution plates. Extract preparation and anticandidal activity testing were conducted at the laboratories of the Interdisciplinary Research Platform at 'King Michael I' University of Life Sciences in Timișoara, Romania.

Table 2.

Preparation procedure for hydroalcoholic extracts

sample	ratio	solvent	extraction time
1	1:10	ethanol 70%	48h
2	1:10	methanol 70%	48h
3	1:2	ethanol: water 1:1	72h
4	1:2	methanol: water 1:1	72h

The extract samples were coded according to the extraction solvent, dilution ratio, and extraction technique as follows: 101C/1 = hydroethanolic extract (1:10), 101C/2 = hydromethanolic extract (1:10),

Preparation of hydroalcoholic extracts

Compared to laboratory standards and literature, two types of dilutions (1:2 and 1:10) were performed for the preparation of the hydroalcoholic extracts (Table 2) (Floares *et al.*, 2023).

Essential Oil Extracts

The essential oil was extracted using steam distillation with a Clevenger-type apparatus and hydrodistillation method (Beicu *et al.*, 2021). For hydrodistillation, 30 g of plant material was placed in a distillation flask containing 1000 mL of distilled water. For steam distillation, 300 g of plant material was positioned above heated water, producing steam that passed through the plant matrix. As the steam moved through the plant material, it captured the volatile compounds (Floares *et al.*, 2023). The essential oil was collected, transferred into colored glass vials, and stored at 4°C. The yield of essential oil (Y) was expressed as a percentage, calculated using the following formula: $Y = (\text{Me}/\text{Mp}) \times 100$ (1), where Y represents the percentage yield of essential oil, Me is the mass of the essential oil in mg, and Mp is the mass of the plant material in grams.

The yields of essential oils obtained from the plant material were $2.07 \pm 0.07\%$ for hydrodistillation and $0.17 \pm 0.01\%$ for steam distillation.

In vitro Anti-Candida Effect

The anticandidal activity of the samples was evaluated using the microdilution method and spectrophotometric optical density measurement. Preparations of various ATCC strains at an optical density (OD) of 0.5 McFarland standard (1.5×10^8 CFU/mL) using 100 µL of freshly cultured, diluted fungal suspension broth were used to test different concentrations of *X. aethiopica* extract samples. Several dilution series were performed. The two essential oil samples (101C/5 and 101C/6) were tested at concentrations of 0.5 mg/mL, 0.7 mg/mL, 1 mg/mL, 2 mg/mL, 4 mg/mL, and 8 mg/mL. For the hydroalcoholic samples (101C/1 to 101C/4), the concentrations tested were 10 µL/mL, 15 µL/mL, 30 µL/mL, 45 µL/mL, 60 µL/mL, and 75 µL/mL.

The plates were covered and incubated for 24 hours at 37°C. Following incubation, absorbance was measured at 540 nm using an ELISA microplate reader (BIORAD PR 1100, Hercules, CA, USA). The OD of each sample was measured in triplicate (n = 3). Strain

101C/3 = hydroethanolic extract (1:2), 101C/4 = hydromethanolic extract (1:2), 101C/5 = essential oil obtained by hydrodistillation, and 101C/6 = essential oil obtained by steam distillation.

suspensions and BHI (Brain Heart Infusion) broth were used as the negative control (NC), while miconazole (0.5 µg/mL) served as the positive control (PC) (Obistioiu *et al.*, 2023).

Evaluation of the Capacity to Potentiate the Anti-Candida Activity of miconazole

The same ATCC strain culture used for the anti-Candida activity assessment was employed. In each well of a 96-well plate, 100 µL of a fungal suspension at 1.5×10^8 CFU/mL (0.5 McFarland standard) were inoculated. Miconazole (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) at a concentration of 0.5 µg/mL, corresponding to the minimum inhibitory concentration (MIC) determined according to CLSI recommendations (Clinical and Laboratory Standards Institute, 2002), was dissolved in a NaCl solution (0.5 mg/mL) and added to each well. The same concentration series used for the anticandidal test were performed (Obistioiu *et al.*, 2023).

Data analysis

For the ethnopharmacological survey results, qualitative data were converted into quantitative data during entry into GraphPad Prism 6 software. The data were illustrated using bar charts and tables.

Calculated Ethnopharmacological Indices

The ethnopharmacological indices used were: Total Number of Reported Pathologies (NPR), Total Number of Reported Plant Utilization Forms (NFuR) (Issa, 2018), Citation Frequencies (CF) and Informant Consensus Factors (ICF) (Dassou *et al.*, 2014).

Microbiological potency indices

Percentage of mycelial growth (MGP%), the percentage of mycelial inhibition (MIP%), Minimum Inhibitory Concentration (MIC) were the microbiological potency indices used in this study.

RESULTS AND DISCUSSION

Ethnomedicinal importance of *Xylopi* *aethiopica* fruits

Eight different sociolinguistic groups encountered during the survey are familiar with, utilize, and designate *Xylopi aethiopica* fruits under various appellations (Table 3). The use of plants as medicines is a long-standing tradition dating back to the dawn of humanity (Chude *et al.*, 2020).

Table 3.

Vernacular names of *Xylopi* *aethi* *opica* fruits in local languages

Local languages	Vernacular names
Kotokoli	Souzi
Kabyè	Koyèkirariè/Koyè-billé/Soussi
Losso ou Naodéba	Selm-bim
Ifè	Kpédjékoum
Agnanga	Insinsrin
Kambolé	Aroun/Oroun
Fon	Kpédjékoum
EWé	Etsoo

The aromatic medicinal plants like *Xylopi* *aethi* *opica* are widely used in African traditional medicine for its different therapeutic virtues (Kola *et al.*, 2020). The pronunciation of the name for *Xylopi* *aethi* *opica* fruits involves homophonous homonyms in Kotokoli and Kabyè (“Souzi” / “Soussi”) and homographic homonyms in Ifè and Fon (“Kpédjékoum”).

Certain vernacular names in some villages of Cové, in southern Benin, are similar (Houehouha *et al.*, 2020), to those found in our study. These include “Esso” in Adja, “Kpédjékoum” in Mahi and Fon, and “Oroun” in Yoruba. This correlation in the naming of *Xylopi* *aethi* *opica* fruits highlights the diversity and the shared origins of sociolinguistic groups across sub-Saharan Africa. As reported in the literature, dried fruits of *Xylopi* *aethi* *opica* possess nutritional and medicinal values (Oloyede *et al.*, 1996), and that all its organs

(roots, leaves, bark, and pods) are utilized in pharmacopoeia (Houehouha *et al.*, 2020).

Diseases, disorders, and ailments treated using the dried fruits of *Xylopi* *aethi* *opica*

Approximately 30 disorders, ailments, and diseases, categorized into 9 types of conditions, were reported (Table 4). Regarding chronic conditions, mystical or spiritual ailments, and other types of uses, *Xylopi* *aethi* *opica* fruits are used in combination with other medicinal plants (Table 4).

The fruits, in particular, are involved in the treatment of diseases and disorders such as cough, painful menstruation, fungal infections, fibroids, diarrhea, stomach aches, hiccups, and sexual weakness (Houehouha *et al.*, 2020); this confirms why the populations in the Central Region of Togo, extensive use *Xylopi* *aethi* *opica*, particularly in the management of gynecological, obstetrical.

Table 4.

Condition types, pathologies, ailments, and disorders

Types of conditions	Pathologies, disorders and/or ailments
Gynecological and obstetrical conditions	Vaginal infections, postpartum recovery, milk ejection, post-menstrual intimate wash, post-parturition cleansing
Oral and dental conditions	Dental caries, oral wounds
Respiratory conditions	Asthma attacks, common cold, cough
Skin conditions	Scabies [kizaou (Kotokoli)], unknown skin rashes
Chronic conditions or NCDs*	Diabetes, hypertension, tumors
Inflammations	Bee stings, physical trauma, muscle aches
Asthenic conditions	Muscle pain (myalgia), sprain, strain, rheumatism, lower back pain (lumbago), headache
Neurological conditions	Epilepsy, convulsions, headache, mental disorders
Mystical or spiritual conditions and other uses*	Exorcism (cleansing), expelling evil spirits, protective magic (return-to-sender), dietary use (spice), female sterility, aphrodisiac

*: condition types treated or relieved by combining *Xylopi* *aethi* *opica* fruits with other plant species.

Utilization forms of *Xylopi* *aethi* *opica* dried fruits and modes of administration

The dried fruits of *Xylopi* *aethi* *opica* are prepared in several forms (e.g., decoctions, powders, and infusions) and administered through different routes including oral, topical, nasal, and other localized applications. The ICF1 and ICF2 values range from 0.1 to 0.91. For gynecological and obstetrical conditions, as well as asthenic conditions, the ICF1 values are close to

1 (Table 5), indicating a very high informant agreement regarding the use of *Xylopi* *aethi* *opica* fruits in the management of these conditions. Six out of nine (6/9) types of conditions have an ICF1 greater than 0.5, which demonstrates a moderately high consensus regarding the information provided. Indeed, the populations of the Central Region of Togo are highly dependent on natural resources, utilizing these plant resources for economic, medicinal, and dietary purposes (Atakpama *et al.*, 2012).

Table 5.

Informant Consensus Factor (ICF) according to condition types and different utilization forms

Types of conditions	ICF1	Preparation forms	ICF2
Gynecological and obstetrical conditions	0.91	Decoction + infusion	0.91
Oral and dental conditions	0.62	Infusion + decoction	0.62
Respiratory conditions	0.61	Incense + fumigation	0.61
Skin conditions	0.14	Infusion + paste	0.14
Chronic conditions or NCDs	0.55	Infusion + decoction	0.47
Inflammations	0.69	Maceration + paste	0.69
Asthenic conditions	0.86	Infusion + decoction + paste	0.54
Neurological conditions	0.1	Infusion + decoction + paste + powder	0.1
Mystical or spiritual conditions and other uses	0.39	Incense + fumigation + whole dried fruits	0.39

Types of conditions frequently treated or relieved using *Xylopiya aethiopic* (Fig.3 A.) and different utilization forms of *Xylopiya aethiopic* (Figure 3 B)

With the exception of poultice as a utilization form and rectal irrigation as a mode of administration, these same forms and modes have been cited in previous studies (Houhouha *et al.*, 2020). In Benin, for instance,

dried fruits are commonly used as a component of bathing extracts and as a potion administered to newborns (Ayedoun *et al.*, 1996). The seeds are crushed and applied topically to the forehead for the treatment of headaches and neuralgia. They can also be taken as a decoction or concoction or even chewed and swallowed for the management of various aches and pains (Igwe *et al.*, 2003).

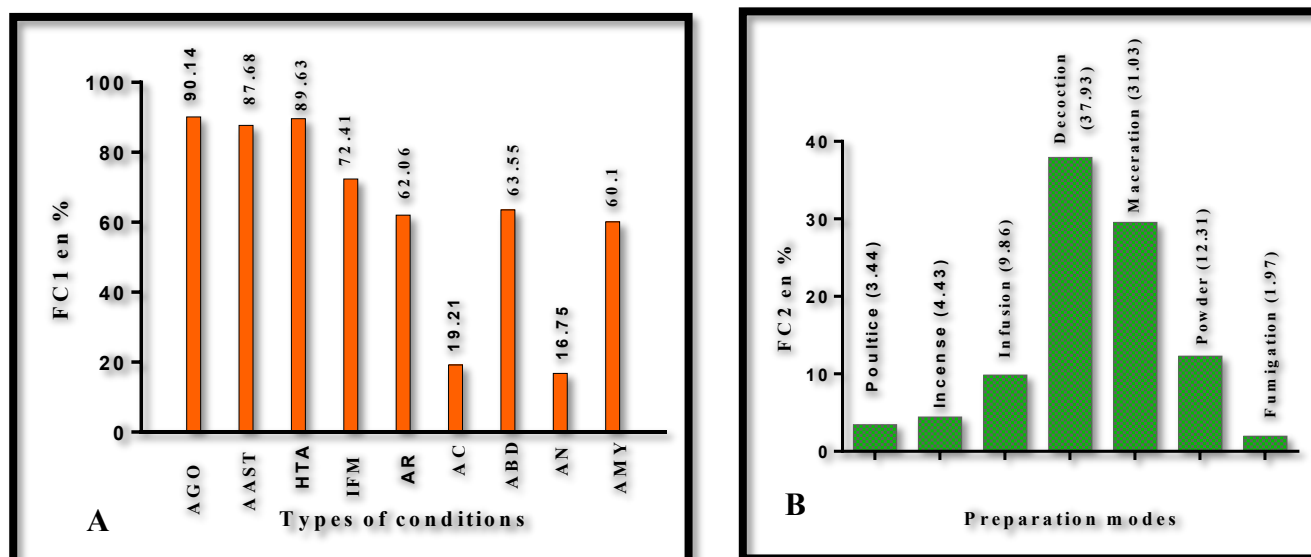


Fig. 3. Frequently treated or relieved condition types using *Xylopiya aethiopic*-based preparations (A). Citation frequencies (FC2) of the various utilization forms of *Xylopiya aethiopic* (B).

FC1: Citation frequency of a pathology, disorder, or ailment belonging to a specific condition type. GOC: Gynecological and Obstetrical Conditions. ASC: Asthenic Conditions. HTN (hypertension): Chronic conditions or NCDs. INF: Inflammations. RC: Respiratory Conditions. SC: Skin Conditions. ODC: Oral and Dental Conditions. NC: Neurological Conditions. MSA: Mystical or Spiritual Affections/Other uses (A).

In vitro anti-*Candida* effect and miconazole-potentiating capacity of *Xylopiya aethiopic* dried fruit extracts

Table 6 presents the results regarding anti-*Candida* activity, mycelial growth percentage (MGP%) and mycelial inhibition percentage (MIP%).

The experimental results showed that the hydroethanolic and hydromethanolic extracts (101C/1–101C/4) exhibited high mycelial growth percentages (MGP%), reaching values up to 242.75% for *C. albicans* and 251.12% for *C. parapsilosis*, accompanied by predominantly negative MIP% values, indicating a lack of antifungal activity and a possible growth stimulation. On the other hand, the essential oils (101C/5 and 101C/6) exhibited a clear concentration-dependent antifungal effect, with MGP% decreasing to 24.30% (*C.*

albicans) and 13.14% (*C. parapsilosis*) at 8 mg/mL. Correspondingly, the MIP% values increased significantly, reaching 75.70% and 86.86% for *C. albicans* and *C. parapsilosis*, respectively. It can be observed that *C. parapsilosis* appeared more susceptible, as evidenced by the higher inhibition percentages in all concentrations tested.

Capacity of the extracts to potentiate miconazole, percentage of mycelial growth (MGP%) and the percentage of mycelial inhibition (MIP%) is present in table 7.

The research revealed that the hydroethanolic and hydromethanolic extracts (101C/1–101C/4) showed limited and inconsistent potentiation effects of miconazole against both *C. albicans* and *C. parapsilosis*, as indicated by persistently high MGP%

values, reaching up to 254.19% and 240.59%, respectively. These values were accompanied by predominantly negative MIP% values, suggesting not only the absence of synergistic activity but also a potential antagonistic effect at higher concentrations. In contrast, the essential oils (101C/5 and 101C/6) demonstrated a pronounced, concentration-dependent potentiation effect. For *C. albicans*, MGP% values decreased significantly from 44.19% to 24.93% for 101C/5 as the concentration increased from 0.5 to 8 mg/mL, while MIP% increased correspondingly to 75.07%. A similar result was observed for *C. parapsilosis*, where the MGP percentage decreased to 27.53% and the MIP percentage reached 72.47% at the highest concentration tested. It was noted that the essential oil obtained by hydrodistillation (101C/5) consistently exhibited stronger potentiating activity compared to the essential oil obtained by steam distillation (101C/6), which exhibited delayed or weaker

synergistic effects, especially at lower concentrations. The stronger antifungal and miconazole-potentiating activity observed for the essential oil obtained by hydrodistillation may be associated with differences in extraction yield and the recovery of volatile bioactive compounds. Nevertheless, because no phytochemical characterization was conducted in the present study, these assumptions remain hypothetical. Additional phytochemical studies, particularly GC-MS profiling, are necessary to identify the compounds potentially involved in the observed biological activity. In addition, the slightly higher susceptibility of *C. parapsilosis* suggests species-specific responses to the combination treatment. These findings suggest a possible synergistic interaction between the essential oil constituents and miconazole, which may increase membrane permeability or interfere with fungal resistance mechanisms, improving antifungal efficacy.

Table 6.

MGP and MIP values according to different assays and extract samples

MGP%					MIP%				
Anti-Candida activity									
<i>C. albicans</i> : MGP%/NC					<i>C. albicans</i> : MIP%/NC				
μL/mL	Extracts				μL/mL	Extracts			
	101C/1	101C/2	101C/3	101C/4		101C/1	101C/2	101C/3	101C/4
10	160.40	127.54	97.48	61.03	10	-60.40	-27.54	2.52	38.97
15	163.37	133.03	121.24	53.56	15	-63.37	-33.03	-21.24	46.44
30	166.25	140.77	145.36	60.67	30	-66.25	-40.77	-45.36	39.33
45	173.45	150.32	220.70	84.97	45	-73.45	-50.32	-120.70	15.03
60	180.11	156.08	231.32	113.14	60	-80.11	-56.08	-131.32	-13.14
75	184.25	156.26	242.75	140.14	75	-84.25	-56.26	-142.75	-40.14
mg/mL	101C/5		101C/6		mg/mL	101C/5		101C/6	
0.5	118.18		83.71		0.5	-18.18		16.29	
0.7	47.43		73.81		0.7	52.57		26.19	
1	40.77		71.47		1	59.23		28.53	
2	39.06		61.12		2	60.94		38.88	
4	28.35		59.32		4	71.65		40.68	
8	24.30		55.18		8	75.70		44.82	
<i>C. parapsilosis</i> : MGP%/NC					<i>C. parapsilosis</i> : MIP%/NC				
10	145.13	113.32	101.52	55.41	10	-45.13	-13.32	-1.52	44.59
15	146.02	130.74	106.08	63.72	15	-46.02	-30.74	-6.08	36.28
30	148.70	137.98	177.48	71.05	30	-48.70	-37.98	-77.48	28.95
45	149.96	152.37	217.16	88.38	45	-49.96	-52.37	-117.16	11.62
60	150.13	158.00	237.53	130.21	60	-50.13	-58.00	-137.53	-30.21
75	164.70	165.06	251.12	165.15	75	-64.70	-65.06	-151.12	-65.15
mg/mL	101C/5		101C/6		mg/mL	101C/5		101C/6	
0,5	109.65		105.18		0,5	-9.65		-5.18	
0,7	45.67		98.39		0,7	54.33		1.61	
1	23.95		67.38		1	76.05		32.62	
2	21.98		58.18		2	78.02		41.82	
4	17.96		47.54		4	82.04		52.46	
8	13.14		40.57		8	86.86		59.43	

Note: MGP% = Percentage of mycelial growth; MIP% = percentage of mycelial inhibition 101C/1 = hydroethanolic extract (1:10); 101C/2 = hydromethanolic extract (1:10); 101C/3 = hydroethanolic extract (1:2); 101C/4 = hydromethanolic extract (1:2); 101C/5 = essential oil obtained by hydrodistillation; 101C/6 = essential oil obtained by steam distillation; NC = negative control (without miconazole).

Table 7.

MGP and MIP values according to different assays and extract samples

MGP%					MIP%				
Miconazole-potentiating capacity									
<i>C. albicans</i> : MGP%/NC					<i>C. albicans</i> : MIP%/NC				
$\mu\text{L/mL}$	Extracts				$\mu\text{L/mL}$	Extracts			
	101C/1	101C/2	101C/3	101C/4		101C/1	101C/2	101C/3	101C/4
10	152.39	101.44	87.94	59.68	10	-52.39	-1.44	12.06	40.32
15	154.37	139.24	100.90	59.86	15	-54.37	-39.24	-0.90	40.14
30	160.49	164.45	147.07	87.04	30	-60.49	-64.45	-47.07	12.96
45	163.19	172.46	221.51	91.99	45	-63.19	-72.46	-121.51	8.01
60	170.57	174.08	252.03	121.60	60	-70.57	-74.08	-152.03	-21.60
75	181.01	175.70	254.19	123.13	75	-81.01	-75.70	-154.19	-23.13
mg/mL	101C/5		101C/6		mg/mL	101C/5		101C/6	
0.5	44.19		127.99		0.5	55.81		-27.99	
0.7	44.10		125.83		0.7	55.90		-25.83	
1	39.87		114.58		1	60.13		-14.58	
2	34.56		85.87		2	65.44		14.13	
4	29.43		77.32		4	70.57		22.68	
8	24.93		52.66		8	75.07		47.34	
<i>C. parapsilosis</i> : MGP%/NC					<i>C. parapsilosis</i> : MIP%/NC				
10	145.10	115.82	87.62	59.33	10	-45.10	-15.82	12.38	40.67
15	153.22	130.46	108.95	63.60	15	-53.22	-30.46	-8.95	36.40
30	157.82	146.53	152.05	73.14	30	-57.82	-46.53	-52.05	26.86
45	160.75	162.59	217.32	78.16	45	-60.75	-62.59	-117.32	21.84
60	163.26	165.27	231.46	112.05	60	-63.26	-65.27	-131.46	-12.05
75	163.26	172.13	240.59	125.27	75	-63.26	-72.13	-140.59	-25.27
mg/mL	101C/5		101C/6		mg/mL	101C/5		101C/6	
0.5	105.94		91.30		0,5	55.94		8.70	
0.7	42.85		78.24		0,7	57.15		21.76	
1	38.16		62.93		1	61.84		37.07	
2	29.04		62.09		2	70.96		37.91	
4	27.62		38.74		4	72.38		61.26	
8	27.53		37.24		8	72.47		62.76	

Note: MGP% = Percentage of mycelial growth; MIP% = percentage of mycelial inhibition 101C/1 = hydroethanolic extract (1:10); 101C/2 = hydromethanolic extract (1:10); 101C/3 = hydroethanolic extract (1:2); 101C/4 = hydromethanolic extract (1:2); 101C/5 = essential oil obtained by hydrodistillation; 101C/6 = essential oil obtained by steam distillation; NC = negative control (without miconazole).

The results regarding the minimum inhibitory concentration (MIC) values are presented in Table 8. The differential antifungal efficacy of the tested *Xylopi* *aethiopic* extracts is further confirmed. The hydroethanolic and hydromethanolic extracts (101C/1–101C/3) did not show a detectable MIC against either *C. albicans* or *C. parapsilosis*, which is consistent with the negative MIP% values observed previously. In contrast, the hydromethanolic extract (101C/4) showed moderate antifungal activity, with MIC values of 15 $\mu\text{L mL}^{-1}$ for *C. albicans* and 10 $\mu\text{L mL}^{-1}$ for *C. parapsilosis*, although the inhibitory effect remained limited and unstable, as indicated by the decline phase at higher concentrations. The essential oils (101C/5 and 101C/6) demonstrated potent antifungal potential, with minimum inhibitory concentration (MIC) values exceeding 8 mg mL^{-1} and high inhibition percentages, reaching up to 75.70% for *C. albicans* and 86.86% for *C. parapsilosis*. A similar trend was observed for miconazole potentiation capacity, where only 101C/4 showed detectable MIC values (10 $\mu\text{L mL}^{-1}$), while the essential oils demonstrated substantial synergistic effects at higher concentrations, especially for 101C/5. These results highlight the superior efficacy of essential oils compared to solvent-based extracts and support their

potential role as adjuvant agents in antifungal therapy. The enhanced activity may be attributed to the presence of lipophilic bioactive compounds capable of disrupting fungal cell membranes and facilitating the absorption of antifungal drugs.

The *in vitro* microbiological evaluation demonstrated that the essential oils of *Xylopi* *aethiopic* possess significant and dose-dependent anti-*Candida* effect against *C. albicans* and *C. parapsilosis*. Among the hydroalcoholic extracts, the hydromethanolic (1:2) extract showed the most promising results, whereas other solvent extracts (1:10) and hydroethanolic (1:2) tended to promote yeast proliferation at the tested doses. Indeed, lower optical density (OD) values for sample, indicate reduced yeast (*Chryssanthou et al.*, 2006); biological or pharmacological activity is frequently influenced by the nature of the solvent and the concentration (Floares *et al.*, 2023). The increase in OD values observed at higher concentrations (for instance, from 1.782 to 2.047 for 101C/1) could be attributed to either the inherent opacity of the extract at high doses or a lack of marked inhibitory effect at the highest concentrations (CLSI, 2008). Indeed, *Xylopi* *aethiopic* extracts are highly colored and rich in polyphenols (Samba *et al.*, 2022).

Furthermore, it has been experimentally demonstrated that the seeds possess significant anthelmintic activity against *Nippostrongylus brasiliensis* (Suleiman *et al.*, 2005). Various extracts of *Xylopi*a *aethi*o*pica* have also shown promising results for use as an adjunct therapy in the management of sickle cell disease (Uwakwe, 2013). The essential oil extracts (101C/5 and 101C/6) were the most effective, showing a dose-dependent effect; with MIP (%) values reached 72.47% and 62.76%, respectively against *C. parapsilosis* and *C. albicans*, at a concentration of 8 mg/mL and with a MIC near 8 mg/mL, an inhibition rate of approximately 70%. Indeed, the Annonaceae family is renowned for oils rich in terpenoid compounds (such as germacrene or pinene) that alter the fungal membrane (Samba *et al.*, 2022), which confirms the inhibitory activity of *Xylopi*a *aethi*o*pica* essential oils observed in this study (MIP = 86.86% against *C. parapsilosis* at 8 mg/mL). These results prove that the essential oils, especially 101C/5, exert a synergistic effect on the anticandidal activity of miconazole. The sensitivity of *C. albicans* to *Xylopi*a *aethi*o*pica* essential oils is superior to that of *E. coli* and *E. faecalis*, whereas *P. aeruginosa* remained insensitive (Samba *et al.*, 2022).

No phytochemical profiling was performed in the present study; therefore, direct correlations between chemical composition and antifungal activity cannot be conclusively established. The stronger activity observed for the essential oils, particularly for the hydrodistilled oil, may be partially related to differences in extraction yield and the recovery of volatile lipophilic compounds. Nevertheless, the statistically superior antifungal and miconazole-potentiating effects observed for the essential oils compared to the hydroalcoholic extracts suggest that volatile constituents may play a major role in the anti-*Candida* activity of *Xylopi*a *aethi*o*pica*. In contrast, most hydroalcoholic extracts showed limited inhibitory activity and, at higher concentrations, even promoted fungal growth, possibly due to the presence of non-volatile constituents or extract turbidity interfering with OD measurements. Consequently, the present study primarily demonstrates the biological potential of *Xylopi*a *aethi*o*pica* extracts, while the exact phytochemical determinants underlying these effects remain to be elucidated.

Minimum Inhibitory Concentrations (MICs)

Table 8.

MIC values of the various *Xylopi*a *aethi*o*pica* extract samples

Anti- <i>Candida</i> effect			
Extracts	Strains	MIC	Hight MIP %
101C/1, 101C/2 and 101C/3 101C/4	<i>C. albicans</i>	(-)	MIP% negatives
	<i>C. parapsilosis</i>	(-)	
	<i>C. albicans</i>	15 µLmL ⁻¹	
101C/5	<i>C. parapsilosis</i>	10 µLmL ⁻¹	46, 44 (Before the decline phase or the reverse)
	<i>C. albicans</i>	> 8 mgmL ⁻¹	44,59 (Before the decline phase or the reverse)
101C/6	<i>C. albicans</i>	> 8 mgmL ⁻¹	75,70
	<i>C. parapsilosis</i>	> 8 mgmL ⁻¹	86,86
101C/6	<i>C. albicans</i>	> 8 mgmL ⁻¹	44,82
	<i>C. parapsilosis</i>	> 8 mgmL ⁻¹	59,43
Miconazole-potentiating capacity			
101C/1, 101C/2 and 101C/3 101C/4	<i>C. albicans</i>	(-)	MIP negatives
	<i>C. parapsilosis</i>	(-)	
	<i>C. albicans</i>	10 µLmL ⁻¹	
101C/5	<i>C. parapsilosis</i>	10 µLmL ⁻¹	40, 32 (Before the decline phase or the reverse)
	<i>C. albicans</i>	> 8 mgmL ⁻¹	40,67 (Before the decline phase or the reverse)
101C/6	<i>C. albicans</i>	> 8 mgmL ⁻¹	75,07
	<i>C. parapsilosis</i>	> 8 mgmL ⁻¹	72,47
101C/6	<i>C. albicans</i>	> 8 mgmL ⁻¹	47,34
	<i>C. parapsilosis</i>	> 8 mgmL ⁻¹	62,76

Note. (-): MIC not detected. MIC: minimum inhibitory concentration. MIP: mycelial inhibition percentage. 101C/1 = hydroethanolic extract (1:10); 101C/2 = hydromethanolic extract (1:10); 101C/3 = hydroethanolic extract (1:2); 101C/4 = hydromethanolic extract (1:2); 101C/5 = essential oil obtained by hydrodistillation; 101C/6 = essential oil obtained by steam distillation.

CONCLUSION

This study highlights the ethnopharmacological importance and the microbiological potential of *Xylopi*a *aethi*o*pica* fruits within the Togolese flora. The ethnopharmacological survey conducted in the Central Region of Togo confirmed the extensive use of this plant, particularly in the management of gynecological, obstetrical (90.14%) and asthenic conditions. Decoction

and maceration emerged as the primary traditional preparation methods, with oral administration being the most frequent route (65.02%). The *in vitro* microbiological evaluation demonstrated that the essential oils of *Xylopi*a *aethi*o*pica* possess significant and dose-dependent anti-*Candida* effect against *Candida albicans* and *Candida parapsilosis*. Among the hydroalcoholic extracts, the hydromethanolic (1:2)

extract showed the most promising results. The essential oil extracts exhibited significant and dose-dependent anti-*Candida* activity, with a MIC near 8 mg/mL and an inhibition rate of approximately 70%. Consequently, *Xylopiya aethiopyca* essential oils possess therapeutic potential against candidiasis and represent the most bioactive forms. Moreover, the essential oils, especially the one obtained by hydrodistillation, exhibited a synergistic capacity to potentiate the effect of miconazole, suggesting their potential as adjunct therapies in the treatment of candidiasis. These anticandidal findings may partially support the traditional use of *Xylopiya aethiopyca* in gynecological and obstetrical conditions associated with microbial infections. These findings corroborate the traditional use of *Xylopiya aethiopyca* in the management of microbial and parasitic infections. Further studies are required to identify the secondary metabolites or the specific molecules responsible for this antifungal activity, synergy and to evaluate their safety through *in vivo* models.

AUTHORS CONTRIBUTIONS

Conceptualization: T.K., P.K., D.F., D.O. and I.I.; methodology: T.K., D.F., D.O., and A.H.; Data collection: T.K., P.K., D.F., D.O. and A.H. Data validation was conducted by D.F., D.O., A.H., I.I., and A.D. Data processing: T.K., P.K., and B.B. The original draft: T.K., D.O. and P.K., while review and editing were performed by T.K., P.K., D.F., D.O., A.H., A.D., B.K., C.S. and I.I.

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CONFLICT OF INTEREST

The authors have not any competing financial, professional, or personal interests from other parties. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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