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Comprehensive Evaluation of Antifungal Activity of *Argania spinosa* (L.) Skeels Kernels of the Hot and Cold Extraction and the Effect of Solvents Varying Polarities and the Heat Treatment on Yield and Activity Variance

Aziza Lfitat[1*], Imad Ed-dahmani[1], Fatima Zohra Bousraf[1], Abdelhaq Belhaj[2], Tarik Ainane[3], Mustapha Taleb[1], Abdelkader Gourch[1], Abdelfattah Abdellaoui[1]

[1]Laboratory of Engineering, Organometallic, Molecular Materials and Environment (LIMOME), Department of Chemistry, Faculty of Sciences, Sidi Mohamed Ben Abdellah University, P.O. Box 1796 (Atlas), 30000 Fez, Morocco.
[2]Laboratory of Environment, Ecology, and Health, Department of Biology, Faculty of Sciences, Moulay Ismail University, B.P. 11201 Zitoune, Meknes, Morocco.
[3]Superior School of Technology, University of Sultan Moulay Slimane, B. P. 170, 54000 Khenifra, Morocco.
*Corresponding author: lfitat.aziza@gmail.com

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ABSTRACT: A comparative antifungal efficacy study of argan kernels was performed using different solvent fractions obtained by the two extraction methods: cold extraction by maceration at room temperature (1) and hot extraction by Soxhlet apparatus (2). Results demonstrated that the two extraction methods induce different yields among each solvent used. Maceration promoted the maximum contents of crude extract in hexane (42.15%), diethyl ether (2.59%), dichloromethane (4.22%), and ethyl acetate (2.52%). Whereas the hot extraction yielded higher extractives in the case of methanol (22.97%) and water (5.88%) compared to cold extraction. The MIC values of the fractionated extracts obtained by maceration were in the range of 0.051 ± 0.002 and 0.223 ± 0.005 g/ml, whilst they were in the range of 0.101 ± 0.002 and 0.286 ± 0.005 g/ml for the Soxhlet extracts. According to total activity (TA) interpretation, dichloromethane was the most effective solvent to extract active antifungal components, revealing the strongest ability to suppress the growth of fungi at much lower MIC values (0.051 ± 0.002 to 0.156 ± 0.005 g/ml) than the rest of fractions. The effect of heating resulted in an increase of the MICs values of the Soxhlet extracts from 10 to 25 times higher than macerated ones resulting in lower antifungal activity.

Keywords: argan kernel; *Aspergillus fumigatus*; *Aspergillus niger*; *Plasmodium brasilianum*; *Fusarium dimerium*.

INTRODUCTION

Argania spinosa (L.) Skeels (syn. Argania syderoxylon L., Sideroxylon spinosum L.), is a multipurpose perennial tree (*Sapotaceae*) endemic to Morocco, mainly located in the southwest region (Bani-Aameur and Ferradous, 2001) and globally known for its production of a high-quality oil used for both cosmetic and culinary purposes (Metougui *et al*., 2017). The argan tree constitutes an essential element of the biodiversity of agroforestry ecosystems in Morocco (Ait Aabd *et al*., 2019). For the southwestern Moroccan population, argan production (cosmetic oil, edible oil, and byproducts) is a pivotal leverage for building sustainable social, economic, and human development (Kharbach *et al*., 2020).

Despite the fact that synthetic drugs have been actively involved in recent decades in health treatments, replacing traditional medicine, medicinal plants are still widely used because their safe and proven beneficial by virtue of their dynamic constituents named phytochemicals that are the basis of many modern pharmaceuticals we use today (Qadir and Ahmad, 2017; Jasuja *et al*., 2013; Chhetri *et al*., 2008). Recently, the demand for natural products has continuously increased, and so is people's returning to completely natural and plant-based recipes (Choi *et al*., 2019), putting forward the view that more plants and plants-by-products need to undergo screening for phytochemical and biological activities as they remain a major source of new drugs discovery and to achieve positive and safe therapeutic results.

The kingdom of Fungi represents a large proportion of the genetic diversity on Earth (Peay *et al*., 2016). Many global bio-diverse fungal species are an increasing cause of severe disease and mortality (Nicoletti and White, 2022). With the largely increasing microbial infections to a great extent and with the resistance against antibiotics becoming an ever-increasing therapeutic problem, fungal infection has become a significant event leading to over 1.5 million deaths annually worldwide (Venkatesan and Karrunakaran, 2010; Jasuja *et al*., 2013; Deaguero *et al*., 2020). The impact of mycoses has increased across the globe due to the increased morbidity and mortality caused by invasive fungal infections where the situation is getting more alarming, especially in patients with immunodeficiency disorders who have undergone transplant surgery,

chemoradiotherapy, hemodialysis, or the treatment with immunosuppressive agents (Drgona *et al.*, 2014).

Also, many pathogenic fungi cause diseases in fruits and vegetables, leading to crop spoilage and high economic loss annually. (Al-Zubaidi *et al.*, 2019).

Due to the increased resistance of many microorganisms towards established antimicrobials, several scientific studies have been carried out on extracts and active principles isolated from plants and plants byproducts, as there is a tremendous need for novel antifungals from different sources (Karaalp, 2009). The emergence of drug-resistant or multidrug-resistant waves has led to a challenge in treatment due to the limitations of the classes of synthetic drugs available (Zhang *et al.*, 2021). Hence, there is an urgent need to find a lasting solution to the problem of drug resistance, resulting in the emergence of alternative medicine as a possible option for improving the current situation (Laxminarayan *et al*., 2013).

The argan tree is considered, above all, an oilseed and fodder tree. Yet, the argan kernel is still the most important component of the argan fruit for its richness in its well-known valuable oil, which only accounts for about 3% of the fruit's mass (El Alaoui, 1999). As argan oil production results in huge quantities of extrusion paste (press-cake), we considered valorizing it as rich in biologically active components by revealing its antimicrobial activity. Enlightening and encouraging researchers to explore new and favorable characteristics of argan kernel byproduct (press-cake), finding ways to capitalize on the expansion opportunities and creative ways to repurpose argan dough.

Currently, the argan production sector is maintained mainly by women's cooperatives in Morocco using semi-industrial mechanical extraction and production processes. Many of the products (edible oil, cosmetic oil, creams, soaps, etc.) are manufactured and marketed nationally by Moroccan distributors and internationally exported through different companies in Europe and North America that distribute the products (with a predominance of bulk). In comparison, for the present, there are rare to absent Indonesian distributors or commercial product developers interested in incorporating argan products (kernels, oil) or byproducts (press-cake, shells) in their production or merchandising plan.

The present work aimed to gain insight into the antifungal activity of argan kernel extracts. The experiment involves the extraction of bioactive molecules of antifungal activities from the argan kernels with various solvents using conventional low-pressure standard methods (maceration and Soxhlet) for extraction. Maceration and Soxhlet are conventional methods that have widely been used in laboratories and industry in a wide variety of official methods (Palmieri *et al.*, 2020). As they are one of the traditionally and practically relevant techniques for their simplicity, inexpensiveness, and ease of handling, as well as when it comes to their environmentally friendly aspect, they are the target (Sridhar *et al.*, 2021). Cold maceration is a simple procedure that does not require any special equipment (apparatus) and consistently results in a non-degradation of the thermolabile compounds present in the fraction due to the low extraction temperature, similar to cold pressing (Sankeshwari *et al.*, 2018). On the other hand, hot Soxhlet extraction has the advantage of the fresh solvent being constantly in contact with plant particles and using high temperatures, which leads to increased solubility of low-soluble compounds at low temperatures (Farahani, 2021). Now, it is often used as a reference method for comparing the yield of an advanced extraction technique (Ngamwonglumlert *et al.*, 2017).

The objective of the present work was to explore and compare the antimicrobial properties of argan kernels fractionated extracts against some important fungal strains (*Aspergillus fumigatus*, *Aspergillus niger*, *Plasmodium brasilianum*, *Fusarium dimerum*), and investigate the heating effect on the antifungal potential of the antifungal agents present in the fractions by accentuating the effect of the heating during the extraction process on the variation of the activity. This is the first-ever study on *Argania spinosa* (L.) Skeels kernel extracts tested against a collection of certified pathogens for antifungal activity.

MATERIALS AND METHOD

Chemicals and reagents

Solvents used for extraction and fractionation were of HPLC grade. Sabouraud dextrose agar and Malt extract were purchased from Sigma-Aldrich (St. Louis, MO, US). All the other chemicals used were of analytical grade.

Preparation of plant material

Ripe and manually peeled *Argania spinosa* (L.) Skeels (var. apiculata maire) fruits collected from the Agadir argan forest by an indigenous inhabitant of the forest population who has the « right to use » of this natural resource; afforded argan nuts whose shell was manually broken to obtain kernels. Then, the kernels (almonds) were ground by an electric blender to obtain a paste-like material.

Preparation of extracts (bioassay-guided fractionation)

Exhaustive maceration extraction

Ground argan kernels (100 g of paste) collected from fully ripe fruits, placed in a 0.5 L reagent bottle (media bottle), and soaked in 200 ml of hexane as a first extraction, as shown in Figure 1. Then, a successive extraction, as shown in flow chart N°1 illustrates the experimental design in detail, describing the procedures of extracting bioactive substances from argan paste and filter cake (residue) each time with 200 ml of diethyl ether,

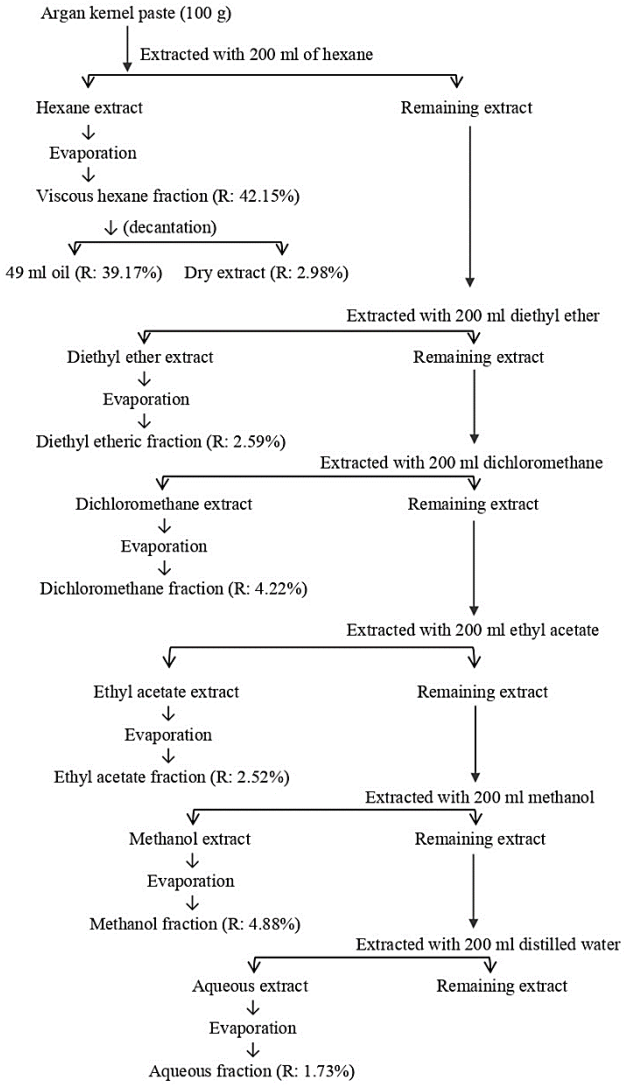

Figure 1. Extraction procedure of argan kernel paste by maceration.

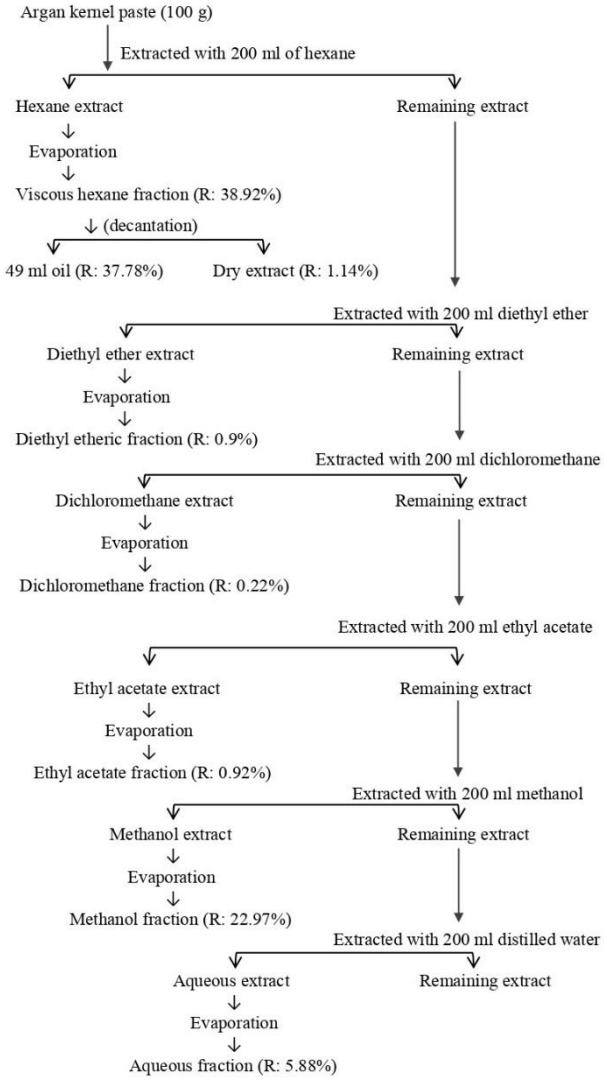

Figure 2. Extraction procedure of argan kernel paste by Soxhlet extractor.

dichloromethane, ethyl acetate, methanol, and distilled water. The extraction was carried out in the dark by covering the media bottle and at room temperature for 48h for high material depletion where the stirring was applied. Each time, the liquid extract was separated from the solid residue by filtration through Whatman paper N°1. The final extracts were concentrated (supernatant evaporation) by removing solvents under reduced pressure on a rotary vacuum evaporator at 40 °C to obtain concentrated extracts. The crude extracts obtained were kept in sterile tubes and stored in the refrigerator at 4 °C until further use in antifungal activity testing.

Exhaustive soxhlet extraction

Exhaustive Soxhlet extraction (Figure 2) was performed using classical apparatus for argan seed paste (dough), precisely weighing as much as 100 g. The kernels collected from fully ripe fruits were packed in a cellulose cartridge (extraction thimble), placed in the extraction tube of a Soxhlet apparatus, and extracted with 200 ml of analytical grade solvents successively (hexane, diethyl ether, dichloromethane, ethyl acetate, methanol, and distilled water for 48 h, respectively). The extracts were concentrated to dryness using a rotary evaporator, and the crude extracts were stored in an airtight container under refrigerated conditions (4 °C) until further use in antifungal activity testing.

Yield

Under constant vacuum agitation, the extracts were evaporated to dryness and stored in cold until used. The extraction yield was expressed as:

$$Percentage\ yield\ (\%) = \frac{weight\ of\ crude\ obtained\ (g)}{weight\ of\ the\ sample\ used\ for\ the\ extraction\ (g)} \times 100$$

Antifungal susceptibility testing

The *in vitro* antifungal testing of the hexane, diethyl ether, dichloromethane, ethyl acetate, methanol, and the aqueous fractions was determined by the microdilution wells method in terms of minimum inhibitory concentrations (MIC). It was performed according to the

most widely used standard, the 'Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi' published by Clinical Laboratory Standard Institute CLSI (Barbara, 2017), replacing the selective RPMI 1640 (RPMI 2%G) as assay medium with Sabouraud dextrose agar growth medium.

The broth dilution method for MICs calculation was carried out against four pathogenic fungi:
Aspergillus fumigatus 175 134 378-02 (Laboratory of Mycology, CHU-Angers, France).
Plasmodium brasilianum (Laboratory of Plant Biotechnology and Molecular Biology).
Fusarium dimerum 1700 61011-02 (Laboratory of Mycology, CHU-Angers, France).
Aspergillus niger M101 (CNRT-Morocco).

Preparation of inoculum
Stock inoculum suspensions were prepared from 7-day-old cultures as described in the NCCLS M38-P document, grown in non-selective nutritive agar medium (Sabouraud's dextrose agar SDA, pH 5.7) at 35 °C, in physiologic sterile water (NaCl 0.9%). The inoculum suspension was done by vigorous shaking on a vortex mixer for 15 seconds, and the cell density was adjusted to the density of 0.8–0.11 by measuring the absorbance in a spectrophotometer at a wavelength of 530 nm. This will give fungi suspension of 0.4–5×10^4 CFU/ml.

Minimum inhibitory concentration
The MIC was the lowest drug concentration that prevented any discernible growth of a tasted microorganism in the microdilution wells by the microdilution broth assay (Schwalbe *et al.*, 2007). The tested extracts were dissolved in the nutritive medium to disperse the compounds without adding solvent or detergent (Remmal *et al*., 1993). Each well (from the 2nd to the 12th) of a microtitration tray contained 100 µl of Malt extract broth. For the antimicrobial agent serial dilution (1:1) through a sterile Malt extract broth, 200 µl of a known concentration of the tested extract (fraction) was added to the first well and 100 µl of it was diluted to the second well. Then 100 µl of the second is diluted to the third, and so on. Thereafter, wells were inoculated with 100 µl of 0.4–5×10^4 CFU/ml conidial suspension. The growth control wells contained 100 µl of sterile drug-free medium and were inoculated with 100 µl of the same inoculum suspension. The microtitration plate is incubated static and in a humid atmosphere at 35 °C for 48h. The experiment was performed in triplicates under aseptic conditions. At the end of the incubation period, endpoints were read visually. The MIC value is a no-growth visual endpoint. Each extracted fraction (antimicrobial agent) is diluted (1:1) several times through a sterile Malt broth. After the antimicrobial agent has been diluted, a volume of the standardized inoculum equal to the volume of the diluted antimicrobial agent is added to the wells.

Total activity (TA) determination
The total activity of extracts measures the number of antifungal compounds present in a sample. It indicates the volume to which the active compounds extracted from 1 g of primary argan kernel paste can be diluted and still inhibit the growth or kill the microorganism (Eloff, 1999). The total activity is calculated by dividing the number of compounds or crude material extracted from 1 g of argan kernel paste in milligrams (of each specific extraction solvent) by the MIC value in mg/ml of the same test extract (Mahlo *et al.*, 2016), expressed as follows:

Statistical analysis
All analyses were carried out in triplicate, and the result was expressed as arithmetic mean ± standard deviation (SD). The statistical analysis was performed using the statistical package GraphPad Prism 5.03 and by using a one-way ANOVA analysis of variance. Means that were statistically significantly different ($p < 0.05$) were marked with different alphabetical letters.

RESULT AND DISCUSSION

It is widely accepted that fungal pathogens enormously influence plant and animal life (Brown *et al.*, 2012). Some of these fungi have been used for the good of humankind (Cairns *et al*., 2018; Spagnuolo *et al*., 2019). Conversely, a recent report detailed these pathogens' extraordinary and frightening impact on species extinctions, food security, and ecosystem disturbances (Fisher *et al.*, 2012; Brown *et al.*, 2012). Recent studies estimate that fungal infections kill more than 1.6 million people yearly (Bongomin *et al*., 2017). Although these infections contribute substantially to human morbidity and mortality, the impact of these diseases on human health is not widely appreciated (Brown *et al*., 2012). The development of new fungicidal drugs is required to augment the effectiveness of current chemotherapy and counter increasing resistance to pathogens (Nicoletti and White, 2022).

The practices of traditional and complementary medicine play a substantial role in human remedy in world regions of a weak economy. Recently, in economically developed countries, people have returned to alternative medicine based on natural products due to its efficacy in treating and preventing diseases without or with only minor or negligible side effects. The growing international demand for natural medicine (herbs and preparations of botanical origin like dietary supplements or drugs), as a source of health care, has driven an exponential increase in encouraging the bio-metabolites discovery and recovery from natural sources or naturally derived byproducts and environmentally friendly renewable resources of immense benefits socially and environmentally, through sustaining a circular economy, maximizing recycling, and minimizing waste (Sagar *et al.*, 2018; Szabo *et al.*, 2022).

Regarding our study subject, although studies are available in the literature on some of the biological

activities and the potential in dermo-cosmetics that the cosmetic and the alimentary argan oil plus its press-cake have (Guillaume and Charrouf, 2011; Mechqoq *et al*., 2021), no information is currently available on its antifungal potential. With one of the primary goals of the present study being the *in vitro* demonstration of argan kernels' antifungal activity and their crude extracts' approximate antifungal efficacy, we selected four fungal species of the varied *Aspergillus*, *Plasmodium*, and *Fusarium* genera for this study.

It may be interesting to consider the fungi genera's immense genetic and biochemical diversity. According to scientific study reports, the *Aspergillus* genus comprises over 200 species of filamentous fungi (Christensen and Tuthill, 1986). Though normally harmless, several *Aspergillus* species have been described as opportunistic pathogens capable of causing invasive aspergillosis, a severe condition characterized by the germination of spores, growth, and penetration of fungal hyphae into host tissues (Koch *et al*., 2019). Amidst the outstanding opportunistically pathogenic filamentous fungi of the *Aspergillus* genus, *Aspergillus fumigatus* counted as a drastically more prevalent cause of infection than other fungi species of the same genus, along with being a dominating causative agent in invasive aspergillosis with four species (*A. niger*, *A. flavus*, *A. tereus*, and *A. nidulans*) accounting for almost all remaining incidences (Koch *et al*., 2019; O'Gorman, 2011).

Aspergillus fumigatus is a globally distributed fungus that is both an environmental saprobe and an opportunistic human fungal pathogen (Barber *et al*., 2020). It is a common airborne fungal pathogen, with a mortality rate between 15% and > 90%, estimated to be responsible for over half a million deaths per year worldwide (Brown *et al*., 2012). Along similar lines, one of the most life-threatening opportunistic human pathogens from the *Aspergillus* genus species is the filamentous ascomycete fungus *Aspergillus niger* (Andersen *et al*., 2011). A cosmopolitan representative of microscopic filamentous fungi exhibiting a great diversity in its phenotype found globally both as marine and terrestrial strains, it's a strong air pollutant (Pawar and Thaker, 2006; Andersen *et al*., 2011). Stated to be responsible for important seed and soil-borne diseases (Kumari and Singh, 2017), attacking varied types of crops, causing various contaminations and rot diseases for fruits and vegetables, causing heavy losses in yield (Parveen *et al*., 2014). Another important and related fact to the latter is that *A. niger* is a widely used strain in a broad range of industrial processes from food to the pharmaceutical industry, as being a prolific secretor of organic acids, proteins, enzymes, and secondary metabolites (Cairns *et al*., 2018; Yu *et al*., 2021). It may give it advantages, but we cannot ignore that it is a serious pathogenic microorganism.

Like other pathogens, *Plasmodium brasilianum* is a parasite that infects many platyrrhine monkeys in South and Central America (Ramasamy, 2014). In South America, it was first described in monkeys at the beginning of the 20th century and has now been documented in approximately 31 species of New World monkeys (Lourenço-de-Oliveira and Deane, 1995; Alvarenga *et al*., 2017). The simian *Plasmodium brasilianum* causes quartan fever in New World monkeys (Lalremruata *et al*., 2015). Even so, no naturally acquired infections with *Plasmodium brasilianum* parasites has detected in humans until now, non-human primates as a source of Plasmodium infections in humans have received increased attention, fearing the occurrence of a host transfer between humans and primates (Escalante *et al*., 1995; Qari *et al*., 1996; Cormier, 2010; Guimarães *et al*., 2012; Ramasamy, 2014; Lalremruata *et al*., 2015).

The strain of *Fusarium dimerum* is a filamentous mold that belongs to the genus *Fusarium*, a relatively rare plant pathogen and a very rare opportunistic fungus that causes infections in immunocompromised patients, much like other *Fusarium* species (Schroers *et al*., 2009). It can be acquired via inhalation or through skin dehiscence and carries a high risk of dissemination in burn infections (Khalid *et al*., 2021; Salah *et al*., 2015). *F. dimerum* is one of the species identified in keratitis associated with traumatic lesions of the cornea. In fact, one of the main concerns regarding *Fusarium* species is that they are among the most resistant to antifungal agents (do Carmo *et al*., 2016).

Recently, as the term 'argan' world widely became related to the great argan oil extracted from the fruits of the *Argania spinosa* L. (Skeels) tree, the latter became the only main interest for this tree plant in the international market (Khallouki *et al*., 2003). Argan oil is recognized for its high nutritional value, constituting a good source of essential fatty acids and minor compounds like tocopherols (Harhar *et al*., 2011). As presently well known, there are two types of argan oil, cosmetic and alimentary argan oils, that are prepared from unroasted and roasted argan seeds, respectively (Ahansal *et al*., 2008). The extraction processes of edible argan oil by the traditional and press techniques are based on a previous thermal treatment (direct heat) of argan kernels before extraction, while the cosmetic oil is obtained from unroasted kernels (Charrouf and Guillaume, 2014). Roasting is a unit operation very important for the extraction of edible argan oils, as it increases oil yield and allows the development of its specific organoleptic properties such as flavors, aromas, and color (Matthäus *et al*., 2010).

In the traditional extraction, argan kernels are air-dried in clay containers and slowly roasted where the roasting temperature is generally maintained at 110 °C ± 5 °C for 30 to 50 min (Khallouki *et al*., 2003; Matthäus *et al*.,

2010). Usually carried out in clay containers over a wood fire or in rotating oven-applying gas burners (Charrouf and Guillaume, 2014). After that, the roasted kernels are crushed and kneaded into a paste or dough with hot water. The resulting oil/water mixture is separated, furnishing a brown oil with a hazelnut taste. This is termed 'food' or alimentary argan oil, which is used for culinary purposes. An 'aesthetic' variety of argan oil is produced without roasting the kernels for cosmetic purposes and this latter is known as the cosmetic argan oil (Khallouki *et al*., 2003).

There are two types of press-cake produced from argan oil, depending on whether the kernels were roasted or not. (Atifi *et al*., 2019). To conduct a comprehensive evaluation of the effect of the heating on the antifungal performance of argan kernel fractions, bioactive compounds were extracted from argan kernels using two different procedures: cold extraction using maceration and hot extraction using the Soxhlet apparatus. Under almost similar operating conditions, materialized in a similar quantity of the starting plant material that worked with (100 g of argan kernel paste) and similar extraction periods (48h). The only difference is manifested in the temperature maintained at 65 °C (reflux) for the first extraction method and ambient for the second. In effect, exhaustive Soxhlet extraction is usually applied to analytes that are sufficiently thermally stable to avoid thermal degradation.

Based on the literature, the solubility of plant material phytoconstituents depends on the polarity of the extraction medium used, which might result in a difference in extract responses according to the compounds being extracted (Baig *et al*., 2021). Therefore, the step of choosing the solvent for extraction is critical. As Masoko *et al*. (2005) stated, the type of solvent used in the extraction procedure determines the success of isolation of compounds from plant material. Therefore, to extract all compounds, it is important to extract using different solvents of varying polarity to cover the polarity range. Being primordial objective of the extraction process in the present study is to maximize the number of the targeted compounds we are willing to obtain for the fractions to perform the highest biological activity by depleting the primary material. The following solvents have been selected (hexane, diethyl ether, dichloromethane, ethyl acetate, methanol, and water) based on their polarity, seeing that they covered a wide range of polarities (polar, semi-polar, and non-polar) for successive extraction allowing the fractionation of the substances.

Extraction yield

The extraction yield is a measure of the solvent efficiency in extracting specific components from the original material. It gives an idea about the extractability of the plant material studied under different conditions (Adam *et al*., 2019). As cited and remarked in Table 1, extraction yields differed among the extracted fractions depending on the solvents' polarity. The non-polar and polar solvents obtained more yields than the semi-polar ones. Taken altogether, our results indicate that hexane solvent produced the highest extraction yield of (42.15% w/w) in maceration and (38.92% w/w) in Soxhlet extraction, which suggested that most metabolites were highly non-polar fatty acids along with other secondary metabolites in minor quantity. The production yield was higher during maceration extraction for nonpolar to moderately polar solvents (diethyl ether, dichloromethane, ethyl acetate), except for polar ones (methanol and aqueous fractions), which were lower than the fraction obtained by Soxhlet extraction by the same solvents.

Table 1. The extraction yield of argan kernels fraction was obtained by maceration and Soxhlet apparatus.

Fraction	Extraction Technique	Yield (w/w) %
Hexane	Maceration	42.15
	Soxhlet	38.92
Diethyl ether	Maceration	2.59
	Soxhlet	0.9
Dichloromethane	Maceration	4.22
	Soxhlet	0.22
Ethyl acetate	Maceration	2.52
	Soxhlet	0.92
Methanol	Maceration	4.88
	Soxhlet	22.97
Aqueous	Maceration	1.73
	Soxhlet	5.88

Diethyl ether, dichloromethane, and ethyl acetate extraction yielded much higher (2.59%, 4.22%, and 2.52% w/w, respectively) in maceration than Soxhlet method (0.9%, 0.22%, and 0.92% w/w, respectively). The thing that can be explained by, in maceration, these specific latter solvents induce extraction recovery and the extraction selectivity in socking more than the Soxhlet method; whereabouts, largely material being in contact with solvent allows for enough contact between argan paste and these solvents maximizing their yield.

Table 2. Antifungal activity expressed as MIC values in g/ml of different fractions of argan kernels against all test pathogens, obtained by the agar dilution method.

Extraction Technique	Fraction	MIC values in (g/ml)			
		A. fumigatus	*P. brasilianum*	*F. dimerum*	*A. niger*
Maceration	Hexane	-	-	-	-
	Diethyl ether	0.106 ± 0.005^{c}	0.103 ± 0.005^{c}	0.106 ± 0.002^{c}	0.211 ± 0.002^{c}
	Dichloromethane	0.066 ± 0.002^{a}	0.051 ± 0.002^{a}	0.063 ± 0.002^{a}	0.123 ± 0.005^{a}
	Ethyl acetate	0.133 ± 0.005^{d}	0.126 ± 0.005^{d}	0.133 ± 0.005^{d}	0.223 ± 0.005^{d}
	Methanol	0.076 ± 0.005^{b}	0.073 ± 0.005^{b}	0.070 ± 0.010^{b}	0.143 ± 0.005^{b}
	Aqueous	0.146 ± 0.005^{e}	0.136 ± 0.005^{e}	0.133 ± 0.005^{e}	0.223 ± 0.005^{e}
Soxhlet	Hexane	-	-	-	-
	Diethyl ether	0.150 ± 0.010^{b}	0.143 ± 0.005^{b}	0.143 ± 0.005^{b}	0.246 ± 0.005^{c}
	Dichloromethane	0.105 ± 0.005^{a}	0.101 ± 0.002^{a}	0.105 ± 0.005^{a}	0.156 ± 0.005^{a}
	Ethyl acetate	0.193 ± 0.005^{d}	0.193 ± 0.005^{d}	0.193 ± 0.005^{d}	0.256 ± 0.005^{d}
	Methanol	0.166 ± 0.005^{c}	0.163 ± 0.005^{c}	0.163 ± 0.005^{c}	0.223 ± 0.005^{b}
	Aqueous	0.246 ± 0.005^{e}	0.243 ± 0.005^{e}	0.243 ± 0.005^{e}	0.286 ± 0.005^{e}

Note: The results of MIC values are the mean of three replicates. Values in the column followed by a different letter superscript (a, b) are significantly different ($p < 0.05$).

Of the six solvents of different polarities in regards to the two extraction methods used, hexane in maceration was the best extractant, giving the highest yield of (42.15% w/w) of plant substance apropos the initial plant material subjected for extraction. Whereas dichloromethane in the Soxhlet apparatus yielded the least extractives of all the obtained fractions (0.22% w/w). The yield differences are related importantly to the different dissolving powers of different solvents since the compatibility of components with solvent systems and extraction methods will affect the outcome of the content of secondary metabolites that can be extracted (Haslina and Eva, 2017).

Microplates of serially diluted extracts were carried out to determine the extracts' MICs and how potentially effective the antimicrobial agents from argan kernels could be on 48-h-growing fungi, allowing the direct comparison of the antimicrobial effect(s) between extracts. As shown in (Figure 4), white spots on the plate indicate inhibition of growth, and colored spots indicate that organisms are active and resistant at that concentration. Microbial growth of *Aspergillus fumigatus* is indicated by blue-green color, *Plasmodium brasilianum* and *Fusarium dimerum* in gray-green, and *Aspergillus niger* growth in black color.

In general, the results obtained using the broth dilution technique to test extracts' fungal growth inhibition capacity showed good to reasonable inhibitory activity, suggesting that argan kernels display antifungal properties. Five out of six solvent fractions obtained from argan kernels extraction proved the accusation of antifungal activity, the fraction type that gave the highest activity in cold maceration was dichloromethane. The second in the ranking was that obtained by ethanolic fractionation. Whilst, in the hot Soxhlet extraction technique, dichloromethane was first in rank, followed by diethyl ether. As shown in (Table 2), concerning the macerated fractions, dichloromethane exhibited the highest antifungal activity with MICs ranging between 0.051–0.123 g/ml, followed by methanol fraction with MICs ranging between 0.070–0.143 g/ml; diethyl ether fraction ranging between 0.103–0.211 g/ml; ethyl acetate fraction between 0.126–0.223 g/ml; and the aqueous fraction where MIC values were between 0.133–0.223 g/ml.

In hot Soxhlet extraction, the dichloromethane fraction performed the most potent activity based on the obtained MICs, where the ranging MIC values were 0.101–0.156 g/ml. Soxhlet-diethyl ether fraction ranked second in fungal growth inhibition for only three of the four fungal strains tested (*A. fumigatus*, *P. brasilianum*, and *F.*

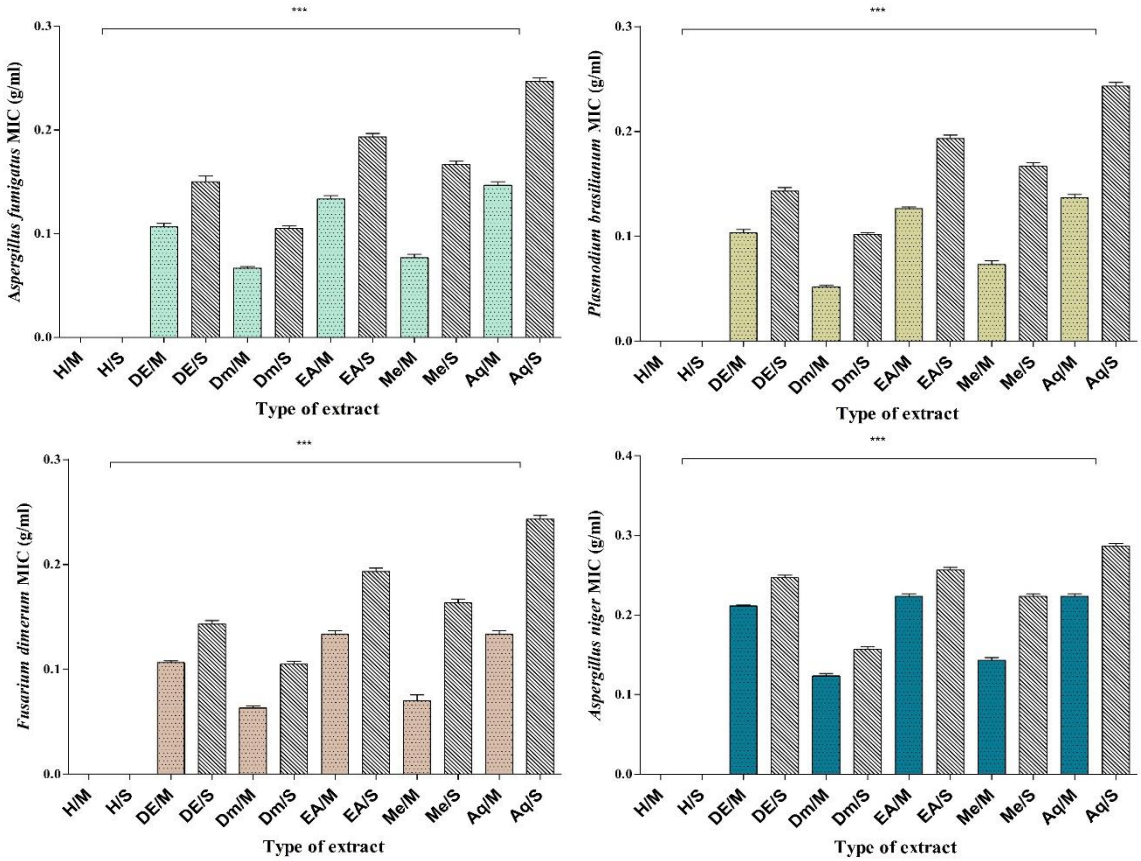

Figure 3. Average MIC values of the six-argan kernel extracts against the four test fungi; the lower the MIC values the most potent the extract. H = Hexane, DE = Diethyl ether, Dm = Dichloromethane, EA = Ethyl acetate, Me = Methanol, Aq = Aqueous, M = Maceration, S = Soxhlet.

dimerum). For *A. niger* growth inhibition, the methanolic fraction ranking second was interestingly different from the previous. Followed in activity in this order of methanol>ethyl acetate>water, for *A. fumigatus*, *P. brasilianum*, *F. dimerum*. Meanwhile, it was in the order of diethyl ether>ethyl acetate>water, for *A. niger*.

Against the four fungi strains and regardless of the effect of heat, aqueous fractions exhibited the lowest MIC values between 0.133 and 0.223 g/ml for cold macerated and between 0.243 and 0.286 g/ml for hot Soxhlet extraction. The weakest MIC values were obtained when testing cold aqueous fraction and hot aqueous Soxhlet on *A. niger* strain (0.223 and 0.286 g/ml, respectively). The negative or non-results implicated in hexane fraction (even with its highest extraction yield) can be explained by the differing amounts of the compounds responsible for the antifungal activity in lipidic and non-lipidic fractions (primarily saponins and phenolic compounds). Saponins are more abundant in argan press-cake than argan oil and increase its antifungal activity. (El Monfalouti *et al.*, 2012); which explains the lower antifungal activity of hexane fraction (primarily fatty acids), whereabouts very low to non-antifungal activity was expected. Referring to El Monfalouti *et al.* (2012) reported results on the nonidentification of a large number of compounds by the use of the LC-MS analytical technique regarding argan kernels and press-cake chemical analysis. Leading us to suggest that much more active components that could be discovered of important antimicrobial activities are not lipidic.

Interestingly, *P. brasilianum* was the most sensitive microorganism to be grow inhibited by argan kernels' extracted fractions. *A. fumigatus*, *P. brasilianum* and *F. dimerum* had slightly close MICs in range but significantly lower than *A. niger*. A noticeable and undeniable difference between the MIC values of the fractions obtained by cold and hot extraction methods was detected (Figure 3), where the fractions of this particular latter showed lower inhibitory activity, which was expressed as an increased MIC value obtained for each fungal strain (Table 2). In line with this observation, a hypothesis of the effect of heating during the extraction process on bioactive compounds reduction or destruction should be highlighted seeing as argan press-cake (APC) owes its beneficial reputation to its richness in bioactive secondary metabolites. Since argan press-cake is the argan press-cake minus approximately 90% of the oil, we can highly envisage that the most active ingredients of argan kernels stay in the argan press-cake.

The phytochemical diversity of antimicrobial compounds that showed remarkable antifungal activities widespread among many plant species are saponins, phenolics, and indoles (Mostafa *et al.*, 2013). In regards to argan kernels

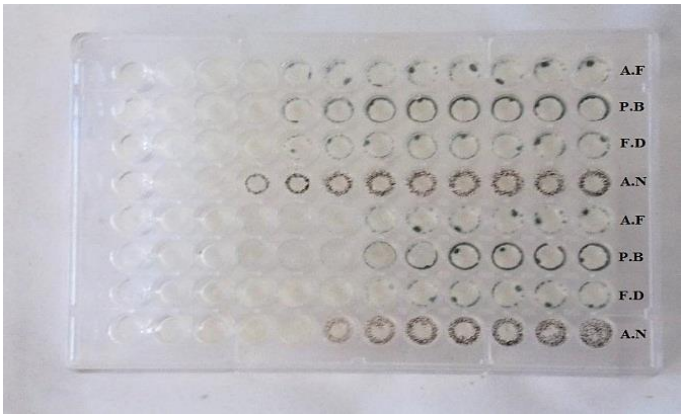

Figure 4. Fungal growth inhibition by argan kernel crude extract tested using serial broth microdilution method and the influence of extracts on 48 h-growing fungi A. F: *Aspergillus fumigatus*, P. B: *Plasmodium brasilianum*, F. D: *Fusarium dimerum*, A. N: *Aspergillus niger*.

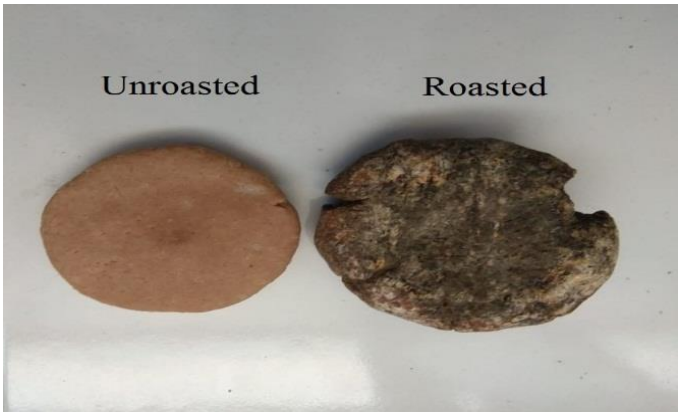

Figure 5. Argan press-cake from unroasted and roasted argan seeds after three successive years of storage in realistic conditions at ambient temperature.

and press-cake chemical analysis, El Monfalouti *et al.* (2012) reported the nonidentification of a large number of compounds in the roasted and unroasted argan kernels as well as for their both press-cakes. LC-MS analytical technique resulted in the detection of 12 components, among which 11 were unambiguously identified and can be categorized into the flavanols, flavonols, and dihydrochalcones groups (El Monfalouti *et al.*, 2012). Others reported seven saponins isolated from the cake of the argan tree. One of the already-known saponins was named "mi-saponin A", the other remained unnamed. The new saponins, along with the latter, have been named "arganin A-F". The genins of the seven saponins isolated from the cake of the argan tree are all of the Δ12-oleanane triterpene type (Guillaume and Charrouf, 2005). Saponins (a group of phytoanticipins) of low-molecular-weight antimicrobial molecules are active against human and plant pathogenic fungi (Favel *et al.*, 1994). Saponins are present constitutively in plants and play important roles in plant defense, acting as the first biochemical barrier against pathogens. The mechanism of antifungal action of saponins is not well understood. However, it is believed that they are complex with sterols in the cell membrane, leading to pore formation and consequent loss of membrane integrity (Mert-Türk *et al.*, 2006).

Argan press-cake ought to be thought of as a high energetic value byproduct material of eminent carbohydrates, proteins, and polyphenols components (Gharby *et al*., 2011). Nuutila *et al*. (2002) stated that prolonged extraction at high temperatures might degrade flavonoid and phenolic acid compounds. In this regard, and according to what Hilali *et al*., (2005) demonstrated, roasting the kernels before argan oil extraction leads to a decrease in the α-tocopherol content compared to extraction from unroasted kernels. Therefore, the high roasting temperature and hot water used during the extraction of edible oil may be an explanation for the reduction in the antimicrobial defensiveness of roasted press-cake attained from roasted kernels in anticipation of preventing foodborne or substrates pathogenic contaminations compared to unroasted press-cake produced from non-heat-treated kernels ensuring its intact state, shown in Figure 5.

Total activity (TA)

The total activity in ml/g was calculated to compare the total activity of the different argan kernels' crude extracts. The total activity that is presented in Table 3 indicates the

Table 3. Total activity of argan kernels of different extracts in ml/g after 48h incubation against

Microorganism	Total activity (ml/g)													
	HF		DEF		DMF		EAF		MF		AF		Average	
	M	S	M	S	M	S	M	S	M	S	M	S	M	S
A. fumigatus	–	–	0.24	0.06	0.63	0.40	0.18	0.13	0.64	0.29	0.11	0.07	0.36	0.19
P. brasilianum	–	–	0.25	0.06	0.82	0.41	0.20	0.13	0.66	0.29	0.12	0.07	0.41	0.19
F. dimerum	–	–	0.24	0.06	0.66	0.40	0.18	0.13	0.69	0.29	0.13	0.07	0.38	0.19
A. niger	–	–	0.12	0.03	0.34	0.27	0.11	0.09	0.34	0.21	0.07	0.06	0.19	0.13
Average	–	–	0.21	0.05	0.61	0.37	0.16	0.12	0.58	0.27	0.10	0.06	0.33	–

Note: HF: Hexane fraction, DEF: Diethyl ether fraction, DMF: Dichloromethane fraction, MF: Methanolic fraction, AF: Aqueous fraction, M: Maceration, S: Soxhlet.

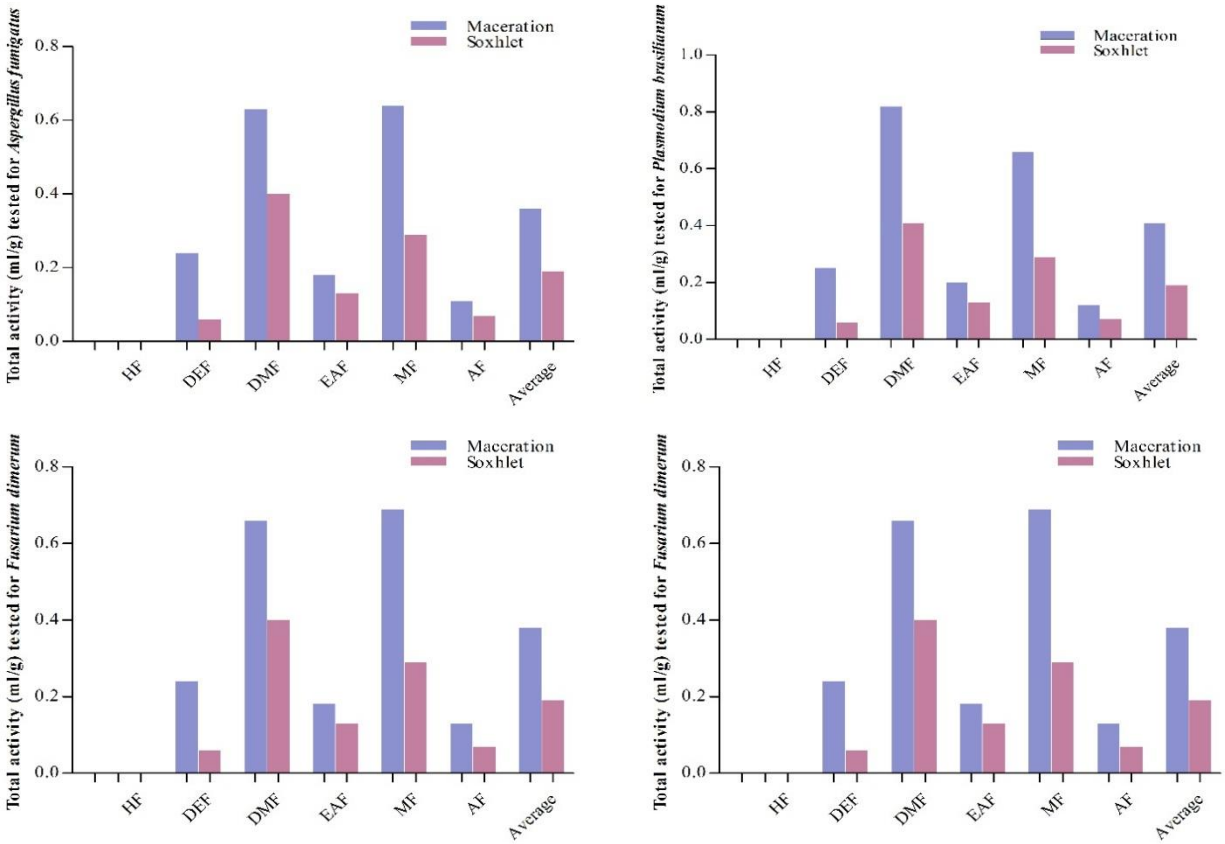

Figure 4. Efficacy (mean TA values, ml/g) of the different argan kernel extracts against all the test fungi strains in maceration and Soxhlet extraction method. The higher the TA value, the more efficacious the plant. HF = Hexane fraction, DEF = Diethyl ether fraction, DMF = Dichloromethane fraction, MF = Methanolic fraction, AF = Aqueous fraction, M = Maceration, S = Soxhlet.

volume at which extract can be diluted while still having the ability to kill the test microorganisms. The concept of total activity is useful when isolating bioactive compounds to determine if there is a loss of activity and if synergism may be involved (Eloff, 2004). Extracts with higher total activity values were considered the best to work with (Masoko *et al.*, 2005). Analyzing the present reported results, the maximum total activity (TA) value was calculated in dichloromethane fractions, followed in descendant order by methanol, diethyl ether, ethyl acetate, and aqueous fractions. The low total activity of aqueous fractions can be ascribed to their high MIC values. These findings call attention to the possible synergism between the separated metabolites in the different obtained crude extracts. The high total activities of extracts could be the result of excellent MIC values (low MIC values) and good extraction yields. As illustrated in Table 3, dichloromethane extracts possess the highest total activity, with an average total activity of 0.61 ml and 0.37 ml in maceration and Soxhlet, respectively. Followed in descendant order by methanol, diethyl ether, ethyl acetate, and the aqueous extracts showing comparatively the lowest total activity. The total activity of the macerated extracts was extensively higher than that of Soxhlet-obtained extracts, as shown in Figure 4. The low total activity of the aqueous fraction can be ascribed to their high MIC values. Inclusively, the remarkable occurrence of antifungal capacity in the different extracts asserted by their shown total activity (TA) calls attention to the possible synergism between the varying bioactive metabolites separately extracted undergoing polarly different extracting solvents.

CONCLUSION

A total of twelve extracts were investigated for their antifungal activity against four pathogenic fungi. As determined from plate-hole diffusion assays, 10 extracts displayed antifungal properties by effectively inhibiting the microorganisms' growth.

The current findings demonstrate noteworthy antifungal properties for argan kernels, where extracts exhibiting notable broad-spectrum activities (MIC values $\leq$ 0.286 g/ml) against the four tested pathogens, including dichloromethane extracts, proved the most active and displaying the greatest antifungal effect (lowest MIC values ranging from 0.051 $\pm$ 0.002 to 0.156 $\pm$ 0.005 g/ml). We also provided the first comparison between cold and hot extracts' fungicidal activity variation referring to a highly possible heat effect on the fungal contamination of

roasted argan press-cake after some period of storage at ambient and realistic conditions as the effect of the heating resulted in an increase of the MICs value of the Soxhlet extracts from 10 to 25 times higher than macerated ones resulting in lower antifungal capacity. characterization to accurately identify the antifungally active bio-molecules in argan kernels are still recommended with corroborating the antifungal potential of argan press-cake biomaterial, ascertaining its usefulness with no longer seen as agro-food production waste.

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REFERENCES

Adam, O. A. O., Abadi, R. S. M. and Ayoub, S. M. H. (2019). The effect of extraction method and solvents on yield and antioxidant activity of certain Sudanese medicinal plant extracts. *The Journal of Phytopharmacology*, *8*(5), 248–252.

Ahansal, L., Ben Sassi, A., Martini, A., Vaughan-Martini, A., Walker, G. and Boussaid, A. (2008). Biodiversity of yeasts isolated from the indigenous forest of argan (*Argania spinosa* (L.) Skeels) in Morocco. *World Journal of Microbiology and Biotechnology*, *24*(6), 777–782.

Ait Aabd, N., Bouharroud, R., Tahiri, A., Wifaya, A., Mimouni, A. and El Mousadik, A. (2019). Genetic diversity and breeding of argan tree (*Argania spinosa* L. Skeels). In "Advances in plant breeding strategies: nut and beverage crops". ed. J.M. Al-Khayri, S.M. Jain, and D.V. Johnson, pp. 31–56. Springer Nature, Cham, Switzerland.

Alvarenga, D.A., Pina-Costa, A., Bianco, C., Moreira, S.B., Brasil, P., Pissinatti, A., Daniel-Ribeiro, C.T. and Brito, C.F. (2017). New potential *Plasmodium brasilianum* hosts: tamarin and marmoset monkeys (family Callitrichidae). *Malaria Journal*, *16*(1), 1–7.

Al-Zubaidi, S., Al-Ayafi, A. and Abdelkader, H. (2019). Biosynthesis, characterization and antifungal activity of silver nanoparticles by *Aspergillus niger* isolate. *Journal of Nanotechnology Research*, *1*(1), 23–36.

Andersen, M. R., Salazar, M. P., Schaap, P. J., van de Vondervoort, P. J., Culley, D., Thykaer, J., Frisvad, J. C., Nielsen, K. F., Albang, R., Albermann, K., Berka, R. M., Braus, G. H., Braus-Stromeyer, S. A., Corrochano, L. M., Dai, Z., van Dijck, P. W., Hofmann, G., Lasure, L. L., Magnuson, J. K., Menke, H., Meijer, M., Meijer, S.L., Nielsen, J.B., Nielsen, M. L., van Ooyen, A.J., Pel, H.J., Poulsen, L., Samson, R. A., Stam, H., Tsang, A., van den Brink, J. M., Atkins, A., Aerts, A., Shapiro, H., Pangilinan, J., Salamov, A., Lou, Y., Lindquist, E., Lucas, S., Grimwood, J., Grigoriev, I.V., Kubicek, C. P., Martinez, D., van Peij, N. N., Roubos, J. A., Nielsen, J. and Baker, S.E. (2011). Comparative genomics of citric-acid-producing *Aspergillus niger* ATCC 1015 versus enzyme-producing CBS 513.88. *Genome Research*, *21*(6), 885–897.

Atifi, H., Jadouali, S.M., Laknifli, A., Bouzoubaâ, Z., Mamouni, R., Faouzi, A. and Achemchem, F. (2019). Effect of ripening degree of argane fruit on the phenolic composition and antioxidant activity of the fruit pulp, kernel, and oil. *Moroccan Journal of Chemistry*, *7*(2), 7–2.

Baig, M. W., Ahmed, M., Akhtar, N., Okla, M. K., Nasir, B., Haq, I. U., Al-Ghamdi, J., Al-Qahtani, W.H. and AbdElgawad, H. (2021). *Caralluma tuberculata* NE Br manifests extraction medium-reliant disparity in phytochemical and pharmacological analysis. *Molecules*, *26*(24), 7530.

Bani-Aameur, F. and Ferradous, A. (2001). Fruits and stone variability in three argan (*Argania spinosa* (L.) Skeels) populations. *Forest Genetics*, *8*(1), 39–43.

Barbara, D.A. (2017). M38 Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. *CLSI*.

Barber, A. E., Riedel, J., Sae-Ong, T., Kang, K., Brabetz, W., Panagiotou, G., Deising, H. B. and Kurzai, O. (2020). Effects of agricultural fungicide use on Aspergillus fumigatus abundance, antifungal susceptibility, and population structure. *MBio*. *11*(6): e02213–20.

Bongomin, F., Gago, S., Oladele, R. O. and Denning, D. W. (2017). Global and multi-national prevalence of fungal diseases–estimate precision. *Journal of Fungi*, 3(4), 57.

Brown, G. D., Denning, D. W., Gow, N. A., Levitz, S. M., Netea, M. G. and White, T. C. (2012). Hidden killers: human fungal infections. *Science Translational Medicine*, *4*(165), 165rv13. https://doi.org/10.1126/scitranslmed.3004404

Cairns, T. C., Barthel, L. and Meyer, V. (2021). Something old, something new: challenges and developments in *Aspergillus niger* biotechnology. Essays in Biochemistry, 65(2), 213–224.

Cairns, T. C., Nai, C. and Meyer, V. (2018). How a fungus shapes biotechnology: 100 years of *Aspergillus niger* research. *Fungal Biology and Biotechnology*, *5*(1), 1–14.

Charrouf, Z. and Guillaume, D. (2014). Argan oil, the 35-years-of-research product. *European Journal of Lipid Science and Technology*, *116*(10), 1316–1321.

Chhetri, H. P., Yogol, N. S., Sherchan, J., Anupa, K. C., Mansoor, S. and Thapa, P. (2008). Phytochemical and antimicrobial evaluations of some medicinal plants of Nepal. *Kathmandu University Journal of Science, Engineering and Technology*, *4*(1), 49–54.

Choi, M. S., Kim, J. K., Kim, D. H., and Yoo, H. H. (2019). Effects of gut microbiota on the bioavailability of bioactive compounds from ginkgo leaf extracts. *Metabolites*, *9*(7), 132.

Christensen, M. and Tuthill, D.E. (1986). *Aspergillus: An overview*. *In "Advances in penicillium and aspergillus systematics"*. ed. R. A. Samson, and J. I. Pitt, pp. 195–209. NATO ASI Series, Springer, Boston, MA.

Deaguero, I. G., Huda, M. N., Rodriguez, V., Zicari, J., Al-Hilal, T. A., Badruddoza, A. Z. M. and Nurunnabi, M. (2020). Nano-vesicle-based anti-fungal formulation shows higher stability, skin diffusion, biosafety, and anti-fungal efficacy *in vitro*. *Pharmaceutics*, *12*(6), 516.

do Carmo, A., Costa, E., Marques, M., Quadrado, M. J. and Tomé, R. (2016). *Fusarium dimerum* species complex (*Fusarium penzigii*) keratitis after corneal trauma. *Mycopathologia*, *181*(11), 879–884.

Eloff, J.N. (2004). Quantifying the bioactivity of the plant extracts during screening and bioassay-guided fractionation. *Phytomedicine. 11*(4), 370–371.

El Alaoui, N. (1999). Paysages, usages et voyages d'*Argania spinosa* (L.) Skeels (IXe-Xe siècles). *Journal d'Agriculture Traditionnelle et de Botanique Appliquée*, *41*(2), 45–79.

El Monfalouti, H., Charrouf, Z., Belviso, S., Ghirardello, D., Scursatone, B., Guillaume, D., Denhez, C. and Zeppa, G. (2012). Analysis and antioxidant capacity of the phenolic compounds from argan fruit (*Argania spinosa* (L.) Skeels). *European Journal of Lipid Science and Technology, 114*(4), 446–452.

Escalante, A. A., Barrio, E. and Ayala, F. J. (1995). Evolutionary origin of human and primate malarias: evidence from the circumsporozoite protein gene. *Molecular biology and Evolution*. *12*(4), 616–626.

Farahani, Z. K. (2021). The effect of extraction method (ultrasonic, maceration, and soxhlet) and solvent type on the extraction rate of phenolic compounds and extraction efficiency of *Arctium lappa* L. roots and *Polygonum aviculare* L. grass. *Food & Health*. *4*(2): 28–34.

Favel, A., Steinmetz, M. D., Regli, P., Vidal-Ollivier, E., Elias, R. and Balansard, G. (1994). *In vitro* antifungal activity of triterpenoid saponins. *Planta Medica*, *60*(01):50–53.

Fisher, M. C., Gurr, S. J., Cuomo, C. A., Blehert, D. S., Jin, H., Stukenbrock, E. H., Stajich, J. E., Kahmann, R., Boone, C., Denning, D. W., Gow, N. A. R., Klein, B. S., Kronstad, J. W., Sheppard, D. C., Taylor, J.W., Wright, G.D., Heitman, J., Casadevall, A. and Cowen, L. E. (2020). Threats posed by the fungal kingdom to humans, wildlife, and agriculture. *MBio*, *11*(3), e00449-20.

Guillaume, D. and Charrouf, Z. (2005). Saponines et métabolites secondaires de l'arganier (*Argania spinosa*). *Cahiers Agricultures*, *14*(6), 509–516.

Guillaume, D. and Charrouf, Z. (2011). Argan oil and other argan products: use in dermo cosmetology. *European Journal of Lipid Science and Technology*, *113*(4), 403–408.

Guimarães, L.O., Bajay, M.M., Wunderlich, G., Bueno, M.G., Röhe, F., Catão-Dias, J.L., Neves, A., Malafronte, R.S., Curado, I. and Kirchgatter, K. (2012). The genetic diversity of *Plasmodium malariae* and *Plasmodium brasilianum* from human, simian, and mosquito hosts in Brazil. *Acta Tropica*, *124*(1), 27–32.

Haldane, D.J. and Robart, E. (1990). A comparison of calcofluor white, potassium hydroxide, and culture for the laboratory diagnosis of superficial fungal infection. *Diagnostic Microbiology and Infectious Disease*, *13*(4), 337–339.

Harhar, H., Gharby, S., Kartah, B., El Monfalouti, H., Guillaume, D. and Charrouf, Z. (2011). Influence of argan kernel roasting-time on virgin argan oil composition and oxidative stability. *Plant Foods for Human Nutrition*, *66*(2), 163–168.

Hilali, M., Charrouf, Z., Aziz Soulhi, A.E., Hachimi, L. and Guillaume, D. (2005). Influence of origin and extraction method on argan oil physico-chemical characteristics and composition. *Journal of Agricultural and Food Chemistry*, *53*(6), 2081–2087.

Jasuja, N. D., Sharma, S. K., Saxena, R., Choudhary, J., Sharma, R. and Joshi, S. C. (2013). Antibacterial, antioxidant, and phytochemical investigation of *Thuja orientalis* leaves. *Journal of Medicinal Plants Research*, *7*(25), 1886–1893.

Karaalp, C., Yurtman, A. N. and Karabay Yavasoglu, N. U. (2009). Evaluation of antimicrobial properties of *Achillea* L. flower head extracts. *Pharmaceutical Biology*, *47*(1), 86-91.

Khalid, S. N., Rizwan, N., Khan, Z. A., Najam, A., Khan, A. M., Almas, T., Khedro, T., Nagarajan, V. R., Alshamlan, A., Gronfula, A., Alshehri, R. and Alshehri, R. (2021). Fungal burn wound infection caused by *Fusarium dimerum*: a case series on a rare etiology. *Annals of Medicine and Surgery*. *70*, 102848.

Khallouki, F., Younos, C., Soulimani, R., Oster, T., Charrouf, Z., Spiegelhalder, B., Bartsch, H. and Owen, R. W. (2003). Consumption of argan oil (Morocco) with its unique profile of fatty acids, tocopherols, squalene, sterols, and phenolic compounds should confer valuable cancer

chemopreventive effects. *European Journal of Cancer Prevention*, 67–75.

Kharbach, M., Marmouzi, I., Kamal, R., Yu, H., Barra, I., Cherrah, Y., Alaoui, K., Vander Heyden, Y. and Bouklouze, A. (2021). Extra virgin Argan oils' shelf-life monitoring and prediction based on chemical properties or FTIR fingerprints and chemometrics. *Food Control*. *121*, 107607.

Koch, B. E., Hajdamowicz, N. H., Lagendijk, E., Ram, A. F. and Meijer, A. H. (2019). *Aspergillus fumigatus* establishes infection in zebrafish by germination of phagocytized conidia, while *Aspergillus niger* relies on extracellular germination. *Scientific Reports*, *9*(1):1–13.

Kumari, M. and Singh, M. (2017). Bio-intensive Management of collar rot disease of groundnut (*Arachis hypogea* L.) caused by *Aspergillus niger*. *International Journal of Chemical Studies*, *5*(4), 80–82.

Lalremruata, A., Magris, M., Vivas-Martínez, S., Koehler, M., Esen, M., Kempaiah, P., Jeyaraj, S., Perkins, D. J., Mordmüller, B. and Metzger, W. G. (2015). Natural infection of Plasmodium brasilianum in humans: man and monkey share quartan malaria parasites in the Venezuelan Amazon. *EBioMedicine*, 2(9), 1186–1192.

Lourenço-de-Oliveira, R. and Deane, L. M. (1995). Simian malaria at two sites in the Brazilian Amazon: I-the infection rates of *Plasmodium brasilianum* in non-human primates. *Memórias do Instituto Oswaldo Cruz*, *90*, 331–339.

Mahlo, S. M., Chauke, H. R., McGaw, L. and Eloff, J. (2016). Antioxidant and antifungal activity of selected medicinal plant extracts against phytopathogenic fungi. African *Journal of Traditional, Complementary and Alternative Medicines*, *13*(4), 216–222.

Matthäus, B., Guillaume, D., Gharby, S., Haddad, A., Harhar, H. and Charrouf, Z. (2010). Effect of processing on the quality of edible argan oil. *Food Chemistry*, *120*(2), 426–432.

Masoko, P., Picard, J. and Eloff, J. N. (2005). Antifungal activities of six South African Terminalia species (*Combretaceae*). *Journal of Ethnopharmacology*, *99*(2), 301–308.

Mechqoq, H., El Yaagoubi, M., El Hamdaoui, A., Momchilova, S., da Silva Almeida, J.R.G., Msanda, F. and El Aouad, N. (2021). Ethnobotany, phytochemistry and biological properties of argan tree (*Argania spinosa* (L.) Skeels) (Sapotaceae)–a review. *Journal of Ethnopharmacology*, *281*, 114528.

Mert-Türk, F. (2006). Saponins versus plant fungal pathogens. *Journal of Cell and Molecular Biology*, *5*, 13–17.

Metougui, M.L., Mokhtari, M., Maughan, P.J., Jellen, E.N. and Benlhabib, O. (2017). Morphological variability, heritability, and correlation studies within an argan tree population (*Argania spinosa* (L.) Skeels) preserved in situ. *International Journal of Agriculture and Forestry*, *7*(2), 42–51.

Mostafa, A., Sudisha, J., El-Sayed, M., Ito, S.I., Ikeda, T., Yamauchi, N. and Shigyo, M. (2013). Aginoside saponin, a potent antifungal compound, and secondary metabolite analyses from *Allium nigrum* L. *Phytochemistry Letters*, *6*(2), 274–280.

Ngamwonglumlert, L., Devahastin, S. and Chiewchan, N. (2017). Natural colorants: Pigment stability and extraction yield enhancement via utilization of appropriate pretreatment and extraction methods. *Critical Reviews in Food Science and Nutrition*, *57*(15), 3243–3259.

Nicoletti, G. and White, K. (2022). The anti-fungal activity of nitropropenyl benzodioxole (NPBD), a redox-thiol oxidant and tyrosine phosphatase inhibitor. *Antibiotics*, *11*(9), 1188.

Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K., Wertheim, H. F., Sumpradit, N., Vlieghe, E., Levy-Hara, G., Gould, I. M., Goossens, H., Greko, C., So, A. D., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A. Q., Qamar, F. N., Mir, F., Kariuki, S., Bhutta, Z. A., Coates, A., Bergstrom, R., Wright, G. D., Brown, E. D. and Cars. O. (2013). Antibiotic resistance–the need for global solutions. *The Lancet Infectious Diseases*, *13*(12), 1057–1098.

O'Donnell, K., Sutton, D.A., Rinaldi, M.G., Gueidan, C., Crous, P.W. and Geiser, D.M. (2009). Novel multilocus sequence typing scheme reveals high genetic diversity of human pathogenic members of the *Fusarium incarnatum-F. equiseti* and *F. chlamydosporum* species complexes within the United States. *Journal of Clinical Microbiology*, *47*(12), 3851–3861.

O'Gorman, C.M. (2011). Airborne *Aspergillus fumigatus* conidia: a risk factor for aspergillosis. *Fungal Biology Reviews*, *25*(3), 151–157.

Palmieri, S., Pellegrini, M., Ricci, A., Compagnone, D. and Lo Sterzo, C. (2020). Chemical composition and antioxidant activity of thyme, hemp, and coriander extracts: A comparison study of maceration, Soxhlet, UAE and RSLDE techniques. *Foods*, *9*(9), 1221.

Pawar, V. C. and Thaker, V. S. (2006). *In vitro* efficacy of 75 essential oils against *Aspergillus niger*. *Mycoses*, *49*(4), 316–323.

Peay, K. G., Kennedy, P. G., and Talbot, J. M. (2016). Dimensions of biodiversity in the Earth mycobiome. *Nature Reviews Microbiology*, *14*(7): 434–447.

Qadir, M. I. and Ahmad, Z. (2017). Advances in hepatoprotective medicinal plants research. *Bangladesh Journal of Pharmacology*, *12*(3), 229–242.

Qari, S. H., Shi, Y. P., Pieniazek, N. J., Collins, W. E. and Lal, A. A. (1996). Phylogenetic relationship among the malaria parasites based on small subunit rRNA gene sequences: monophyletic nature of the human malaria parasite, *Plasmodium falciparum*[1]. *Molecular Phylogenetics and Evolution*, *6*(1), 157–165.

Ramasamy, R. (2014). Zoonotic malaria–global overview and research and policy needs. *Frontiers in Public Health*, *2*, 123.

Remmal, A., Bouchikhi, T., Rhayour, K., Ettayebi, M. and Tantaoui-Elaraki, A. (1993). Improved method for the determination of antimicrobial activity of essential oils in agar medium. *Journal of Essential Oil Research*, *5*(2), 179–184.

Sagar, N. A., Pareek, S., Sharma, S., Yahia, E. M. and Lobo, M. G. (2018). Fruit and vegetable waste: bioactive compounds, their extraction, and possible utilization. *Comprehensive Reviews in Food Science and Food Safety*, *17*(3), 512–531.

Salah, H., Al-Hatmi, A. M., Theelen, B., Abukamar, M., Hashim, S., van Diepeningen, A. D., Lass-Flörl, C., Boekhout, T., Almaslamani, M. and Taj-Aldeen, S. J. (2015). Phylogenetic diversity of human pathogenic *Fusarium* and emergence of uncommon virulent species. *Journal of Infection*, *71*(6), 658–666.

Sankeshwari, R. M., Ankola, A. V., Bhat, K. and Hullatti, K. (2018). Soxhlet versus cold maceration: which method gives better antimicrobial activity to licorice extract against: *Streptococcus Mutans*?. *Journal of the Scientific Society*, *45*(2), 67–71.

Schroers, H. J., O'Donnell, K., Lamprecht, S. C., Kammeyer, P. L., Johnson, S., Sutton, D. A., Rinaldi, M. G., Geiser, D. M. and Summerbell, R. C. (2009). Taxonomy and phylogeny of the Fusarium dimerum species group. *Mycologia*, *101*(1), 44–70.

Schwalbe, R., Steele-Moore, L., and Goodwin, A.C. (2007). *Antimicrobial susceptibility testing protocols*. CRC Press, Boca Raton, Florida.

Sharp, P. M., Plenderleith, L. J. and Hahn, B. H. (2020). Ape origins of human malaria. *Annual Review of Microbiology*, *74*, 39.

Spagnuolo, M., Yaguchi, A. and Blenner, M. (2019). Oleaginous yeast for biofuel and oleochemical production. *Current Opinion in Biotechnology*, *57*, 73–81.

Sridhar, A., Ponnuchamy, M., Kumar, P. S., Kapoor, A., Vo, D. V. N., and Prabhakar, S. (2021). Techniques and modeling of polyphenol extraction from food: a review. *Environmental Chemistry Letters*, *19*, 3409–3443.

Szabo, K., Teleky, B. E., Ranga, F., Roman, I., Khaoula, H., Boudaya, E., Ben Ltaief, A., Aouani, W., Thiamrat, M. and Vodnar, D. C. (2022). Carotenoid recovery from tomato processing by-products through green chemistry. *Molecules*, *27*(12):3771.

Venkatesan, D. and Karrunakaran, C. M. (2010). Antimicrobial activity of selected Indian medicinal plants. *Journal of Phytology*. *2*(2), 44–48.

Yu, R., Liu, J., Wang, Y., Wang, H. and Zhang, H. (2021). *Aspergillus niger* as a secondary metabolite factory. *Frontiers in Chemistry*, *9*.

Zhang, Q., Liu, F., Zeng, M., Mao, Y., and Song, Z. (2021). Drug repurposing strategies in the development of potential antifungal agents. *Applied Microbiology and Biotechnology*, *105*(13), 5259–5279.