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Phytochemical Analysis and Evaluation of the Antifungal Activity of Five Plants against Four Dermatophytes Responsible for Superficial Infections

Agré Don Josette [a*], Kra Adou Koffi Mathieu [a], Konan Gbê Kouakou N'Dri Ange [a] and Bolou Gbouhoury Eric-Kevin [b]

[a] Laboratory of Biology and Health, UFR Biosciences, Félix Houphouët-Boigny University, Côte d'Ivoire.
[b] National Center of Floristics, Félix Houphouët-Boigny University, Côte d'Ivoire.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study is of great interest due to the rise in superficial infections caused by dermatophytes and the need to find natural alternatives to synthetic antifungals. The objective of this research was to determine the phytochemical composition and antifungal activity of total extracts from five plants:

**Corresponding author: E-mail: agrejosette@yahoo.fr;*

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Zanthoxylum gilletii, Distemonanthus benthamianus, Gmelina arborea, Justicia secunda, and *Anacardium occidentale* against four dermatophytes, namely Microsporum canis, Trichophyton mentagrophytes, Trichophyton rubrum, and Trichophyton soudanense.
To achieve this, aqueous and hydroethanolic extracts were prepared and subjected to phytochemical analysis, followed by evaluations of antifungal activity using the slant double-tube method. Antifungal parameters (IC_{50} and MFC) were determined. The results revealed the presence of various bioactive compounds, such as flavonoids, tannins, and alkaloids, in most of the extracts. *Z. gilletii* and *D. benthamianus* showed strong efficacy, with IC_{50} values of 0.004 to 0.00609 mg/mL and 0.018 to 0.155 mg/mL, and MFC values of 0.0975 to 0.39 mg/mL and 0.39 to 12.5 mg/mL respectively, particularly against *M. canis* and *T. soudanense*. In contrast, *A. occidentale* showed no significant antifungal activity. This study thus highlights the potential of plant extracts in the treatment of skin infections and justifies further research to explore their clinical application.

Keywords: Plant extracts; dermatophytes; antifungal activity.

1. INTRODUCTION

Dermatophytes are filamentous fungi responsible for common dermatoses. They cause conditions such as ringworm, which affects hair, skin, and nails. These conditions, known as dermatophytoses, are particularly concerning because they affect approximately 20 to 25% of the global population, with a rising incidence in developing countries and increasing resistance to antifungal treatments [1]. The global prevalence of dermatophytoses varies by region, with higher rates in tropical and subtropical areas due to favorable climatic conditions for fungal growth [2].

In Africa, dermatophytoses are a major cause of fungal skin infections. Studies show that the prevalence of these infections is particularly high in rural areas, where hygiene conditions are often poor and access to medical care is limited [3]. The species *Trichophyton rubrum, Trichophyton mentagrophytes*, *Trichophyton soudanense*, and *Microsporum canis* are among the most frequently isolated in sub-Saharan Africa. In Côte d'Ivoire, the prevalence of dermatophytoses is estimated at around 15%, primarily affecting children and young adults [3,4,5]. Traditional plant-based treatments are common, and these remedies are often the first line of defense against dermatoses.

The interest in studying the anti-dermatophytic activity of medicinal plants lies in several important aspects. First, the growing resistance to synthetic antifungals, such as terbinafine and azoles, makes treating dermatophytoses increasingly difficult. Recent studies show that nearly 20% of Trichophyton rubrum strains are resistant to terbinafine, which complicates the management of these affections [6]. Additionally, current antifungals can lead to undesirable side effects, justifying the exploration of natural alternatives [7,8]. In light of this resistance to synthetic antifungals, it would be wise to explore medicinal plants, many of which have shown strong antimicrobial activity. Among these plants are *Senna podocarpa, Piliostigma thonningii, Terminalia avicennoides, Terminalia ivorensis,* and *Terminalia catappa,* which are traditionally used to treat skin conditions in Africa [9].

This study aims to determine the phytochemical composition of aqueous and hydroethanolic extracts and evaluate the anti-dermatophytic activity of five medicinal plants against four major dermatophytes (*Trichophyton rubrum, Trichophyton mentagrophytes, Trichophyton soudanense*, and *Microsporum canis*) to offer a natural and effective alternative to current antifungal treatments.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material consisted to organs collected from five plants. These different organs were collected in different areas of Côte d'Ivoire (Table 1). After identification of the different species at the national floristic centre of the Université Félix Houphouët-Boigny, the different organs were washed, cut into small pieces and dried in the shade, then ground into a fine powder.

2.2 Fungal Material

The fungal species tested were supplied by the Institut Pasteur de Côte d'Ivoire. These species were collected from various patients attending the Centre University Hospital of Cocody, Ivory Coast.

2.3 Extraction Method

Aqueous and hydroethanol extracts were prepared according to the method of Zirihi et al. [10] using distilled water and an ethanol-water mixture (70/30, v/v) as the solvent. One hundred grams (100 g) of plant powder was dissolved in one litre of solvent, giving a ratio of 1:10 (w/v). The mixture was then homogenised vigorously using a blender. The homogenate obtained was wrung out in a square of percale cloth and then filtered three times on cotton wool and then on filter paper. The filtrate was evaporated at 45°C using a Venticell® type ventilated oven for 24 h for the 70% ethanolic extract and 48 to 72 h for the aqueous extract.

2.4 Phytochemical Sorting

Phytochemical sorting is a method used to characterise the main chemical groups such as sterols, polyterpenes, alkaloids, tannins, phenolic compounds, flavonoids, quinones and saponins. These compounds were identified using appropriate reagents that reacted with chemical compounds to give specific colours or precipitates that attested to the presence or absence of the desired molecules in the extracts. This study was carried out with the total extracts (aqueous and hydroethanolic) of each plant according to the identification protocol described by Békro et al. [11].

2.4.1 Determination of sterols and polyterpenes

For each plant extract taken separately, five (5) mL of each of the two extracts (aqueous and hydroalcoholic) were evaporated over a sand bath. The residue was dissolved while hot in 1 mL of acetic anhydride ; then 0.5 mL of concentrated sulphuric acid was added to the triturate. Whether or not a purple or violet ring appeared at interphase, turning blue and then green, indicated a positive or negative reaction.

2.4.2 Identification of polyphenols

A drop of 2% alcoholic ferric chloride solution was added to two (2) mL of each hydroethanolic and aqueous extract. The presence or absence of polyphenols was indicated by the appearance or absence of a more or less dark blue-black or green colour.

2.4.3 Flavonoid analysis

Two (2) mL of each extract was evaporated and the residue taken up in 5 mL of hydrochloric alcohol diluted 2-fold. By adding 2 to 3 magnesium chips, a release of heat followed by a pink-orange or purplish coloration could be observed. The addition of 3 drops of isoamyl alcohol may or may not intensify this colouration. This confirms the presence or absence of flavonoids.

2.4.4 Identification of tannins

Five (5) mL of each extract was evaporated to dryness. After adding 15 mL of Stiasny's reagent to the residue, the mixture was kept in a water bath at 80°C for 30 min. The presence or absence of catechin tannins was determined by whether or not a coarse flake precipitate was observed. For gallic tannins, the previous solution was filtered. The filtrate was collected and saturated with sodium acetate. The addition of 3 drops of FeCl3 caused the appearance or absence of an intense blue-black colour, indicating the presence or absence of gallic tannins.

2.4.5 Analysis of quinone substances

Two (2) mL of each of the two extracts were evaporated to dryness. The residue was triturated in 5 mL of 1:5 hydrochloric acid. The triturate was transferred to a test tube and heated in a boiling water bath for 30 min. After cooling, it was extracted with 10 mL of chloroform. Twice-diluted ammonia (0.5 mL) was added to the chloroform solution. The presence or absence of quinones was indicated by a red or purple colouration or a lack of colour.

2.4.6 Alkaloid analysis

Six (6) mL of each solution was evaporated to dryness. The residue was taken up with 6 mL of 60° alcohol. The addition of 2 drops of Dragendorff's reagent to the alcoholic solution caused a precipitate or an orange coloration. The addition of 2 drops of Burchard's reagent to the alcoholic solution produced a precipitate or no reddish-brown coloration and indicated a positive or negative reaction.

2.4.7 Identification of saponosides

Ten (10) mL of the total extract was prepared in a test tube. The tube was shaken vertically for approximately 15 seconds and left to stand for 10 to 15 minutes. The height of the foam formed was measured after this period.

Table 1. Plants used

Plants	Collected organ	Harvesting area
Anacardium occidentale	Trunk bark	Abengourou
Distemonanthus benthamianus	Trunk bark	Abengourou
Gmelina arborea	Trunk bark	Abengourou
Justicia secunda	Aerial part	Abengourou
Zanthoxylum gilletii	Trunk bark	Abengourou

Table 2. Clinical information and profile of fungal species

Clinical information	Nature of the sample	isolated species	Antifungal Profile				
			5-FC	AMB	FCA	VRC	ITR
Moth	Hairline	*T. mentagrophytes*	S	S	R	*S*	R
Athlete's foot	Top of the foot	*T. rubrum*	S	S	S	*S*	R
Moth	Hairline	*M. canis*	S	S	S	*S*	S
Moth	Hairline	*T. soudanense*	S	S	S	*S*	R

2.5 Antifungal Activity

The tests were conducted separately on young cultures of dermatophytes grown on slant agar. The various plant extracts were incorporated into the agar before the fungal growth. The incorporation of these extracts into Sabouraud agar was performed using the double dilution method in slant tubes. Each series included 12 test tubes, of which 10 contained the plant extract and 2 were control tubes without the plant extract (one serving as a control for germ growth, the other without germ serving as a control for the sterility of the culture medium). The concentrations in the test tubes ranged from 1000 to 1.52 µg/mL. For the 10 tubes in each series, the concentrations varied according to a geometric progression with a ratio of ½, from tube No. 1 to tube No. 10. After the extract was incorporated, all 12 tubes in each series were autoclaved at 121°C for 15 minutes. The tubes were then tilted with a small base at room temperature to allow for cooling and solidification of the agar [12,10].

Inoculums were prepared separately from young colonies (7 days old) of *Microsporum canis, Trichophyton mentagrophytes, Trichophyton rubrum*, and *Trichophyton soudanense*. A colony of each germ was taken with a loop and homogenized in 10 mL of sterilized distilled water, resulting in the mother suspension (10^0). From this suspension, a second suspension (10^{-1}) was prepared by a tenfold dilution. Thus, for each of the test tubes (except for the control tube for the sterility of the culture medium), the germ culture was performed on the previously prepared media by inoculating 10 µL of the 10^{-1} suspension in cross streaks until exhaustion. The cultures were incubated at 30°C for seven days. After this incubation period, the colonies of each germ were counted. The growth in the ten experimental tubes of each series was evaluated as a percentage of survival, calculated against 100% survival in the growth control tube [13]. The treatment of experimental data allowed for the determination of the following antifungal parameters: the minimum fungicidal concentration (MFC) and the concentration for 50% inhibition (IC50), the latter being determined graphically [14].

3. RESULTS

3.1 Phytochemical Study

Table 3 summarizes the presence of different chemical groups (sterols, terpenes, phenolic compounds, flavonoids, tannins, quinones, alkaloids, and saponins) in aqueous and hydroalcoholic (70% ethanol) extracts of five plants : *D. benthamianus, Z. gilletii, J. secunda, G. arborea,* and *A. occidentale*.

The results show that sterols, polyterpenes, phenolic compounds, flavonoids, and alkaloids are consistently present in almost all extracts, whether aqueous or ethanol-based. Quinones are absent in the majority of extracts, except in *Z. gilletii* and *G. arborea*, where they are present in both types of extracts. Saponins are predominantly found in the aqueous extracts but absent in the hydroalcoholic extracts, particularly in *D. benthamianus, Z. gilletii, J. secunda,* and *A. occidentale.*

Table 3. Secondary metabolites present in plant extracts

		Chemical groups						
Plants	**Extracts**	**Sterols, terpenes**	**Phenolic compounds**	**Flavonoïds**	**Tannins**	**Quinones**	**Saponins**	**Saponins**
D. benthamianus	aqueous	+	+	+	+	-	+	+
	70% ethanol	+	+	+	+	-	+	-
Z. gilletii	aqueous	+	+	+	+	+	+	+
	70% ethanol	+	+	+	+	+	+	-
J. secunda	aqueous	+	+	+	-	-	+	+
	70% ethanol	+	+	+	+	-	+	-
G. arborea	Aqueous	+	+	+	+	+	+	+
	70% ethanol	+	+	+	+	+	+	-
A. occidentale	aqueous	+	+	+	+	-	+	+
	70% ethanol	+	+	+	+	-	+	-

Table 4. Antifungal parameters of the various extracts

Plants extracts		Parameter values for fungal species tested (mg/mL)							
		M. canis		*T. mentagrophytes*		*T. rubrum*		*T. soudanense*	
		IC_{50}	MFC	IC_{50}	MFC	IC_{50}	MFC	IC_{50}	MFC
A. occidentale	Aq	ND	ND	ND	ND	ND	ND	ND	ND
	Eth	ND	ND	ND	ND	ND	ND	ND	ND
D. benthamianus	Aq	0,035	1,56	0,198	6,25	0,292	50	0,035	1,56
	Eth	0,018	0,39	0,155	3,125	0,13	12,5	0,0198	0,78
G. arborea	Aq	0,046	3,125	0,3	12,5	0.26	25	0,045	3,125
	Eth	0,0246	0,78	0,13	3,125	0,14	12,5	0,035	0,78
J. secunda	Aq	0,0487	6,25	0,195	6,25	0,195	ND	0,0487	6,25
	Eth	0,032	0,78	0,1	3,125	0,16	25	0,031	1,56
Z. gilletii	Aq	0,021	0,39	0,13	1,56	0,1	1,56	0,026	0,39
	Eth	0,00609	0,0975	0,04	0,39	0,04	0,39	0,004	0,0975

3.2 Antifungal Activity

The Table 4 shows that ethanolic extracts are generally more effective than aqueous extracts in inhibiting and killing the fungal species tested. *Z. gilletii* and *D. benthamianus* stand out for their strong antifungal activity, with very low IC_{50} and MFC, especially against *M. canis* and *T. soudanense*, while *A. occidentale* showed no measurable activity.

4. DISCUSSION

The chemical composition of the studied plants varies depending on the type of solvent used, particularly with respect to saponins, which are often more present in aqueous extracts than in 70% ethanol extracts. This result aligns with several previous studies that have shown saponins are more soluble in water than in organic solvents. A study on medicinal plants conducted by Rai et al. [15] reported a similar trend for species in the Fabaceae family, where aqueous extracts contained more saponins compared to hydroalcoholic extracts.

As for sterols, polyterpenes, flavonoids, and phenolic compounds, their presence in almost all extracts (aqueous and hydroalcoholic) is consistent with the work of Mahamane et al. [16] which showed that these groups of compounds are widely soluble in polar solvents, whether aqueous or hydroalcoholic. Phenolic compounds and flavonoids tend to be well extracted by polar solvents like water and ethanol, and this extraction is more efficient when ethanol is mixed with water, maximizing the solvent's polarity.

Quinones, present only in *Z. gilletii* and *G. arborea*, are relatively rare in other extracts. This could be explained by the variability in quinone concentrations depending on the plant species. Other authors, such as Akpo et al. [17] have also reported in quinone presence between different plant species, suggesting that these secondary metabolites may be specific to certain plants or growth environments.

In comparison, some studies, including those by Bamba et al. [18] have shown that 70% ethanol effectively extracts tannins from various medicinal plants, which is confirmed by these results, where tannins are present in all extracts except *J. secunda*. However, tannins were absent in the aqueous extract of this plant, which could be related to a lower concentration or a different chemical structure that makes them less soluble in water.

The metabolites identified in these plant extracts exhibit significant pharmacological potential, supported by numerous studies. Sterols and polyterpenes have well-documented antimicrobial, anti-inflammatory, and cholesterol-lowering effects [19]. A recent study by Barkas et al. [20] shows that these compounds help reduce serum cholesterol and improve cardiovascular health by acting on cholesterol absorption in the intestines. Phenolic compounds and flavonoids, present in almost all extracts, are powerful antioxidants. According to Rudrapal et al. [21] these metabolites reduce oxidative damage associated with various chronic diseases, such as diabetes, neurodegenerative diseases, and certain cancers, by neutralizing free radicals.

Tannins, known for their antimicrobial properties, have been recently studied by Trepa et al. [19] who showed they inhibit the growth of several pathogenic bacterial strains and viruses. This study suggests their potential use in developing treatments for gastrointestinal infections and viral diseases. Quinones, found in *Z. gilletii* and *G. arborea*, continue to be studied for their antitumor and antimicrobial properties. Oyenihi et al. [22] demonstrated that these compounds can induce apoptosis in cancer cells and inhibit the growth of drug-resistant pathogens.

Alkaloids, present in most extracts, are still widely recognized for their multiple pharmacological properties. Heinrich et al. [23] highlighted their anticancer, antimicrobial, and analgesic effects, emphasizing their crucial role in modern medicine, particularly due to the chemical diversity of these metabolites. Finally, saponins, abundant in aqueous extracts, are renowned for their anti-inflammatory and expectorant activities. Stan et al. [24] showed that these compounds are particularly effective in treating respiratory conditions and have antimicrobial properties, making them useful against certain skin infections.

Sterols and terpenes are secondary metabolites with well-documented microbiological activities. Sterols, such as β-sitosterol, are known for their antimicrobial and antiviral effects. They act by disrupting the cell membranes of microbes, leading to altered membrane permeability and, consequently, the death of pathogenic cells. A study by Li et al. [25] showed that certain plant-derived sterols inhibit the growth of *Candida*

albicans, suggesting their potential as antifungal and antibacterial agents. Terpenes, particularly monoterpenes and sesquiterpenes, have also demonstrated strong antimicrobial properties. For example, terpenes such as limonene and caryophyllene work by disrupting the cell membrane structures of bacteria and fungi, leading to their lysis. They are especially effective against pathogens such as *Escherichia coli* and *Aspergillus fumigatus*.

Quinones, another important group of secondary metabolites, are widely recognized for their antimicrobial and antitumor activities. These compounds, found in plants like *Zanthoxylum gilletii*, exert their antimicrobial effects by acting as oxidizing agents that generate reactive oxygen species (ROS). This results in oxidative stress in microbes, causing damage to proteins, lipids, and the DNA of pathogenic cells. A study by Oyenihi et al. [22] showed that quinones can inhibit the growth of antibiotic-resistant bacteria, such as *Pseudomonas aeruginosa* and *Staphylococcus aureus*, in addition to their anticancer effects, including inducing apoptosis in tumor cells.

Saponins, on the other hand, are recognized for their antimicrobial properties, particularly their antifungal and antibacterial effects. These compounds, abundant in aqueous extracts of many plants, work by forming complexes with the sterols in microbial cell membranes, which disrupts their integrity and leads to the leakage of intracellular components. This makes them particularly effective against pathogenic fungi such as *Trichophyton mentagrophytes* and *Candida albicans*. A study by Stan et al. [24] revealed that saponins exhibit significant activity against several strains of bacteria and fungi responsible for skin infections, highlighting their potential as natural therapeutic agents.

The results obtained show significant variations in the antifungal activity of aqueous and ethanolic extracts of the tested plants, depending on the fungal species. Unlike the other plants, extracts of *Anacardium occidentale* showed no measurable antifungal activity (no data for IC_{50} and MFC). This could be explained by the absence of active antifungal compounds in the parts used for extraction or a concentration too low to produce an effect. Other studies, such as that of Quejada et al. [26] have reported moderate activity of some *A. occidentale* extracts on *Candida albicans* species, suggesting that antifungal activity might be specific to certain conditions or extraction methods.

Ethanolic extracts, in almost all cases, show superior antifungal activity compared to aqueous extracts. This observation is consistent with numerous studies, such as that of Sepehri et al. [27] which show that ethanol is a better solvent for extracting bioactive compounds, such as polyphenols and flavonoids, responsible for antifungal effects. Indeed, the CI50 values of ethanolic extracts of *D. benthamianus* and *Z. gilletii* against *M. canis* and *T. soudanense* are significantly lower than those of aqueous extracts, indicating more potent activity.

The results show that *Z. gilletii* and *D. benthamianus* exhibit excellent antifungal activity, particularly with very low CI50 values (e.g., 0.00609 mg/mL for *Z. gilletii* on *M. canis*) and MFC values that reflect fungicidal action at low concentrations. These results suggest that these plants may contain powerful antifungal compounds, such as quinones and flavonoids, already known for their effects on pathogenic fungi [28].

Antifungal activities vary depending on the fungal species. For instance, *G. arborea* is more effective against *C. albicans* and *T. rubrum*, while its activity on *T. mentagrophytes* and *M. canis* is more moderate. This suggests possible specificity of the bioactive compounds extracted, depending on the membrane or defense mechanisms specific to each fungal species. This phenomenon is often observed in plant pharmacology, as discussed by Zhou et al. [29] who found differences in fungal species' sensitivity to plant extracts.

These results highlight the therapeutic potential of the tested plants, particularly for fungal skin infections, such as those caused by *T. mentagrophytes* and *T. rubrum*, which are responsible for ringworm and other dermatophytoses. The fungicidal activity at low concentrations suggests that these plants could be used to develop natural treatments for these infections, thus reducing reliance on synthetic drugs, which can lead to resistance. These findings are supported by recent studies, such as that of Ivanov et al. [30] which demonstrate that plant extracts offer a viable alternative in treating resistant fungal infections.

5. CONCLUSION

This study combined phytochemical analysis and antifungal activity. The results revealed a strong therapeutic potential for the tested plant extracts. Phytochemical analyses highlighted the presence of bioactive metabolites such as sterols, polyterpenes, phenolic compounds, flavonoids, tannins, alkaloids, and saponins in the majority of the extracts. These compounds are well-known for their antifungal properties, as confirmed by the IC_{50} and MFC values obtained from tests on various fungal species.

Ethanolic extracts proved particularly effective, especially those of *Z. gilletii*, *D. benthamianus*, and *G. arborea*, which showed strong inhibition at low concentrations, particularly against *M. canis*, *T. soudanense*, and *T. mentagrophytes*. These results suggest that ethanol, as a solvent, allows for better extraction of the bioactive compounds responsible for antifungal activity, such as flavonoids and quinones. In contrast, *A. occidentale* showed no significant activity, which could be due to the absence of certain antifungal metabolites in the tested parts or a low concentration of these compounds.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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