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Study of the Antimicrobial Activity of Leaf Extracts of *Moringa Oleifera* Lam and *Combretum Micranthum* G.Don in the Regions of (Kita) Mali and Thiès (Senegal)

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ABSTRACT

In developing countries where medicines are very expensive, research on the antimicrobial activities of ethnomedicinal plants may still be necessary.

The objective of this study was to evaluate the antimicrobial activity of leaf extracts from *Moringa oleifera* Lam and *Combretum micranthum* G.Don. Different extracts and fractions were tested against four bacterial strains from the ATCC collection (*Pseudomonas aeruginosa, Straphylococcus aureus, Escherichia coli, Enterococcus faecalis*). The extracts were also tested against yeast strains, including Candida albicans and Candida krusie. The results showed the efficacy of the various *Moringa oleifera* Lam extracts against all bacterial strains tested except Enterococcus faecalis, with most inhibition zones ranging from 7 to 11 mm. The various extracts were effective against *Candida albicans*, while *Candida krusie* was resistant. As for *Combretum micranthum* G.Don, we found that extract M1 was sensitive to *Pseudomonas aeruginosa*, with a zone of inhibition of 14 mm and a MIC of 9.71 mg/ml, and the other extracts were resistant to the strains. The results of the study confirmed the chemotherapeutic value of plant extracts in ethnomedicine for the treatment of various conditions.

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INTRODUCTION

The use of plants as remedies for the treatment of numerous diseases dates back to prehistoric times, and this tradition is present on every continent. Despite remarkable advances in synthetic organic chemistry in the 20th century, more than 25% of drugs prescribed in industrialized countries are derived directly or indirectly from plants (Newman et al., 2000). However, plants used in traditional medicine are still understudied, particularly in clinical microbiology (Kirby, 1996). It is reported that most antibiotics are derived from microorganisms, and that one to three antibiotics are marketed each year (Clark, 1996). In developing countries where drugs are very expensive, research on the antimicrobial activities of ethnomedicinal plants may still be needed. It is clear that these phytochemicals will find their place in the arsenal of antimicrobial drugs prescribed by physicians (Cowan, 1999). All antibiotics have a limited duration of effectiveness, and the public is increasingly aware of the problems associated with overprescribing these antibiotics. Furthermore, many people, particularly in developed countries, desire greater autonomy in their medical care, making self-medication common (Eisenberg et al., 1993). In developing countries, particularly in West Africa, new drugs are often inaccessible. Thus, up to 80% of the population uses medicinal plants as remedies (Kirby, 1996; Hostettmann and Marston, 2002). The Moringa tree is presented as an exceptional indigenous source of highly digestible protein, carotenoids, and vitamin C suitable for use in many so-called developing regions of the world where malnutrition is a major concern (Fugile, 2001). Recently, this plant has attracted a lot

of interest due to the results of numerous relevant researches and publications on the subject that highlight its nutritional and medicinal properties (Olson and Fahey, 2011). Plants have always had an important place in the therapeutic arsenal of humanity. They are a potential source of bioactive molecules namely polyphenols, alkaloids, terpenes, flavonoids, tannins, steroids, which are at the origin of several biological activities such as anti-inflammatory, antimicrobial, antiseptic, diuretic and antioxidant activity (Haddouchi et al., 2014). They take their therapeutic capacity as the antimicrobial and antioxidant power, from its primary and secondary metabolites or by the synergy between the two or between the different compounds of each type of metabolite. Among the secondary metabolites, phenolic compounds are considered strong antioxidants and antimicrobials (Zeghad, 2009; Lehout and Laib, 2015; Bourgou et al., 2016; Bendif, 2017). The *Combretum micranthum* G.Don is common in cultivated and fallow lands throughout the continent, but it appears to be dominant in sub-Saharan Africa, from Sudan to Nigeria, from Gambia to Congo, with higher concentrations in Senegal, Mali, and Burkina Faso (Iwu, 1993). It is currently being introduced, in the form of tea, in other African countries, Europe, and the American market (Juliani et al., 2009). Although its therapeutic properties are known and appreciated in African savannah cultivation, the chemistry and validation of its therapeutic use underlying these health benefits remain poorly understood. The leaves of *Combretum micranthum* G.Don have a high content of total phenols and can be considered a potent antioxidant (Juliani et al., 2009; Karou et al., 2005). This activity can be preliminarily attributed to the polyphenols previously identified in the leaves by a number of scientists (Agostino et al. 1990; Paris, 1942; Bassene et al. 1986; Karou et al. 2005) who studied the antimicrobial activity of *Combretum micranthum* G.Don leaves against pathogenic bacteria, trying to illustrate a link between antioxidant capacity and antimicrobial activity. Leaf extracts of *Combretum micranthum* G.Don demonstrated microbicidal activity against *Shigella dysenteriae*, *Salmonella parathyphi* B. and *Staphylococcus aureus* and microbiostatic activity against Shigella flexneri, Shigella boydii, *Salmonella typhi*, *Klebsiella ozenae* and *K. pneumoniae*. Benoit et al. (1996) confirmed the traditional use of *Combretum micranthum* G.Don stems and leaves in the treatment of malaria by inhibiting the in vitro growth of chloroquine-sensitive and -resistant *Plasmodium falciparum* strains and by reducing parasitemia after 30 hours of contact, indicating activity of the extract on the parasite reinvasion process. Ancolio et al. (2002) and Karou et al. (2003) both confirmed that *Combretum micranthum* G.Don leaf extract had moderate antiplasmodial activity. Thus, the objective of this study was to investigate the antimicrobial activity of *Moringa oleifera* Lam and *Combretum micranthum* G.Don leaf extracts.

MATERIALS AND METHODS

MATERIALS

Plant Materials

The samples used in this study came from the localities of Kita and Landaré (Kita region of Mali) and Mbour and Thiès (Thiès region of Senegal). Harvests were made on April 7 and 8 in Mali and on May 20 and 21, 2022, in Senegal. After being sorted and lightly cleaned, the leaves were left to dry in the open air, away from light. They were then ground using an electric grinder. The resulting powder was then sieved (electric sieve (Retsch-AS 200)) and stored in glass jars away from light to prevent deterioration before use. The acronyms M1, M2, and S1 and S2 refer to the samples collected in Kita and Landaré in Mali and in Thiès and Mbour in Senegal, respectively.

METHODS

Methanolic Extraction

The extraction was performed by macerating 50 g of the plant powder in 500 mL of a methanol-water mixture (70/30, v/v) (Fathiazad et al., 2011). The mixture was stirred vigorously for 48 hours using a stirrer. After vacuum filtration (using a pump) through Whatman filter paper, the resulting hydroalcoholic phase was evaporated to dryness under reduced pressure in a rotary evaporator at 50°C to remove the methanol.

Liquid/Liquid Fractionation of Ethyl Acetate and Butanol Extract

The aqueous phase obtained by hydromethanolic extraction was fractionated by liquid-liquid extraction with ethyl acetate, then butanol, using a separating funnel. The fraction was then concentrated to dryness under reduced pressure using a rotary evaporator at 40°C (Bekkara et al., 1998).

Antimicrobial Activity

The antimicrobial activity of the various extracts and fractions was evaluated using the solid-state diffusion method to determine inhibition diameters. Sabouraud medium was used for *Candida albicans* and *Candida krusie* yeasts, and Mueller-Hinton medium was used for the bacterial strains.

Determination of Inhibition Diameters

Media Preparation

Mueller-Hinton and Sabouraud media were prepared according to the manufacturers' instructions: 38 g of Mueller-Hinton agar and 32.525 g of Sabouraud agar were dissolved in 1 L and 500 mL of distilled water, respectively. Both solutions were brought to a boil before autoclaving at 121°C for 15 minutes. The media were then placed in Petri dishes homogeneously for solidification at room temperature and then stored in the refrigerator before use.

Preparation of microbial inocula

The different microbial strains were subcultured and incubated at 37°C for 18 to 24 hours to obtain young cultures and well-isolated colonies. These were used to prepare inocula with cell density adjusted to 0.5 McFarland.

Inoculation

A swab was dipped into the tubes containing the inocula and then its contents spread over the entire surface of each plate containing Mueller-Hinton or Sabouraud media, ensuring homogeneous distribution of the inoculum. In each plate, four samples of extracts and fractions, at 15 and 30 mg/mL in DMSO for *Moringa oleifera* Lam and 80 mg/mL for *Combretum micranthum* G.Don, were added in a ratio of 100 µL. The plates were incubated at 37°C for 24 hours. The absence of microbial growth was indicated by the presence of an inhibition zone around the wells, the diameter of which was measured using a caliper.

Statistical Analysis

All analyses were performed in three replicates and the results were presented as the mean ± standard deviation of the mean (SEM). Statistical analysis of the results was performed using EXCEL 2019.

RESULTS

Antimicrobial Activity The results shown in Tables 1, 2, and 3 indicate the values of the different inhibition diameters induced by the hydromethanolic, butanoic, and ethyl acetate extracts of *Moringa oleifera* Lam in the four samples. For *Combretum micranthum* G.Don, the results are shown in Tables 5 and 6. The antimicrobial activity of the different extracts from our two plants was evaluated using the agar diffusion method. Antimicrobial potency is determined by measuring the inhibition diameters (mm) of each bacterium or yeast against the tested extracts. The results of the antibacterial test show a variation in the inhibition efficacy of our different extracts against the bacterial strains tested.

Table 1: Antibacterial activity of hydro-methanolic extracts of *Moringa oleifera* Lam leaves against bacterial strains.

Souches bactériennes	Extraits							
	M1		M2		S1		S2	
	Concentrations mg/mL							
	15	30	15	30	15	30	15	30
	Diamètres des souches microbiennes en mm							
Escherichia coli ATCC 25922	-	-	-	-	-	8	-	7
Enterococcus faecalis ATCC 29212	-	-	-	-	-	-	-	-
Staphylococcus aureus ATCC 29231	-	-	-	7	-	9	-	-
Pseudomonas aeruginosa ATCC 27853	-	-	-	7	-	10	-	7

Table 2: Antibacterial activity of butanolic *Moringa oleifera* Lam leaf extracts against bacterial strains.

Souches bactériennes	Extraits							
	M1		M2		S1		S2	
	Concentrations mg/mL							
	15	30	15	30	15	30	15	30
	Diamètres des souches microbiennes en mm							
Escherichia coli ATCC 25922	-	7	-	-	-	9	-	8
Enterococcus faecalis ATCC 29212	-	-	-	-	-	-	-	-
Staphylococcus aureus ATCC 29231	-	7	-	6	-	10	-	8
Pseudomonas aeruginosa ATCC 27853	-	-	-	8	-	11	-	9

Table 3: Antibacterial activity of ethyl acetate extracts of Moringa oleifera Lam leaves against bacterial strains.

Souches bactériennes	Extraits							
	M1		M2		S1		S2	
	Concentrations mg/mL							
	15	30	15	30	15	30	15	30
	Diamètres des souches microbiennes en mm							
Escherichia coli ATCC 25922	-	7	-	7	-	11	-	8
Enterococcus faecalis ATCC 29212	-	-	-	-	-	-	-	-
Staphylococcus aureus ATCC 29231	-	8	-	7	-	9	-	7
Pseudomonas aeruginosa ATCC 27853	-	9	-	8	-	10	-	9

Table 4: Inhibition diameter of ethyl acetate extracts of *Combretum micranthum* G.Don leaves against bacterial strains.

Souches bactériennes	Extraits			
	M1	M2	S1	S2
	Concentrations mg/mL			
	80	80	80	80
Diamètres des souches microbiennes en mm				
Escherichia coli ATCC 25922	-	-	-	-
Enterococcus faecalis ATCC 29212	-	-	-	-
Staphylococcus aureus ATCC 29231	-	-	-	-
Pseudomonas aeruginosa ATCC 27853	14	-	-	-

Table 5: Minimum inhibitory concentration (MIC) of ethyl acetate extract from *Combretum micranthum* G.Don leaves against *Pseudomonas aeruginosa.*

Souches bactériennes	Extraits			
	M1	M2	S1	S2
	CMI (µg/mL)			
Pseudomonas aeruginosa ATCC 27853	9,71	-	-	-

Antifungal Activity

The results shown in Tables 6, 7, and 8 demonstrated the antifungal activity of the tested extract against two fungal strains (*Candida albicans, Candida krusie*).

An increase in the diameter of the inhibition zones of our extracts was proportional to the increase in concentration.

Table 6: Antifungal activity of hydromethanolic extracts of *Moringa oleifera* Lam leaves against Candida species.

Genres Candida	Extraits							
	M1		M2		S1		S2	
	Concentrations mg/mL							
	15	30	15	30	15	30	15	30
	Diamètres des souches microbiennes en mm							
Candida albicans ATCC 14053	-	7	-	-	-	10	-	7
Candida krusie ATCC 34135	-	-	-	-	-	-	-	-

Table 7: Antifungal activity of butanolic extracts of *Moringa oleifera* Lam leaves against Candida species.

Genres Candida	Extraits							
	M1		M2		S1		S2	
	Concentrations mg/mL							
	15	30	15	30	15	30	15	30
	Diamètres des souches microbiennes en mm							
Candida albicans ATCC 14053	-	-	-	8	-	11	-	9
Candida krusie ATCC 34135	-	-	-	-	-	-	-	-

Table 8: Antifungal activity of ethyl acetate extracts of *Moringa oleifera* Lam leaves against Candida species.

Genres Candida	Extraits							
	M1		M2		S1		S2	
	Concentrations mg/mL							
	15	30	15	30	15	30	15	30
	Diamètres des souches microbiennes en mm							
Candida albicans ATCC 14053	-	7	-	8	-	12	-	8

Candida krusie ATCC 34135	-	-	-	-	-	-	-	-

DISCUSSION

The antimicrobial activity of the various *Moringa oleifera* Lam extracts allowed us to obtain inhibition diameters of 7 and 8 mm against *Escherichia coli* with samples S2 and S1, respectively, compared to samples M1 and M2, which did not exhibit an inhibition zone with the hydromethanolic extract. With the same bacterial strain, we obtained inhibition diameters of 7, 8, and 9 mm with samples M1, S2, and S1, respectively, compared to sample M2, which did not exhibit any inhibition zone with the butanolic extract. However, with the ethyl acetate extract, we obtained inhibition diameters of 7, 7, 8, and 11 mm with all our different samples, M2, M1, S2, and S1, respectively. Our data are consistent with the work of Pawaskar and Sasangan. (2017) on aqueous extracts of *M. oleifera* leaves but with low concentrations ranging from 0.25 to 5 mg/mL. However, Vinoth et al., (2012); Udosen et al., (2016) who worked under the same study conditions did not obtain any inhibitory effect on *Escherichia coli*. Regarding *Staphylococcus aureus*, we obtained inhibition diameters between 7 and 9 mm with samples M1 and S1 respectively compared to samples M2 and S2 which had no inhibition zone with the hydro-methanolic extract. Furthermore, the butanolic extract gave us inhibition diameters between 7, 8 and 10 mm with samples M1, S2 and S1 respectively compared to sample M2 which had no inhibition zone. Regarding the ethyl acetate extract, we obtained inhibition diameters of 7, 7', 8 and 9 mm with all four of our samples. These results are also consistent with those found by Houfani and Kihal. (2022) who worked on the same microbial strains but with different extract concentrations ranging from 50-200 mg/mL. However, Millogo et al., (2012) found results higher than those found in our study, this could be explained by the use of another different extract. Regarding *Pseudomonas aeruginosa*, we note that samples M1, S2 and S1 have inhibition diameters of 7, 7' and 10 mm with the hydro-methanolic extract and sample M2 had no inhibition diameter. On the other hand, samples M1, S2 and S1 have inhibition zones of 8, 9 and 10 mm respectively with the butanolic extract compared to samples M2 which had no inhibition zone. However, samples M1, M2 and S1 have inhibition zones of 8, 9 and 10 mm respectively compared to samples S2 which had no inhibition zone with the ethyl acetate extract. The authors Pawaskar and Sasangan. (2017) as well as Fella and Hafida. (2018) found similar results to that found in our study with aqueous extracts with lower concentrations ranging from 0.25 to 5 mg/mL. As for Singh and Tafida. (2013) obtained inhibition diameters lower than those found in our study with the methanolic extract at a concentration of 30 mg/mL against *Pseudomonas aeruginosa*. Furthermore, Mashiar et al. (2009) worked with the cold water extract of fresh leaves, which showed a relatively better antibacterial effect against *Pseudomonas aeruginosa* and *Pseudomonas spp*., with recorded inhibition zones of 15 and 27.5 mm, respectively, which are also higher than the inhibition zones of our different extracts. Indeed, with *Enterococcus faecalis*, we did not obtain any inhibition diameter in all our different samples.

The antimicrobial activity of extracts differs in the nature of their chemical compounds, such as polyphenols, flavonoids, and tannins, as well as their levels, which can be considered highly active antibiotic compounds. Phenolic compounds are well known for their antibacterial activity, which has been reported by several authors (Cowan, 1999; Okuda et al., 2005; Taguri et al., 2006). Some authors have noted that the antibacterial activity of phenolic compounds is likely due to their ability to bind to soluble extracellular proteins and thus to bacterial cell walls (Tsuchiya et al., 1996). Variations in chemical composition therefore explain the observed variations in the antimicrobial activity of extracts from one plant to another. The optimal efficacy of an extract may not be due to a single main active constituent, but to the combined action of different compounds found in the extract (Yakhlef, 2010). Similarly, a trend was shown indicating that extracts richest in flavonoids possess the highest antibacterial activities (Beddou, 2015). To this end, we found that considerable antibacterial activity was recorded in samples S1 and M1 which are rich in flavonoids and tannins compared to samples M2 and S2. Thus, the absence of inhibition is probably due to the low levels of flavonoids and tannins in these different samples. Regarding *Candida albicans*, we note that samples M1, S2 and S1 possess almost similar inhibition zones of 7 and 10 mm respectively with the same concentration of extracts at 30 mg/mL with the butanolic extract compared to samples M2 which had no inhibition zone. With the butanolic extracts, we obtained inhibition diameters of 8, 9, and 10 mm with samples M1, S2, and S1, respectively. Sample M2 did not obtain an inhibition zone. Regarding the ethyl acetate extracts, we obtained inhibition diameters of 7, 8, and 10 mm with all of our different samples. As for *Candida krusie*, we obtained no inhibition diameters against all of our different samples. Our extracts demonstrated antifungal activity against *Candida albicans*, which is consistent with the results found by Houfani and Kihal (2022). However, Millogo et al. (2012) obtained more effective results against *Candida albicans*, even better compared to bacteria. Bacterial sensitivity to *Combretum micranthum* G.Don extract is classified according to the diameters of the inhibition zones. The antibacterial effect was observed only on *Pseudomonas aeruginosa* among all the bacterial strains tested. The results of the antibacterial test show the inhibition efficacy of the M1 extract alone against the 14 mm *Pseudomonas aeruginosa* compared to the other extracts, where we obtained no inhibition diameter and an MIC = 9.71 µg/mL. This value demonstrates the sensitivity of

the M1 extract against *Pseudomonas aeruginosa*, thus demonstrating its strong antimicrobial activity. Furthermore, with the other bacterial strains, no inhibition diameter was found with any of the extracts. This could be explained by the high content of flavonoids and polyphenols in the M1 sample, particularly their antioxidant properties. According to the literature, polyphenols exhibit antibacterial activities with distinct characteristics in their reactivity with proteins related to polyamide polymers (Haslam, 1996). The inhibition of microorganisms by phenolic compounds could be due to iron deficiency or hydrogen bonding with vital proteins such as microbial enzymes (Scalbert, 1991). Phenolic compounds, especially proanthocyanidins (often referred to as condensed tannins), are vulnerable to polymerization in air by oxidation reaction. Therefore, an important factor determining their toxicity is the size of their polymerization. Oxidized condensation of phenols can lead to toxicity of microorganisms. Conversely, polymerization can lead to detoxification of phenols (Scalbert. 1991; Fiel and Lettinga. 1992). These results support the hypothesis that polyphenols may be responsible for the antimicrobial activities of M1 extracts.

Our results are consistent with the study conducted by Karou et al., (2005) on the antimicrobial activity of *Combretum micranthum* G.Don leaves against pathogenic bacteria, thus illustrating a link between antioxidant capacity and antimicrobial activity as demonstrated in our study. However, leaf extracts of *Combretum micranthum* G.Don demonstrated microbicidal activity against *Shigella dysenteriae*, *Salmonella parathyphi* B. and *Staphylococcus aureus* and microbiostatic activity against *Shigella flexneri*, *Shigella boydii*, *Salmonella typhi*, *Klebsiella ozenae* and *Klebsiella pneumoniae*, unlike that obtained by our study for which we found antimicrobial activity with *Pseudomonas aeruginosa*. The variability of results may be due to the influence of several factors, such as the microorganisms tested, according to Pattnaik et al. (1996), however, Suhr and Nielsen (2003) mentioned that the antifungal effects of plant extracts depend on the application method.

CONCLUSION

The objective of this study was to evaluate the antimicrobial activity of leaf extracts from *Moringa oleifera* Lam and *Combretum micranthum* G.Don. The results of the different contents clearly indicate that the plant leaves are rich in nutrients and have the potential to be used as a food additive for multiple purposes. This abundance of nutrients and macronutrients in the dried leaves is an important nutritional indicator of the plant's potential use as a food resource. The plant derives its therapeutic capacity, such as antimicrobial and antioxidant power, from its primary and secondary metabolites or from the synergy between the two or between the different compounds of each type of metabolite. Among the secondary metabolites, phenolic compounds are distinguished, which are considered strong antioxidants and also antimicrobials. These two plants are said to have certain medicinal properties; their inclusion in diets could have a curative and therapeutic effect. As such, they can be used to improve health and nutrition in sub-Saharan countries.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest regarding this work.

AUTHOR CONTRIBUTION

MElBN, AD, and HT initiated the work to evaluate the antimicrobial activity of Moringa oleifera Lam and Combretum micranthum G.Don leaf extracts. MElBN wrote the article. SOS and DF supervised the laboratory work. YMD and BYK authorized the laboratory work. SOS, DF, YMD, RS, BYK, and AD facilitated the implementation of the work. AD reviewed and edited the article.

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