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Exploring the Bioactive Potential of *Tamarix africana*: Phytochemical Profiling, Antioxidant and Antibacterial Activities Assessment

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ABSTRACT

In Morocco, the *Tamaricaceae* family is represented by six species belonging to the *Tamarix* genus. including *Tamarix africana* which is utilized in traditional medicine to treat various ailments. This study aims to compare and evaluate the total polyphenol and flavonoid contents, as well as the antioxidant and antibacterial activity of *Tamarix africana* leaf and flower extracts obtained by Soxhlet extraction using five solvents of increasing polarity. The highest extraction yield was obtained with methanol for the leaves and flowers. Indeed, the results indicate that methanolic extracts contained the highest concentration of polyphenols and flavonoids for both organs (Polyphenols: 101.80 mg GAE/g DW in the leaf extract and 50.55 mg GAE/g DW in the flower extract. Flavonoids: 990.723 µg RE/g DW in the leaf extract and 630.84 µg RE/g DW in the flower extract). The results of antioxidant activity revealed that the aqueous extract of leaves and flowers of *T. africana* (IC_{50}: 1.89 µg/mL and 3.175 µg/mL respectively) had higher antioxidant activities than ascorbic acid. Concerning the antibacterial study, *Bacillus subtilis* showed resistance to the tested extracts. However, for the *Citrobacter freundii* strain, inhibition zones of 14 mm were recorded by the aqueous extract of flowers. On the other hand, the strong inhibition zones recorded against the *Enterococcus faecalis* strain, were 13 mm recorded by the leaves methanolic extract. Regarding the MIC, it is 6.25 mg/ml for the two strains. Concerning MBC, the results showed that the extracts are bacteriostatic in nature against *Citrobacter freundii* and *Enterococcus faecalis*. Thus, *Tamarix africana* seems to be a potential source of active molecules that could constitute a new alternative for medical and industrial use.

Keywords: *Tamaricaceae* family, active constituents, polyphenols, flavonoids, extraction, Morocco.

INTRODUCTION

Since time immemorial, medicinal plants have been utilized in almost every civilization as a source of medication (Devhade, 2015), and have helped many improve their health (Gezici and Sekeoglu, 2019). Plants have been and always will be a source of molecules used in therapy. The significance of plants in medicine is becoming increasingly pertinent, especially with the current global trend towards sourcing medicines from plant sources (Aliyu et al., 2008). Medicinal plants offer an excellent source of natural compounds known for their therapeutic properties (Bouyahya et al., 2017). Extensive research has highlighted the antioxidant (Ayda et al., 2012), anti-inflammatory, anti-diarrheal (Ksouri et al., 2009), and antibacterial properties (Adnan et al., 2015) of plant extracts. These diverse activities are attributed to the presence of phytochemical

compounds such as alkaloids, tannins, flavonoids, and terpenes (Samejo et al., 2013), each contributing to the plant's medicinal potential. Variations in biological activities observed among extracts can be attributed to differences in their chemical composition (Ghareeb et al., 2011, cited by Mgamat et al., 2024). Among these plants, certain species within the *Tamarix* genus have long been utilized in traditional medicine for their recognized astringent properties, alongside their ability to stimulate perspiration and act as diuretics (Lefahal et al., 2010; Abo-Dola et al., 2015; Bibi et al., 2015). Nonetheless, further comprehensive studies are warranted to delve into additional *Tamarix* species, elucidating their diverse biological activities and potential health benefits.

Morocco is home to six species of the genus *Tamarix* (*Tamaricaceae* family) that are distributed throughout the country (Fennane et al., 1999; Bihaoui et al., 2020a; 2020b; 2020c). Among these species, some are known in the southern regions of Morocco for their therapeutic virtues. Thus, the leaves and flowers of species of the *Tamarix* genus have been used in some Asian and African civilizations as anti-inflammatory, antidiarrheal, wounds, healing, and antiseptic agents, astringent, diuretic and sweat stimulants (Karker et al., 2016; Bahramsoltani et al., 2020). Sqalli et al. (2007) reported, the ability of leaf extracts of *Tamarix africana* to inhibit the growth of several mycobacteria responsible for tuberculosis. According to some previous studies, *T. africana* is rich in phenols (phenolic acids, tannins and flavonoids) (Benabdallah et al., 2014). In Algeria, these leaves have a traditional use in treating digestive system disorders and gastroduodenal diseases (Benabdallah et al., 2014; Hassiba et al., 2014). Indeed, the study conducted by Bechlaghem et al. (2019) unveiled the presence of numerous polyphenol compounds in the leaves of *T. africana*, including gallic acid, caffeic acid, rutin, chlorogenic acid, myricetin and quercetin. However, the effectiveness of these activities varies depending on the characteristics of the environment (attitude, climate) and human activity (Labhar et al., 2023). In addition, to our knowledge, no study has been conducted to evaluate the biological properties of *T. africana* extracts in Morocco. In other countries, the study conducted in this respect does not concern the extracts of the flowers. Therefore, additional studies are needed on this species.

This study was carried out to study the variations in the contents of extracts of leaves and flowers of *T. africana* in total polyphenols, total flavonoids, antioxidant activity, and to test these extracts on the growth of three bacteria: *Bacillus subtilis*, *Enterococcus faecalis* and *Citrobacter freundii*. In addition, this study attempts to compare these results with those of previous studies carried out in other localities in order to provide a database for future studies or potential applications in various fields.

MATERIAL AND METHODS

Plant material

The plant material (leaves and flowers) of *Tamarix africana* was collected in Ait Oudi (Tagzirt), 10 km from Beni Mellal. Identification of the species was conducted utilizing various identification keys (Baum, 1978; Fennane et al., 1999; Valdes et al., 2003) to ensure accuracy. Subsequently, the collected parts of the plant were meticulously dried and crushed to obtain a fine powder.

Preparation of extracts

A 20 gram portion of vegetable powder from leaves or flowers is enclosed in filter paper and inserted into the Soxhlet extractor. Subsequently, 120 mL of one of the designated extraction solvents (such as petroleum ether, dichloromethane, chloroform, methanol, or distilled water) is added to the flask before heating to boiling. The extraction temperature is adjusted according to the boiling temperature of each solvent. The extracts were filtered, concentrated by a rotary evaporator at 40 **°C,** and evaporated in a ventilated oven (40 °C) for 2 hours (Nasri, 2016). These extracts were then dried by leaving them in the fume hood for 24 hours.

Extraction yield

The extraction yield (%) was estimated as a percentage using the following equation (Boubekri, 2014; Nasri, 2016; Benamari et al., 2024):

$$Yield\ (\%) = \frac{m}{M} \times 100 \quad (1)$$

where: m – mass of dry matter, M – mass of extract obtained

Determination of the total phenolic content

According to the method described by Slinkard and Singleton in 1977, total phenolics

were evaluated using the Folin-Ciocalteu reagent. This involves adding 250 µL of each extract to 1.25 mL of the Folin-Ciocalteau reagent (10%) and adding 1 mL of sodium carbonate (7.5%). Thereafter, a 30 min incubation. The absorbance at 765 nm was determined. A blank underwent the same procedure, replacing the extract with 80% methanol. Total polyphenol content is reported in mg gallic acid equivalent per gram of dry weight (mg GAE/g DW) (Khoudali et al., 2014).

Determination of the total flavonoid content

The determination of flavonoid content followed the protocol outlined by Dewanto et al., 2002 (as cited by Boulaaba et al., 2013). This involved taking 75 µL of 5% $NaNO_2$ and adding it to 250 µL of each extract. After a 6 min delay, 150 µL of freshly prepared aluminum chloride ($AlCl_3$, $6H_2O$, 10%). Following a second incubation of 5 minutes, a volume of 500 µL of NaOH (1 M) was added, then 1.525 mL of distilled water was added to adjust the final volume to 2.5 mL. The absorbance at 510 nm was determined. The content was expressed in µg rutin equivalent per gram of dry weight (µg RE/g DW) (Boulaaba et al., 2013; Karker et al., 2016).

Antioxidant activity (DPPH)

A 50 µL portion of methanolic DPPH solution (0.2 mM) is combined with 200 µL of the extract at various concentrations (from 1.87 to 200 µg/mL). After incubating for 30 min in darkness (Bourgou et al., 2016), the absorbance at 540 nm was determined (Boly et al., 2016). Simultaneously, a negative control lacking extract (50 µL of 80 % methanol) and a positive control containing ascorbic acid are also subjected to analysis for comparison, following the same procedure as with the extracts. The anti-radical activity was calculated according to the following formula: (Linda and Manef, 2018; Jdey et al., 2017):

$$DPPH\ (\%) = \left(\frac{A_0 - A_1}{A_0}\right) \times 100 \qquad (2)$$

where: A_0 – absorbance of the black, A_1 – absorbance of the sample

Evaluation of antibacterial activity

Preparation of bacterial suspensions

The selected bacterial strains were subcultured using the streak method and subsequently incubated in the oven at 37 °C for 24 hours on a solid medium (Muller Hinton) to obtain a young culture for the preparation of the bacterial inoculum (Ben Tabet, 2015; Saffidine, 2015). Indeed, the concentration of the inoculum prepared is 0.5 Mc Farland (1–3 x 10^8 bacteria/mL; 10^8 CFU/mL) (Laouini, 2014).

Aromatogram

Sterile filter paper discs, each with a diameter of 6 mm, were positioned within petri dishes. These discs were then loaded with varying amounts of the extract under examination: 15 µL (equivalent to 1.5 mg of the extract), 10 µL (1 mg), and 5 µL (0.5 mg). Additionally, a disc soaked with 10 µL of DMSO served as a negative control. To serve as positive controls, discs of Gentamicin (10 µg) and Erythromycin (15 µg) were also included.

Determination of the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC)

The determination of MIC was done by micro-dilution on a plate (Carson et al., 1995 cited by Clément et al., 2009). The assay was conducted on a 96 well microtiter plate, Brifly, the plate was filled with 100 µL of sterile Mueller Hinton broth. Then 100 µL of each extract (100 mg/mL) was added to the first well of each row, and Cascade dilutions were conducted to generate 8 concentrations, ranging from 0.39 to 50 mg/mL. For the negative control, 100 µL of DMSO was utilized. Then, 100 µL of 10^8 CFU/mL inoculum was added to each well (Esmail et al., 2015; Bachiri et al., 2016). Then, the micro-dilution plate was placed in an incubator. The MIC is evaluated following an incubation period of 18 to 24 hours by direct reading with the naked eye. It corresponds to the small concentration at which turbidity induced by germ growth at the bottom of the wells is absent (Toty et al., 2013). The MBC was determined from the wells, which show no visible growth after incubation. For the determination of MBC, 5 µl of each well's contents were collected and plated on Mueller-Hinton agar. The antibacterial activity is indicated by the MBC/MIC ratio. Referring to the study of Fauchere and Avril (2002, cited by Ouattara et al., 2017): MBC/MIC ≤ 2, MBC/MIC > 2 represent bactericidal and bacteriostatic activities, respectively.

STATISTICAL ANALYSIS

The resulting data were transferred to SPSS software and further analyzed using a one-way ANOVA. Data are expressed as means ± standard

deviation (SD) of two separate experiments. When the p-value was less than 0.05, the differences were deemed statistically significant.

RESULTS AND DISCUSSION

Extraction yields

Five increasing polarity solvents were utilized to extract active components from *T. africana* leaves and flowers: petroleum ether, dichloromethane, chloroform, methanol, and distilled water. Once the extracts were dry, their yields were determined in relation to the initial quantity used. The results in Figure 1 represent the yield as a function of the extraction solvent used for each organ. These results showed that the organs of this species are richer in polar than apolar compounds. The results show that the highest extraction yield for leaves and flowers was obtained with methanol (11.355% and 8.445%, respectively), while the lowest extraction yield (1.16% and 1.325%, respectively) was recorded in petroleum ether extracts. However, the study of Bechlaghem et al. (2019), carried out on the leaves of *T. africana* in Algeria, showed that extraction by maceration presents the highest yield with 14.60 %. The differences in yield reported between our results and those of Bechlaghem et al. (2019), may be due to the variation in climatic conditions or the composition of the soil where the plants are developed (Linda and Manef, 2018). These differences may also be due to the solid/liquid extraction ratio (Wollinger et al., 2016), which is 0.166 in our case (1 gram of the plant material, leaf, or flower powder, extracted by 6 mL of the solvent), whereas in the study of Bechlaghem et al. (2019) it is only 0.05 (1 g/20 mL). Indeed, the larger the solid/liquid ratio, the smaller the extraction yield, which can be explained by the saturation of the solvent. These results are similar to those of Cacace and Mazza (2003, cited by Hyardin, 2008) who showed that a larger solvent/sample ratio indicates a higher extraction yield.

Determination of total phenolic content

In this part of the study, the evaluation of polyphenols in the extracts, indicates the existence of these phytochemical compounds in variable quantities. The analysis of variance (anova with one factor) carried out by the SPSS software, showed that the compound contents of the 10 extracts were significantly different. The phenolic contents of *T. africana* extracts show that the methanolic extracts have the highest polyphenol contents. This is consistent with the study of Chater et al. (2023), as phenolic chemicals are often polar. The maximum value is 101.80 mg GAE/g DW in the leaf extracts and 50.55 mg GAE/g DW in the flower extracts (Table 1) (Nounah et al., 2019).

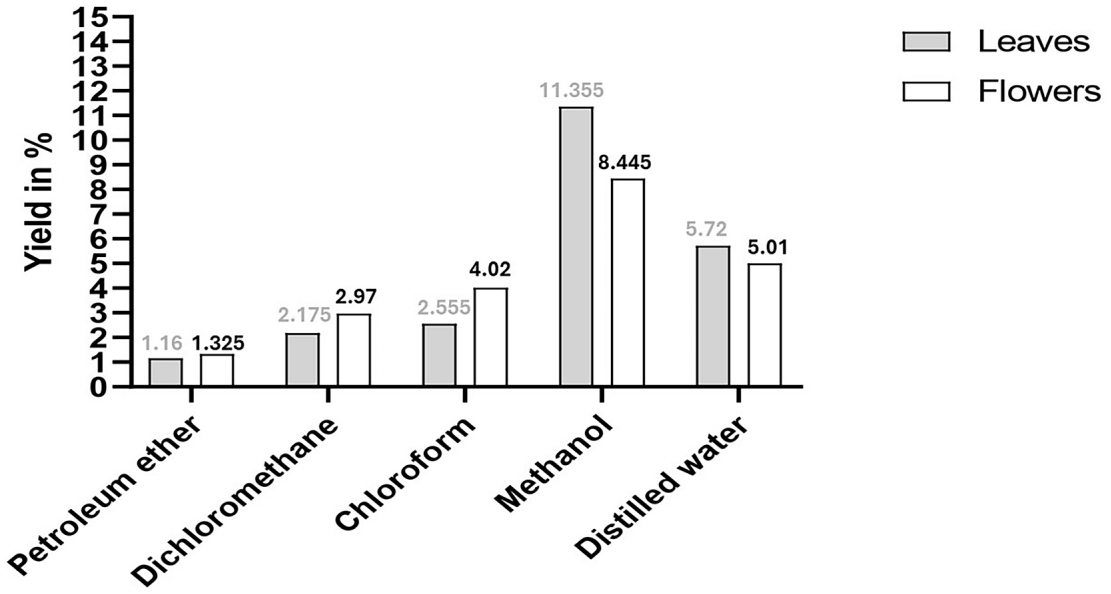

Figure 1. Percentage yield of plant extracts

Table 1. Total phenolic content of leaf and flower extracts of *Tamarix africana*

Extraction solvent	Leaves (mg GAE/g DW)	Flowers (mg GAE/g DW)
Petroleum ether	8.89 ± 1.42	6.25 ± 0.27
Dichloromethane	1.54 ± 0.09	1.11 ± 0.09
Chloroform	2.3 8± 0.07	3.05 ± 0.14
Methanol	101.8 ± 2.78	50.55 ± 0.58
Distilled water	53.03 ± 0.47	46.23 ± 0.02

For leaves, the differences in polyphenol content according to the solvents have been reported in other studies, but with different solvents. Thus, the study of Bechlaghem et al. (2019) indicates the best concentrations with aqueous extracts (61.06 mg GAE/g DW) and 19.75 mg GAE/g DW with methanolic extracts, which is in the order of 101.80 mg GAE/g DW in our study. However, this value is still lower than the value obtained in the methanolic extracts of *T. africana* shoots reported by Karker et al. (2016) (151.1 mg GAE/g DW).

The variations could be attributed to environmental conditions in the habitat, including factors like elevated temperatures, intense sunlight, drought, and salinity. These conditions encourage the production of secondary metabolites, including polyphenols (Falleh et al., 2008). Let us also add the effect of the development stage of the plant material used in the extraction. In this study, the extraction was performed on the leaves and flowers of *T. africana*, unlike the study of Karker et al. (2016), who studied on the shoots of this species without defining the organ used in the extraction. Thus, the effect of the combination of extraction solvents in the study of Bechlaghem et al. (2019).

Determination of total flavonoid content

The results of the flavonoid determination of the extracts obtained by Soxhlet show that all the extracts contain flavonoids, but with different contents. For the leaves, they vary between 170,752 µg RE/g DW recorded in the petroleum ether extract and 990,723 µg RE/g DW in the methanolic extract, while the results of the extracts of the flowers show weaker values of 153,53 µg RE/g DW to 630,84 µg RE/g DW (Table 2). This suggests that non-polar solvents may not be effective for extracting phenolic chemicals (Chater et al., 2023). The differences obtained in phenolic compound concentration between the two organs can be attributed to differences in the composition of phenolic compounds in different parts of the plant (Chater et al., 2023).

Nevertheless, methanolic and aqueous extracts of *T. africana* leaves recorded weaker flavonoid contents than those reported by Bechlaghem et al. (2019) (2.21 and 1.05 milligram quercetin equivalents per gram dry weight, respectively). The same difference is noted in dichloromethane extract. In addition, the study conducted by Karker et al. (2016) on *T. africana* shoots showed that the flavonoid content of the methanolic extract is very high, with a concentration of 23.9 milligrams of catechin equivalent per gram of dry weight. The disparity between these results and those published in earlier research can be attributed to the same factors mentioned in the case of the determination of polyphenols without neglecting the effect of using different standard ranges. In our study, we used rutin, while Karker et al. (2016) Bechlaghem et al. (2019) used catechin and quercetin, respectively. This hypothesis can be reinforced, by the results of Dghim et al. (2014) study, which demonstrated that some extracts have much higher condensed tannin contents than total polyphenol contents.

Antioxidant activity of extracts

DPPH is the most fluently used substrate for rapid and direct measurement of antioxidant activity, due to its free radical stability and ease of analysis (Haddouchi and Halla, 2016). Various medicinal plants have been studied as potential sources of natural antioxidants (Nekhla et al., 2023). In methanolic and aqueous extracts of *T. africana* leaves, antioxidant compounds such as chlorogenic acid, caffeic acid and quercetin were identified by Bechlaghem et al. (2019). In this part of the study, the DPPH inhibition results of the various extracts show that the majority of the

Table 2. Flavonoid content of leaf and flower extracts of *Tamarix africana*

Extraction solvent	Leaves (ug RE/g DW)	Flowers (ug RE/g DW)
Petroleum ether	170.752 ± 5.741	153.53 ± 1.35
Dichloromethane	517.831 ± 5.126	403.92 ± 1.75
Chloroform	390.063 ± 7.527	332.66 ± 17.77
Methanol	990.723 ± 66.909	630.84 ± 11.94
Distilled water	384.380 ± 4.044	258.015 ± 30.997

Table 3. Antioxidant activity (DPPH) of leaf and flower extracts of *Tamarix africana*

Extraction solvent	Leaves (IC_{50}, µg/mL)	Flowers (IC_{50}, µg/mL)
Petroleum ether	11.315 ± 0.190	18.625 ± 0.308
Dichloromethane	124.085 ± 0.714	173.625 ± 14.10
Chloroform	79.725 ± 7.035	121.825 ± 12.551
Methanol	129.99 ± 1.172	6.825 ± 0.207
Distilled water	1.89 ± 0.339	3.175 ± 0.31

extracts exhibit the capacity to scavenge free radicals. The IC_{50} values of *T. africana* extracts are shown in Table 3. These results indicate that the aqueous extracts of the leaves and flowers (IC_{50}: 1.89 and 3.175 µg/mL respectively) are more effective than ascorbic acid (IC_{50} = 5.23 µg/mL). For the extracts obtained from the petroleum ether of the leaves and the flowers, as well as the chloroformic extract of the leaves and the methanolic extract of the flowers, they also show an important activity that varies between 6.825 µg/mL and 79.725 µg/mL. Regarding the extracts from dichloromethan and methanol extraction of leaves, dichloromethane, and chloroform extracts of flowers, show average activities that vary between 121.825 and 173.625 µg/mL.

These results show that the extracts of *T. africana* leaves exhibit variable capacities to scavenge DPPH radicals, depending on the solvent used. In addition, the antioxidant activities recorded are higher compared to those reported in the work of Bechlaghem et al. (2019) (between 0.74 and 4.83 mg/mL).

Regarding the relationship between polyphenol content and radical scavenging activity (DPPH), research has indicated that there is a close relationship between the antioxidant activity of extracts and their polyphenol content (Suleiman et al., 2019). In addition, Dillard and German (2000; cited in Tabet et al., 2018) suggest that the high polyphenol content may also be responsible for the high DPPH radical scavenging action, but this assumption should not be generalized. However, Bourgou et al. (2008), Zeng et al. (2013), Kasangana et al. (2015) and Khiraoui et al. (2018), have shown different results. This applies to what we found in this study.

In 2011, Bouzid et al. (2011) showed that for the β-carotene bleaching test, extracts with low polyphenol content show significant antioxidant activity compared to high content extracts, which, according to the author, is related to qualitative differences in polyphenol compounds in the extracts. Furthermore, this can be explained by the fact that the extract may contain minor polyphenol compounds that should not be neglected, as the synergy between them must be considered in the biological activity. Moreover, the antioxidant activity is affected by both the molecular structure and variations in the quantity and placement of hydroxyl groups on the aromatic ring (Bourgou et al., 2008). Phenolic-OH groups can give oxygen atoms, forming pairs with unpaired electrons. This action reduces the overall number of unpaired electrons and aids in the scavenging of DPPH (Benamari et al., 2024). On the technical side, the difference between the results obtained and the research results of Bechlaghem et al. (2019), may result from the application of different measurement and testing techniques to evaluate antioxidant activity, which reduces the reliability of the comparison of values (Popovici et al., 2009).

Antibacterial activities: disc diffusion method

The disc diffusion technique was used to examine the antibacterial activity of the ten extracts from the leaves and flowers of *T. africana*. Which provides a preliminary idea about the ability of an extract to inhibit microbial growth (Abdallah et al., 2019). The results obtained show an inhibition of bacterial growth that is proportional to the diameter of the inhibition zone and an uneven activity of the extracts used on the bacterial strains tested. Overall, the extracts were more active against *Citrobacter freundii* than *Enterococcus faecalis*. On the other hand, *Bacillus subtilis* showed resistance to the tested extracts, this is due to several factors, such as: the type of microorganism, the concentration, the type of extract and especially the nature and molecular structure of the biologically active molecules of the secondary metabolites (Ghedadba et al., 2014). Tables 4 to 7 show the relationship between concentrations and antibacterial effects and compare the inhibition zones of the extracts to those of the antibiotics. For *Citrobacter freundii*: among the 10 extracts tested, 7 extracts showed a remarkable effect, they exerted antibacterial activity at most of the concentrations used, which manifests itself with a diameter of the inhibition zone which is between 6.25 mm and 14 mm,

Table 4. Diameters of the inhibition zones (mm) illustrating the antibacterial activity of *T. africana* extracts against *Citrobacter freundii*

Extraction solvent	Leaves (inhibition diameter in mm)			Flowers (inhibition diameter in mm)		
	15 µL (1.5 mg/mL)	10 µL (1 mg/mL)	5 µL (0.5 mg/mL)	15 µL (1.5 mg/mL)	10 µL (1 mg/mL)	5 µL (0.5 mg/mL)
Petroleum ether	13.25 ± 1.75	10 ± 1	9.5 ± 0.5	9.75 ± 0.25	7.75 ± 0.25	8.25 ± 0.75
Dichloromethane	9.5 ± 1	7.75 ± 0.25	6.5 ± 0	10 ± 1	7.75 ± 0.25	6.25 ± 0.25
Chloroform	–	–	–	–	-	–
Methanol	13.75 ± 1.25	12 ± 1	9.5 ± 1.5	11.25 ± 1.25	10.25 ± 1.75	7.25 ± 0.75
Distilled water	–	–	–	14 ± 1.5	9.75 ± 0.75	7 ± 0.75

Table 5. Diameters of the inhibition zones (mm) illustrating the antibacterial activity of some antibiotics against *Citrobacter freundii*

Microorganism	Antibiotic	
	Gentamicin (10 µg/disc)	Erythromycin (15 µg/disc)
Citrobacter freundii	16.1	–

Table 6. Diameters of the inhibition zones (mm) illustrating the antibacterial activity of *T. africana* extracts against *Enterococcus faecalis*

Extraction solvent	Leaves (inhibition diameter in mm)			Flowers (inhibition diameter in mm)		
	15 µL (1.5 mg/mL)	10 µL (1 mg/mL)	5 µL (0.5 mg/mL)	15 µL (1.5 mg/mL)	10 µL (1 mg/mL)	5 µL (0.5 mg/mL)
Petroleum ether	11.75 ± 0.25	9.5 ± 0.5	8.25 ± 0.25	-	-	-
Dichloromethane	11.5 ± 1	9 ± 0.5	-	11.75 ± 0.75	10.25 ± 0.75	8.75 ± 0.75
Chloroform	-	-	-	8	7.75 ± 0.25	-
Methanol	13 ± 1.5	11 ± 1	7.75 ± 0.25	10.75 ± 1.25	9.25 ± 0.75	7.75 ± 0.25
Distilled water	-	-	-	-	-	-

Table 7. Diameters of the inhibition zones (mm) illustrating the antibacterial activity of some antibiotics against *Enterococcus faecalis*

Microorganism	Antibiotic	
	Gentamicin (10 µg/disc)	Erythromycin (15 µg/disc)
Enterococcus faecalis	22.4	16.5

allowing this strain to be classified as sensitive and very sensitive according to Himed et al., (2016). The effect of these extracts is slightly less significant than that recorded by Gentamicin (10 µg/disc; 16.1 mm). The greatest inhibition zones were obtained for volumes of 15 µL (1.5 mg). This agrees with the results obtained by Ogbulie (et al., 2007). Testing the ethanolic extract of *Euphorbia hirta* on *E. coli*, *S. aureus* and *P. aeruginosa*, the authors found that increasing the concentration in the disc from 50 to 250 mg/mL was accompanied by a notable rise in the inhibitory zone's diameter. *Citrobacter freundii* can be qualified as sensitive to all extracts at 1.5 mg/disc and sensitive to some extracts for volumes of 0.5 mg/disc and 1 mg/disc. On the contrary, Erythromycin (15 µg/disc) showed no activity. According to the study carried out by Mignanwandé et al. (2023) on the aqueous extract of Crateva adansonii, the *Citrobacter freundii* strain showed resistance to the extracts at concentrations of 5 mg/ml and 10 mg/ml. But for a concentration of 20 mg/ml, the strain is considered very sensitive with

an inhibition zone of around 14 mm. Equal to the value obtained with the aqueous extract of *T. africana* flowers, but with a lower concentration (15 ul = 1.5 mg/ml). Regarding *Enterococcus faecalis*: among the 10 extracts tested, only 6 were found to be active. Concerning the antibacterial activity of most of the concentrations used, the bacterial strain is classified as sensitive to very sensitive, according to Himed et al. (2016). For 1.5 mg/disc of the extracts, *T. africana* leaves showed the highest zone of inhibition (13 mm) by the methanolic extract. For the extracts obtained from the flowers of this species, it varies between 8 mm for the methanolic extract and 11.75 mm for the dichloromethane extract. These antibacterial activities are low compared to those recorded by Erythromycin (15 µg/disc; 16.5 mm) and Gentamicin (10 µg/disc; 22.4 mm). While that, most of the values obtained in our study are high compared to those obtained by Yazdi (et al., 2014), where different concentrations (from 5 mg/ml to 15 mg/ml) of Lavandula stoechas leaf extracts have inhibited the bacterial growth of Enterococcus faecalis with a diameter that is between 8 mm and 10.9 mm for aqueous extracts and 7.3 mm to 10 mm for ethanolic extracts.

Determination of MIC and MBC

The microplate method allowed us to determine the MIC in concentrations ranging from 6.25 mg/mL. According to the results obtained in our study, the bacteria present a different sensitivity towards the different extracts (Tables 8 and 9). For *Citrobacter freundii*, three extracts presented the highest activity with MIC values of 6.25 mg/mL, while only one extract for the *Enterococcus faecalis* strain.

We found that the MIC values are equal between the two strains (from 6.25 mg/mL) *Citrobacter freundii* and *Enterococcus faecalis*. Therefore, based on Toyang et al., (2012 cited by Saffidine, 2015), extracts with MICs greater than 1 mg/mL are considered inactive. According to this classification, all the extracts tested, can be considered inactive extracts.

The high MIC value obtained is due to several factors, including the effect of the concentration of the bacterial suspension used. In the present study, it is 10^8 bacteria/mL (10^8 CFU/mL), while in the study reported by Chaouche (2014), Abedini (2013) and Ouattara et al., (2017) it is 5×10^5 CFU, 10^4 bacteria/mL, and 10^6 CFU/mL respectively. Let us also add the

Table 8. Minimum inhibitory concentrations of *T. africana* extracts against *Citrobacter freundii*

Organ	Extract	Concentration in mg/mL							
		50	25	12.5	6.25	3.125	1.56	0.78	0.39
Leaves	Petroleum ether	-	-	-	-	+	+	+	+
	Dichloromethane	-	-	-	+	+	+	+	+
	Methanol	-	-	-	-	+	+	+	+
Flowers	Petroleum ether	-	-	+	+	+	+	+	+
	Dichloromethane	-	-	+	+	+	+	+	+
	Methanol	-	-	-	+	+	+	+	+
	Distilled water	-	-	-	-	+	+	+	+

Table 9. Minimum inhibitory concentrations of *T. africana* extracts against *Enterococcus faecali*

Organ	Extract	Concentration in mg/mL							
		50	25	12.5	6.25	3.125	1.56	0.78	0.39
Leaves	Petroleum ether	-	-	-	-	+	+	+	+
	Dichloromethane	-	-	-	+	+	+	+	+
	Methanol	-	+	+	+	+	+	+	+
Flowers	Dichloromethane	-	-	+	+	+	+	+	+
	Methanol	-	-	+	+	+	+	+	+
	Distilled water	-	-	-	+	+	+	+	+

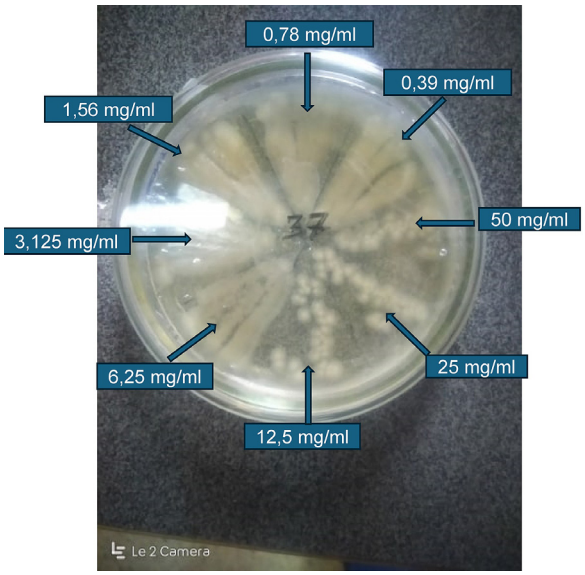

Figure 2. Determination of the minimum bactericidal concentration (MBC) of *T. africana* extracts

effect of the volume of extracts, all these factors can affect the value of MBC.

For MBC, the results obtained in this study, we find that the bacteria were not inactivated in the presence of the plant extracts (Figure 2). The MBC/MIC ratio is higher than 2 for both strains studied. Therefore, the extracts of both species exert a bacteriostatic action against these strains.

CONCLUSIONS

The present study reports for the first time the quantification of some chemical entities (polyphenols and flavonoids), antioxidant, and antibacterial activities of 10 extracts obtained by Soxhlet extraction of leaves and flowers of *T. africana*. The results obtained in this study show that the leaves and flowers are rich in polyphenol compounds, and that several extracts possess antioxidant activities superior to those of ascorbic acid.This study also evaluated the antibacterial activity of the two aerial parts of *T.africana* on three bacterial strains and determined the MIC and MBC. The marked differences may be due to the phytochemical nature of the substances they contain. Based on these findings, *T. africana* emerges as a promising reservoir of natural bioactive compounds, indicating its potential for medicinal applications. Further investigations are imperative, necessitating purification or fractionation processes to isolate and identify the specific bioactive molecules accountable for the observed activities.

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