Contribution to phytochemical, antioxidant and antibacterial studies of extracts of crushed, sieved and rejected organs of two medicinal plants from Côte d'Ivoire

ABSTRACT

Aims : This research work was carried out on the Broyat, Tamisat and Refus of the whole plant *Heterotis rotundifolia* and the leaves of *Manihot esculenta*, two medicinal plants from Côte d'Ivoire, with the aim of establishing their phytochemical, antioxidant and antibacterial profiles.
Methodology : To this end, crude extracts obtained by Soxhlet extraction and selective liquid-liquid contact were prepared. For phytochemical screening, the selective extracts were used for TLC, while the ethanolic crude extracts were used for color reaction identification tests (in tube), total polyphenols, total flavonoids and condensed tannins, as well as for their antioxidant potential and antibacterial action. Antioxidant activity was assessed spectrophotometrically using the DPPH radical, while antibacterial activity was estimated against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa strains.*
Results : Phytochemical screening results show that plant extracts contain polyphenols, flavonoids, tannins, terpenes, sterols and sugars. The Broyat yields a greater number of extractable phytocompounds than the Tamisat and the Refus, regardless of the type of extraction solvent and plant species used. Tamisat contains the highest number of compounds of interest, and therefore displays greater antioxidant potential against DPPH and antibacterial activity against *Staphylococcus aureus.*
Conclusion : At the end of this study, it emerged that it would be preferable to use the Broyat, for phytochemical studies, and the Tamisat for biological studies.

Keywords : Heterotis rotundifolia ; Manihot esculenta ; antioxidant ; antibacterial ; studies

1. INTRODUCTION

Plant extracts have long been used in various medicinal and therapeutic applications. The growing emphasis on natural compounds and their health-promoting properties has led to a significant increase in studies aimed at better understanding their chemical composition and biological activities [1]. A crucial step in the analysis of organic compounds present in plants is extraction. Different extraction methods and techniques have been developed to obtain extracts rich in bioactive compounds. Extraction is influenced by the type of solvent, temperature, liquid-solid ratio, pH, pressure, number of extraction steps and particle size [2, 3]. Indeed, particle size refers to the size of the particles in a material, and in this specific case, it is the size of the particles of crushed and sieved plant organs used in the extraction. When plant organs are ground, they can be reduced into different particle sizes which can then be sieved. In order to optimize the yield of the extraction of secondary metabolites from plant organs, some experimenters prefer to use Broyat to carry out the extraction while others opt instead for Tamisat. Therefore, we were interested in knowing which of the plant meshes (Broyat, Tamisat, Refus) would be likely to give the best results both in terms of the extraction of phytocompounds and the biological profile. It is in this context that this study was initiated. The present work aims to evaluate the influence of the size of particles from plant matrices on the extraction of organic phytocompounds and their biological activity. To achieve this, it was specifically necessary to extract the phytoconstituents from Broyat, Tamisat and Refus from the study plants; to carry out qualitative and quantitative phytochemical screening then to evaluate the antioxidant and biological potential of the plant mesh.

2. MATERIAL AND METHODS

2.1 Material

2.1.1 Plant material

The plant material consists of the leaves of *Manihot esculenta* and the whole plant *Heterotis rotundifolia.* The harvest was made on the site of Nangui ABROGOUA University (UNA) (Abidjan / Ivory Coast). The plant material collected was identified by referring to the species in the herbarium of the National Floristic Center (CNF) of the Félix HOUPHOUËT-BOIGNY University (Cocody / Abidjan), then authenticated by Professor MALAN Djah François (Systematic botanist at the UNA). The plant material was cleaned and dried for 2 days away from the sun in a ventilated room then in a room with air conditioning (18°C) for 7 days and in an oven (45°C) for 3 days.

2.1.2 Laboratory equipment

The laboratory equipment consists of usual laboratory glassware, an oven, a UV-254/366 nm lamp (VL-6.LC), an electronic balance (XY6002C), a rotary evaporator brand (BÜCHI type EL-131), a refrigerator, a dryer, a UV-visible spectrophotometer (Spectro AL 800), a hood, an electric grinder (Nutri Blitzer GS 158- V1457). To carry out Thin Layer Chromatography (TLC), chromatoplates (silica gel 60 F254, aluminum support, 20 × 20) (Merck) were used.

2.1.3 Chemicals

The chemicals used were purchased from Ryca Pharma, Polychemistry and Chimtec. The organic solvents used have a purity of 96%.

2.1.4 Biological material

The bacterial strains (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*) were provided by the biobank of the Pasteur Institute of Côte d'Ivoire (IPCI). They were selected because of their recurrent involvement in a good number of human pathologies.

2.2 Methods

2.2.1 Acquisition of powders of different particle sizes

After drying, the plant material was pulverized using an electric grinder (nutri blitzer GS 158-V1457) to provide powders called “Broyat”. Subsequently, the Broyat was passed through a sieve with a diameter of less than 1 mm to obtain on the one hand, "Tamisat", and on the other hand "Refus" which constitutes the powder having been retained in the sieve. The vegetable meshes (Broyat, Tamisat, Refus) were used to prepare the different extracts.

2.2.2 Soxhlet extraction

A Whatman cartridge containing 5 g of vegetable powder is introduced into the extraction chamber, connected at the top to a condenser, and at the bottom to a ground-neck flask containing 300 mL of solvent. Petroleum ether, ethyl acetate and ethanol were used as extraction solvents, respectively. This operation was repeated 3 times. After vacuum filtration, the crude ethereal, ethyl acetate and ethanolic extracts are concentrated using a rotary evaporator at 40°C, then stored in vials protected from light. The yields of the different extractions are calculated.

For the rest of the work, the crude hydroethanolic extracts were used on the one hand for the preparation of selective extracts and on the other hand, for phytochemical screening by colored reactions, the quantification of phenolic compounds (polyphenols, flavonoids and total tannins), and for the evaluation of the antioxidant and antibacterial potential.

2.2.3 Preparation of selective extracts

A volume of 15 mL of the crude hydroethanolic extract was exhausted by successive fractionations with (3 × 10 mL) hexane, (3 × 10 mL) dichloromethane and (3 × 10 mL) ethyl acetate. The different selective organic filtrates are concentrated in the oven then stored in the refrigerator at 4°C. They were used for phytochemical screening by TLC.

2.2.4 Qualitative tests in tube and on TLC plate

To carry out colored tube tests on crude hydroethanolic extracts, the analytical techniques used are those described in the literature [4-6].

The TLC screening of the selective extracts was carried out following the methods described in the literature [7].

2.2.5 Quantitative analysis

2.2.5.1 Dosage of total phytophenols

Total polyphenol contents were determined using the Folin-Ciocalteu colorimetric method [8]. To 1 mL of each extract are added 1.5 mL of sodium carbonate (Na_2CO_3) (17%, m/v) and 0.5 mL of Folin-Ciocalteu reagent (0.5N). The whole is incubated at 37°C for 30 min and the absorbance is read at 760 nm on the spectrophotometer against a blank without plant extract taken as a reference. The quantification of total phytophenols is carried out according to a linear calibration line (y= ax + b) carried out by a standard extract of gallic acid at different concentrations (0 to 1000 µg/mL) under the same conditions that the sample.

2.2.5.2 Dosage of total flavonoids

This assay was carried out according to the method used by Arvouet-Grand and colleagues [9]. 500 µL of 2% aluminum chloride ($AlCl_3$) in methanol was added to an equal volume of plant extract. After 10 min of incubation, the reading was taken with a spectrophotometer at 415 nm. Quercetin (0-100 µg/L), used as a standard, allowed the establishment of the calibration curve. Three (3) tests were carried out for each plant extract, and the result given is an average of the 3 readings.

2.2.5.3 Dosage of condensed tannins

The determination of condensed tannins in the different plant extracts was carried out according to the method described by Heimler and colleagues [10]. For 400 µL of each plant or standard sample, 3 mL of a 4% methanolic vanillin solution and 1.5 mL of concentrated HCl are added. The mixture is incubated for 15 min and the absorbance is read at 500 nm on the spectrophotometer. Condensed tannin contents are deduced from the calibration curve established with catechin.

2.2.6. Evaluation of antioxidant potential by the DPPH test

The method used is that of Brand-Williams and colleagues [11] with slight modifications in terms of concentrations. DPPH is solubilized in absolute ethanol to obtain a solution with a concentration of 0.03 mg/mL. Different concentration ranges of each plant extract are prepared with the same solvent (1 mg/mL, 0.5 mg/mL, 0.25 mg/mL; 0.125 mg/mL; 0.0625 mg/mL; 0.03125 mg /mL; 0.015625 mg/mL; 0.007812 mg/mL; ; 0.003906 mg/mL and 0.001953 mg/mL). In dry and sterile tubes, 1 mL of plant extract solution to be analyzed and 2 mL of DPPH solution are introduced. After shaking, the tubes are placed away from light for 30 min. The absorbance of the mixture is measured at 517 nm with a spectrophotometer against a blank (2 mL of DPPH solution + 1 mL of absolute ethanol). The positive reference control used is ascorbic acid (vitamin C) prepared under the same conditions as the samples.

2.2.7. Biological study

2.2.7.1. Preparation of the inoculum for the efficacy test

Preparation of the bacterial inoculum began with the collection of a well-isolated bacterial colony 18 to 24 hours later. It was homogenized in 2 mL of sterile physiological water in order to obtain an optical density of 0.5 Mc Farland, with a bacterial population of approximately 106 CFU/mL. This inoculum was used to inoculate the surface of the Mueller Hinton agar plates in tight streaks using a swab to carry out the tests [12].

2.2.7.2. Testing the effectiveness of extracts

On the agar previously inoculated, the extracts to be tested with a concentration of 100 mg/mL were placed in the previously prepared wells. The antibiotics (Cefotaxime (COX), Ceftazidime (CZD) and Cefoxitin (FOX)) involved in the treatment of pathologies linked to the bacterial strains to be tested served as positive controls. They were also placed on the agar. Sterile distilled water constituted the negative control [13,14].

2.2.7.3. Determination of the Minimum Inhibitory Concentration (CMI)

One hundred microliter (100 µL) of bacterial inoculum is distributed into the microplate wells. To these quantities are added 100 µL of the different concentrations (C_1 to C_6) of the extracts to be tested, from the highest concentration to the lowest with the exception of the wells of the growth control (Tc) in which only 100 µL of sterile distilled water (the total volume of each well being 200 µL). Concerning the wells reserved for sterility witnesses (Ts), only 200 µL of distilled water are added. The microplate is subsequently incubated at 37°C for 24h. The minimum inhibitory concentration (CMI) corresponds to the concentration of the first experimental well from which no disorder is observed with the naked eye. This operation was repeated 3 times in a row [15].

2.2.7.4. Determination of the minimum bactericidal concentration (CMB)

First, 4 successive dilutions at 1/10 from 10^{-1} to 10^{-4} are made from the starting inoculum. These dilutions and the inoculum are inoculated in 5 cm streaks on the different MH agar plates, then incubated at 37°C for 24 h. These Petri dishes constituted the A dishes [14]. Then, the wells of the microplate in which no disorder is observed with the naked eye are seeded in 5 cm streaks on the MH agar plates, starting with the CMI tube. They are then incubated at 37°C for 24 hours. These boxes constituted boxes B. The CMB corresponds to the lowest concentration of extract for which there is at most 0.01% of surviving bacteria. It is read by comparing box B to box A [15].

3. RESULTS AND DISCUSSION

3.1. Extraction yields

The phytocompounds present in the different powders studied were extracted using a Soxhlet with 3 types of solvents (petroleum ether, ethyl acetate and ethanol). The yields of these extractions are recorded in Tables 1, 2 and 3.

Table 1. Percentages obtained from petroleum ether extracts

Plant species	Yield (%)		
	Broyat	**Tamisat**	**Refus**
M. esculenta	17.40 ± 0.00	16.00 ± 0.00	17.20 ± 0.09
H. rotundifolia	15.73 ± 0.31	7.87 ± 0.09	11.80 ± 0.13

Table 2. Percentages obtained from ethyl acetate extracts

Plant species	Yield (%)		
	Broyat	**Tamisat**	**Refus**
M. esculenta	13.80 ± 0.00	13.40 ± 0.27	11.80 ± 0.00
H. rotundifolia	16.07 ± 0.22	10.60 ± 0.27	10.40 ± 0.27

Table 3. Percentages obtained from ethanol extracts

Plant species	Yield (%)		
	Broyat	**Tamisat**	**Refus**
M. esculenta	20.47 ± 0.36	14.67 ± 0.22	18.53± 0.22
H. rotundifolia	17.60 ± 0.27	10.60 ± 0.40	13.53 ± 0.09

The Soxhlet extractions carried out in this work made it possible to obtain variable extract yields depending on the botanical nature of the plant species, the type of solvent and the type of powder (Broyat, Tamisat or Refus) used for the different extractions. Considering the results of the yields obtained, ethanol is the solvent which best extracts the phytocompounds unlike ethyl acetate and petroleum ether. The extractable power of a solvent being linked to its polarity, these results are therefore due to the fact that ethanol is more polar than ethyl acetate and itself more polar than petroleum ether. We note that whatever the plant species and the type of solvent used, the extraction yields of Broyat are higher than those of Tamisat and Refus. Indeed, grinding allows better accessibility to compounds by increasing their bioavailability while sieving induces a differential distribution of active compounds depending on particle size [16].

3.2. Qualitative phytochemical profiles

The results of the detection tests by color tube reactions and by TLC of the secondary metabolites of the extracts of *M. esculenta* and *H. rotundifolia* powders are recorded in Table 4.

Table 4. Summary of qualitative analyzes in tube and by TLC

	PP	Flav	Coum	Tan	Ste/Ter	Sap	Suc	Cp. Red	Ac. Ph	Pro
BMe	+	+	+	+	+	-	+	-	-	+
TMe	+	+	+	+	+	-	+	-	-	+
RMe	+	+	+	+	+	-	+	-	-	+
BHr	+	+	+	+	+	-	+	-	+	+
THr	+	+	+	+	+	-	+	-	+	+

RHr	+	+	+	+	+	-	+	-	+	+

PP : Polyphenols; Flav: Flavonoids; Coum: Coumarins; Tan: Tannins; Ste/Ter: Sterols/Terpenes; Sap: Saponins; Suc: Sugars; Cp. Red : Reducing compounds ; Ac. Ph : Phenolic acids; Pro: Protein; BMe: Broyat of *M. esculenta*; TMe: Tamisat of *M. esculenta*; RMe: Refus of *M. esculenta*; BHr: Broyat of *H. rotundifolia*; THr: Tamisat of *H. rotundifolia*; RHr: Refus of *H. rotundifolia*

Taking into account the results obtained at the end of the qualitative study, we note that the extracts of *M. esculenta* and *H. rotundifolia* contain polyphenols, flavonoids, coumarins, tannins, sterols, terpenes and proteins. Phenolic acids were observed only in *H. rotundifolia*. Furthermore, the qualitative analysis carried out by Etekpo [17] on the plant species *H. rotundifolia* indicated the presence of sterols, steroids, terpenes, triterpenes, 1,8-diantracene, coumarins, anthocyanins, flavonoids, tannins, phenolic acids, saponins and alkaloids.
In addition, the Broyat, Tamisat and Refus of the study extracts are made up of the same phytoconstituents. This qualitative study therefore does not make it possible to predict which Broyat, Tamisat or Refus contains the most phytocompounds.

3.3. Contents of phytophenols, total flavonoids and condensed tannins

Tables 5-7 present varied contents of phytophenols, total flavonoids and condensed tannins of the hydroethanolic extracts obtained from Broyat, Tamisat and Refus from the leaves of *Manihot esculenta* and the whole plant *Heterotis rotundifolia*.

Table 5. Total phytophenol contents of hydroethanolic extracts

Extracts	Total phytophenol contents (mg EAG/g DM)
BMe	146.24 ± 3.67
TMe	84.10 ± 2.23
RMe	92.85 ± 0.12
BHr	53.29 ± 0.60
THr	43.98 ± 0.44
RHr	57.13 ± 1.31

Table 6. Total flavonoid contents of hydroethanolic extracts

Extracts	Total flavonoid contents (mg EQ/g DM)
BMe	20.77 ± 1.74
TMe	23.49 ± 1.15
RMe	7.68 ± 0.62
BHr	3.76 ± 0.47
THr	1.42 ± 0.04
RHr	4.45 ± 0.16

Table 7-. Condensed tannin contents of hydroethanolic extracts

Extracts	Condensed tannin contents (mg EC/g DM)
BMe	51.09 ± 0.98
TMe	66.78 ± 3.16
RMe	20.82 ± 1.34
BHr	9.59 ± 0.39
THr	3.18 ± 0.24
RHr	14.26 ± 0.61

BMe: Broyat of *M. esculenta;* TMe: Tamisat of *M. esculenta*; RMe: Refus of *M. esculenta*; BHr: Broyat of *H. rotundifolia*; THr: Tamisat of *H. rotundifolia*; RHr: Refus of *H. rotundifolia*

The quantification of phenolic compounds in the leaves of *M. esculenta* revealed that the Broyat presents a better content of total polyphenols with a value of 146.24±3.67 mg EAG/g DM followed by the Refus with 92.85±0.12 mg EAG/g DM while Tamisat had the lowest content 84.10±2.23 mg EAG/g

DM. Furthermore, Refus signed the highest content of total polyphenols (57.13±1.31 mg EAG/g DM) in the whole plant of *H. rotundifolia* followed by Broyat (53.29±0.60 mg EAG/ g DM), as for Tamisat, it presented the lowest content (43.98±0.44 mg EAG/g DM); It appears that following the quantitative analysis of phenolic compounds, Tamisat presented the lowest content of total polyphenols regardless of the plant species used. Regarding the quantification of total flavonoids and condensed tannins in the leaves of *M. esculenta*, Tamisat manifested the highest contents respectively (23.49±1.15 mg EQ/g DM and 66.78±3. 16 mg EC/g DM) followed by Broyat with respective values of 20.77±1.74 mg EQ/g DM and 51.09±0.98 mg EC/g DM. Note that the lowest contents were observed with Refus with respective values of 7.68±0.62 mg EQ/g DM and 20.82±1.34 mg EC/g DM. On the other hand, regarding the determination of the quantities of total flavonoids and condensed tannins in the whole plant *H. rotundifolia*, Refus presented the highest contents (4.45±0.16 mg EQ/g DM and 14.26±0.61 mg EC/g DM). Broyat for their part, showed total flavonoid contents of the order of 3.76±0.47 mg EQ/g DM and those of condensed tannins worth 9.59±0.39 mg EC/g DM. Tamisat presented the lowest contents of respective values 1.42±0.04 mg EQ/g DM and 3.18±0.24 mg EC/g DM.
It appears from this quantitative analysis of total flavonoids and condensed tannins that the *M. esculenta* Tamisat had the highest contents compared to the Broyat and Refus coming from the same plant matrix. On the other hand, in the case of the plant species *H. rotundifolia*, it was rather the Refus which showed the highest contents of total flavonoids and condensed tannins.

3.4. Antioxidant profiles with respect to DPPH

3.4.1. Percentage reduction (PR)

Figures 1 and 2 present the PR values obtained from *M. esculenta* leaf powders with concentrations ranging from 0.031 mg/mL to 0.500 mg/mL and those from the whole *H. rotundifolia* plant for concentrations between 0.016 mg /mL and 0.130 mg/mL.

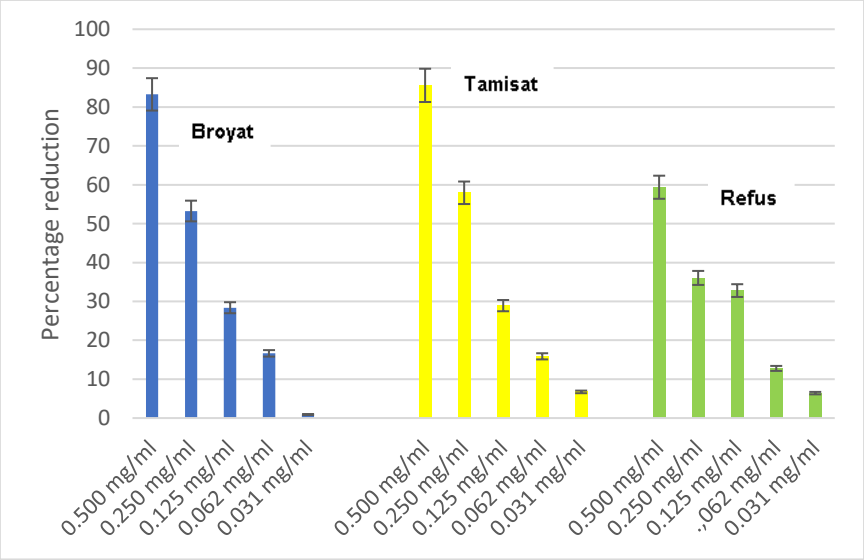

Fig. 1. Percentage reduction in hydroethanolic extracts from *M. esculenta* leaves

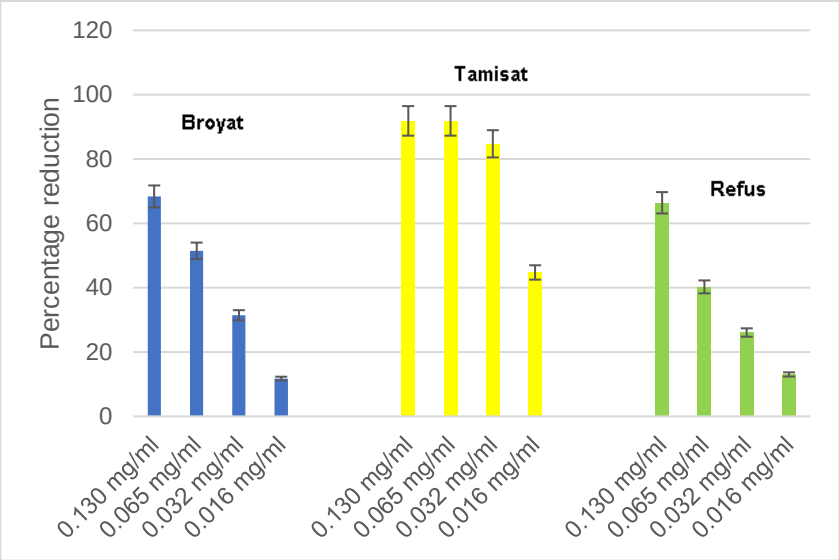

Fig.2. Percentage reduction in hydroethanolic extracts from the whole *H. rotundifolia* plant

The hydroethanolic extracts of the powders of the two plant species show an antioxidant potential whatever the concentration of the extract. The synergistic action of the reducing secondary metabolites present in the study extracts could be the origin of their antioxidant activity when analyzing the results of the quantitative tests. Indeed, certain secondary metabolites such as flavonoids, terpenes, etc. are known for their ability to reduce free radicals [18,19].
Furthermore, the highest PR values are observed in Tamisat for the two plant matrices. For a better assessment of the effectiveness of said extracts, their median concentration (CR_{50}) [20] was determined graphically using the EXCELL software.

3.4.2. Median reduction concentration (CR_{50})

DPPH radical using ascorbic acid (vitamin C) as a reference antioxidant. The CR_{50} of the plant extracts studied and vitamin C were determined graphically using EXCELL software. Each CR_{50} is calculated by solving the equation y= ax+b of each trend curve with: y=50. The average CR_{50} values obtained are shown in Table 8.

Table 8. CR_{50} values of *M. esculenta* leaf powders, whole *H. rotundifolia* plant and vitamin C

	BMe	TMe	RMe	BHr	THr	RHr	Vit. C
CR_{50} (mg/mL)	0.229	0.215	0.403	0.059	0.02	0.088	0.002

BMe: Broyat of *M. esculenta*; TMe: Tamisat of *M. esculenta*; RMe; Refus of *M. esculenta*; BHr: Broyat of *H. rotundifolia*; THr: Tamisat of *H. rotundifolia*; RHr : Refus of *H. rotundifolia;* Vit.C : Vitamine C

The antioxidant activities of the different plant extracts were evaluated based on their CR_{50} in comparison with that of vitamin C. Thus, the lower the value of the CR_{50}, the greater the antioxidant power of the extract [21]. The evaluation of the antioxidant activity by spectrophotometry which is intended to be quantitative showed that vitamin C presents the best antioxidant power with a CR_{50} equal to 0.002 mg/mL. However, the antioxidant activity of the different plant extracts is not negligible compared to that of the reference molecule (vitamin C). This is because vitamin C is a pure molecule while plant extracts are complex in terms of chemical composition. On the other hand, the Tamisat extracts showed greater antioxidant activity with respect to DPPH than the Broyat and Tamisat. In addition, Broyat have a greater activity than Refus. In the case of *M. esculenta* leaf extracts, the sieved particles are richer in total flavonoids and condensed tannins. This would justify the fact that Tamisat have greater antioxidant activity than those of Broyat and Refus. Indeed, flavonoids and tannins, more precisely proanthocyanidins, are powerful antioxidants [22]. Furthermore, with regard to the whole plant *H. rotundifolia*, the highest antioxidant activity observed in Tamisat could be due to its phytochemical composition, in particular to the presence of phenolic acids detected only in said plant species.

3.5. Antibacterial profile

3.5.1. Antibacterial activity in solid media

The antibacterial activity in a solid medium made it possible to research the sensitivity of bacteria to hydroethanolic extracts of powders (Broyat, Tamisat, Refus) from the leaves of *M. esculenta* and the whole plant of *H. rotundifolia*. It also made it possible to check their behavior in the face of the different antibiotics used. The choice of antibiotics was made according to their specificity in relation to the different groups of bacteria used for the tests. Thus, cefotaxime (COX) was used for *Escherichia Coli*, cefoxitin (FOX) was used for *Staphylococcus aureus* and finally ceftazidime (CZD) was tested on *Pseudomonas aeruginosa*. These antibiotics played the role of positive controls and sterile distilled water (EDS) that of negative control. The results obtained are recorded in Table 9.

Table 9. Diameters of inhibition zones The antioxidant potential of Broyat, Tamisat and Refus of *M. esculenta* and *H. rotundifolia* was carried out with the of bacterial strains

	Diameters of inhibition zones (mm)									
	BMe	TMe	RMe	BHr	THr	RHr	COX	FOX	CZD	EDS
E. Coli ATCC	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	18.00 ± 0.00	-	-	06.00 ± 0.00
S. aureus ATCC	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	**11.00 ± 0.00**	**13.00 ± 0.00**	**11.00 ± 0.00**	-	22.00 ± 0.00	-	06.00 ± 0.00
S. aureus 068	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	**10.00 ± 0.00**	**11.00 ± 0.00**	**10.00 ± 0.00**	**-**	18.00 ± 0.00	-	06.00 ± 0.00
P. aeruginosa ATCC	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	-	-	18.00 ± 0.00	06.00 ± 0.00
P. aeruginosa 363	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	06.00 ± 0.00	-	-	06.00 ± 0.00	06.00 ± 0.00

BMe: Broyat of *M. esculenta*; TMe: Tamisat of *M. esculenta*; RMe : Refus of *M. esculenta*; BHr: Broyat of *H. rotundifolia*; THr: Tamisat of *H. rotundifolia*; RHr : Refus of *H. rotundifolia; COX :* cefotaxime; *FOX :cefoxitin; CZD : ceftazidime; EDS :* sterile distilled water

The diameters of the zones of inhibition of bacterial strains by plant extracts vary from one strain to another depending on the extract used. Some powders have shown antimicrobial activity, however, others have not. In fact, the strain is said to be resistant to the substance when the diameter of its inhibition zone is less than 8 mm, sensitive when it is between 9 and 14 mm, very sensitive when it is between 15 and 19 mm, and extremely sensitive when it is greater than 20 mm [23]. Analyzing the values of the diameters of the inhibition zones, it appears that the strains of *Staphylococcus aureus* ATCC and *Staphylococcus aureus* 0.68 are sensitive to the hydroethanolic extracts of *H. rotundifolia* powders. On the other hand, said extracts showed no activity against *Escherichia Coli* ATCC, *Pseudomonas aeruginosa* ATCC and *Pseudomonas aeruginosa* 363. In addition, the antibacterial activity manifested by the powders of *H. rotundifolia* against *Staphylococcus aureus* ATCC and *Staphylococcus aureus* 0.68 would be linked to the presence of flavonoids, tannins, coumarins, sterols and terpenes. Indeed, these above-mentioned compounds are recognized for their biological anti-radical and antibacterial activities [24].

3.5.2. Antibacterial activity in liquid medium

The antibacterial activity in liquid medium made it possible to determine the antibacterial parameters CMI, CMB of the hydroethanolic extracts of *H. rotundifolia* powders. The CMI, CMB and the CMB/CMI ratio of the extracts active against *Staphylococcus aureus* are recorded in Table 10.

Table 10. Antibacterial parameters of plant extracts from *H. rotundifolia* against *Staphylococcus aureus*

Strains	Extracts	CMI (mg/mL)	CMB (mg/mL)	CMB/CMI	Interpretation
Staphylococcus aureus ATCC	BHr	50	>100	>2	Bacteriostatic
	THr	6.25	12.5	2	Bactericidal
	RHr	50	>100	>2	Bacteriostatic
Staphylococcus aureus	BHr	50	>100	>2	Bacteriostatic

068	THr	6.25	12.5	2	Bactericidal
	RHr	50	>100	>2	Bacteriostatic

The results in Table 10 present the quotient of the minimum bactericidal concentration (CMB) by the minimum inhibitory concentration (CMI) of the extracts of *H. rotundifolia* on the strains *Staphylococcus aureus* ATCC and *Staphylococcus aureus* 068. Indeed, the extracts for which this quotient is equal to 2, are called bactericidal while those for which it is greater than 2 are called bacteriostatic. Thus, for the plant active on these two strains, it turns out that the sieved particles (Tamisat) have a bactericidal effect while the Broyat and Refus are both limited only to a bacteriostatic effect. The bactericidal nature of Tamisat from *H. rotundifolia* on the two strains of *Staphylococcus aureus* would be linked to its higher antioxidant potential. In fact, Tamisat demonstrated better antioxidant character than all other extracts studied. Also, Chamayou and colleagues [25] showed that after grinding, significant modifications in texture, structure, shape and surface can occur. The increase in the contact surface due to fragmentation increases the physical, chemical or enzymatic reactivity of the particles. Certain nutritional components can also see their availability increase following the destruction of cellular structures due to the increase in temperature due to the breakdown energy of molecules [26]. This is what would explain the significant biological activity of the finer particles and therefore of Tamisat compared to Broyat and Refus.

4. CONCLUSION

The present work focused on a comparative study of powders of *M. esculenta* and *H. rotundifolia* divided into three categories, namely: Broyat, Tamisat and Refus, the extracts of which were obtained by Soxhlet extraction. The phytochemical composition, the antioxidant activity with respect to the DPPH radical as well as the antibacterial activity of each of these categories of powders were highlighted. The results of the phytochemical screening carried out by color reactions and by means of TLC highlighted the presence of flavonoids, tannins, coumarins, sterols, terpenes and proteins in all the study extracts. Phenolic acids were observed only in *H. rotundifolia*. In addition, Broyat gives a greater number of extractable phytocompounds compared to Tamisat and Refus regardless of the type of extraction solvent and the plant species used. Tamisat contains the most compounds of interest and therefore exhibits greater antioxidant potential against DPPH and greater antibacterial activity against *Staphylococcus aureus*.
It appears at the end of this study that it would be preferable to directly use the powder obtained after grinding, i.e. the Broyat, in the context of a phytochemical study and the Tamisat for biological studies.
As a perspective, we plan to carry out this study on a larger number of plant species; use a better grinding system and several sieves of different mesh sizes in order to have Tamisat of different particle sizes as well as different extraction methods; characterize and quantify the identified active compounds using LC-MS and GC-MS analyses.

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