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Research Article

Optimization of extraction and lyophilization conditions of *Feretia apodanthera* (Rubiaceae) leaf powders for galenic formulation

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Abstract

Feretia apodanthera (Rubiaceae) is used in traditional medicine for the treatment of various pathologies. Its leaves are chewed and swallowed to treat stomach aches, sleep disorders, etc. With the aim of improving the extraction method in order to obtain high contents of phytochemical compounds and good antioxidant activity, this study aimed to highlight the extraction and lyophilization conditions of two extracts (aqueous and hydro-ethanolic) leaves of *Feretia apodanthera.*

An aqueous and hydro-ethanolic extraction was carried out from the leaf powder of *Feretia apodanthera*, after a maceration plan (mass/volume ratio and extraction time). The extracts obtained were freeze-dried (flask size, filling volume and freeze-drying time) in order to determine the optimal extraction conditions. A phytochemical screening of the extracts with extreme yields of different solvents was carried out by TLC, which led to the determination of certains compounds of interest and the evaluation of the antioxidant activity by the DPPH and FRAP method.

The optimal maceration conditions were 02 hours and m/V ratio of 1/20 for the aqueous extract and those of the ethanolic extract were 30 min and m/V ratio of 1/20. The optimal freeze-drying conditions were 60h and a 500ml flask 1/3 filled. The presence of flavonoids, tannins, saponosides, coumarins, triterpenes and sterols has been highlighted. The phenolics compounds contents of hydro-ethanolic lyophilisates were higher than those of aqueous lyophilisates. There was no statistical difference between the antioxidant activity of the different extracts.

The optimal conditions will serve as a standard for the production of the extract necessary for the formulation.

Keywords : *Feretia apodanthera*, extraction, freeze-drying, phenolics compounds, antioxidant.

INTRODUCTION

Plants and herbal products are used for therapeutic purposes around the world and particularly in developing countries for socio-cultural reasons [1]. Plants, in raw or processed form, are widely used in traditional medicine because of their biologically active phytochemicals [2]. These phytochemicals are known to have therapeutic properties. These compounds are obtained by several extraction methods which can constitute the very first step in the research and development process of a drug [3]. The extraction method must be mastered and validated using appropriate solvents because it can influence the composition and content of active substances sought [4]. Indeed, the nature and concentration of the solvent, temperature, time, solid-liquid ratios, etc. are important factors which can constitute limits to the extraction of plant bioactive compounds [5, 6]. One of the main forms of use of products from medicinal plants is the liquid form (decocted, macerated or infused, etc.) [7]. This requires a guarantee of the uniformity of administered doses and their stability during storage through the development of modern and adapted dosage forms [8]. To do this, the extraction method must be optimized and standardized. In this study, it aims to improve the extraction conditions which were chewing of the leaves and/or crushing before administration of *Feretia apodanthera* (Rubiaceae), a plant used in traditional medicine for the management of stomach aches, urinary infections, sleep regulation, etc.[9, 10]. This study therefore aimed to determine the optimal conditions for extraction and freeze-drying of Feretia apodanthera (Rubiaceae) leaf powders with a view to a galenic formulation.

METHODOLOGY

Plant material

The plant material consisted of *Feretia apodanthera* leaf powders. The leaves were pulverized and the resulting powder was used for experimental studies.

Macroscopic and organoleptic characteristics

The examination of the macroscopic characteristics (color, general shape and texture of the drug) was observed with the naked eye and that of the organoleptic characteristics (odor and flavor of the drug) was determined by tasting and sniffing the powder [11].

Determination of residual moisture content (RMC)

The residual moisture levels of the powders were determined by the method described by the European Pharmacopoeia 10th Edition [11]. For each test, the measurement was carried out three times.

Determination of pH

The pH was determined by a pH meter by immersing the electrode in 1% (m/v) aqueous solutions of each powder. For each test, the measurement was performed three (03) times. The mean value and the standard deviation were calculated (m±standard deviation, n = 3) [12].

Particle size distribution

The particle size was determined by the sieving method of the European Pharmacopoeia. A column of ten (10) sieves with a mesh size of 1 ; 0.9 ; 0.71 ; 0.63 ; 0.5 ; 0.4 ; 0.25 ; 0.16 ; 0.1 and 0 mm was used. The vibration duration was 15 min, and the amplitude was 70 vibrations per minute. The rejects from the different sieves were weighed using a precision balance. Histograms of the simple and cumulative particle size frequencies were made to graphically determine the median size (d50) corresponding to the 50% particle size [13].

Preparation of freeze-dried extracts

Choice of maceration parameters

Extraction by maceration of Feretia apodanthera leaf powder in two solvents was carried out in order to be more or less close to the condition of traditional use of the plant which consists of chewing the leaf. This extraction method was carried out according to the protocol described by Owolabi et al., [12] with some modifications. The two extraction solvents used were distilled water and ethanol at 60% (v/v). A test portion of 5 g of Feretia apondanthera leaf powder was dispersed in different volumes of distilled water (50, 100 and 200 mL) and shaken manually. The mixture was macerated for 5 min; 30 mins; 12 hours and 24 hours (Table 1). The maceration conditions were varied in order to determine the optimal extraction conditions.

Table 1 : Maceration conditions for the two solvents

Mass/volume ratio (g/mL)	Time				
5/50	5 min	30 mins	02 hours	12 hours	24 hours
5/100	5 min	30 mins	02 hours	12 hours	24 hours
5/200	5 min	30 mins	02 hours	12 hours	24 hours

After maceration at different times, the liquid extracts were filtered with Wattman number 1 paper. The filtrates of the hydro-ethanolic extracts were passed through a Rotavator to eliminate the ethanol before being taken up with a little distilled water. All extracts (aqueous and hydro-ethanolic) were stored in the freezer at a temperature of -80° C before lyophilization. The freeze-drying conditions were varied in order to determine the optimal freeze-drying conditions, namely the volume of the freeze-drying flask (50 ml; 250 ml and 500 ml), the filling level of the flasks (3/4; 1/2 and 1/3) and finally the freeze-drying time (24; 48; 60 and 72 hours) (Table 2). This comparative study only concerned aqueous extracts; the optimum parameters obtained were used to lyophilize the hydro-ethanolic extract.

Table 2 : Optimization of freeze-drying of extracts

Flask Volume (ml)	Filling the Flask	Time (hours)			
50	**3/4**	24	48	60	72
	1/2				
	1/3				
250	**3/4**	24	48	60	72
	1/2				
	1/3				
500	**3/4**	24	48	60	72
	1/2				
	1/3				

The figure 1 summarizes the maceration and freeze-drying procedures

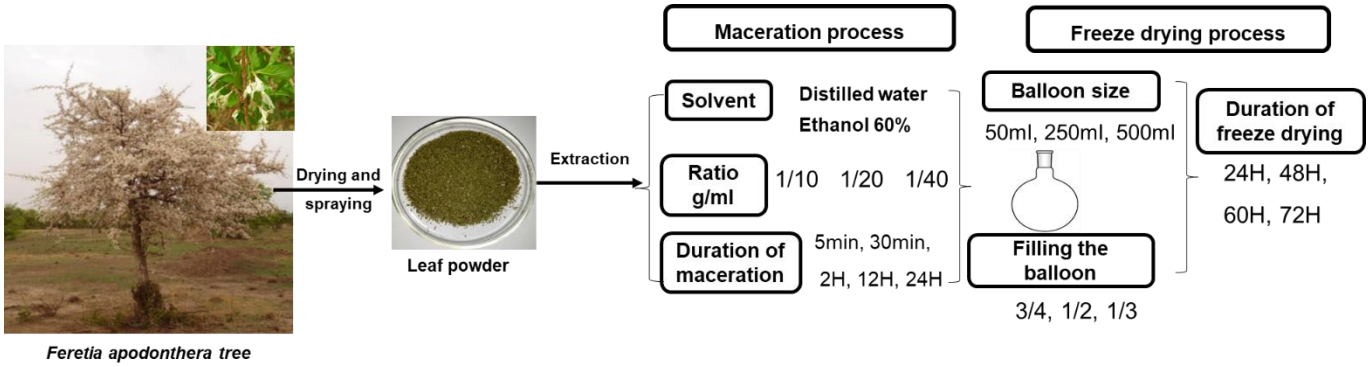


Figure 1: Scheme of maceration and freeze-drying procedures

Chemical screening of extracts

Phytochemical screening

Phytochemical screening of the liquid extracts and the lyophilisate was carried out on chromatoplates (60 F250, 20x20 glass support, Fluka-Silica gel) according to the methods described in the literature [14]. This involved searching for large chemical groups by thin layer chromatography (TLC) such as steroid compounds, terpene compounds, saponosides and phenolic compounds.

Several specific reagents have been used to reveal these groups of compounds: Sulfuric vanillin reagent and Libermann Burchard reagent for terpenes and sterols; methanolic 5% (V / V) KOH reagent for coumarins; Neu reagent for flavonoids; $FeCl_3$ reagent for tannins and phenolic compounds and sulfuric anisaldehyde reagent for saponosides.

Dosage of some chemical molecules of interest

The quantification of phytochemical compounds was carried out on the extracts with the highest yields and the lowest yields in order to see possible differences between the contents of the two yields.

Total phenolics content

Total phenolic compounds were measured according to the method of Singleton and al., [15]. These compounds react with the Folin Ciocalteu Reagent (FCR) in an alkaline medium. The loss of a phenolic proton in an alkaline medium leads to a phenolate anion which is capable of reducing the FCR in which molybdate is reduced, forming a blue colored molybdenum oxide complex whose absorption maximum is at 760 nm. The intensity of the blue color is proportional to the quantity of total phenolics present in the test portion. The reaction mixture consisted of 1 mL of extract (1 mg/ml), 1 mL of FCR (2N) and 3 mL of sodium carbonate solution (Na_2CO_3, 20%). It was left to stand at room temperature for 40 min then the absorbance was measured at 760 nm using the Bio Rad spectrophotometer (model 680). In the white control tube, the extract was replaced by distilled water. The tests were carried out in triplicate. The total phenolic concentration of the extract was determined.

Total flavonoids content

The determination of flavonoids was carried out according to the method of Kumaran et al. [16]. To 100 µL of extract with a concentration of 1 mg/mL, 100 µL of aluminum trichloride (2% in methanol) were added. After 40 minutes of incubation against a blank (100 µL of methanol and 100 µL of extract) and the appearance of a stable yellow color, the absorbance is read at 415 nm by ultraviolet spectrophotometry (Biotek). The flavonoid content of the sample is evaluated in relation to a reference solution of quercetin (0-70 µg/mL). The tests were carried out in triplicate and the results are expressed in grams of quercetin equivalent per g of dry extract (mg EQ/ g). The flavonoid content of the extract was determined.

Evaluation of antioxidant activity

The antioxidant activity of freeze-dried extracts of Feretia apodanthera was evaluated by two techniques such as iron reduction (Ferric Reducing Antioxidant Power or FRAP) and scavenging of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH).

DPPH Antioxidant Test

The method of Kim et al. [17] made it possible to determine the ability of the extracts to reduce DPPH free radicals. A series of eight dilutions from the stock concentration (2 mg/ml) of the samples and Trolox (reference compound) was carried out. On a 96-well microplate, each well was filled with 200 µL of DPPH solution (0.04 mg/ml) and 20 µL of the extract diluted at different concentrations or the reference. After 40 minutes of incubation, the absorbance was read at the wavelength of 490 nm using a spectrophotometer. The blank without sample was prepared under the same conditions and consisted of 200 µL of DPPH and 20 µL of methanol. A curve of percent DPPH inhibition was plotted as a function of sample concentration. On the curve, the concentration necessary to degrade 50% DPPH (IC_{50}) was determined.

FRAP Antioxidant Test

The ability of the extracts to reduce ferric ion is determined according to the Benzie and Strain technic [18]. FRAP reagent is obtained by mixing 2,4,6-Tripyridyl-s-Triazine (TPTZ) solution (10 mM), sodium acetate buffer solution (pH 3.6), and $FeCl_3$ solution (20 mM) in the proportions 1:10:1. An amount of 10 µL of each extract at different concentrations (from a 0.5 mg/ml stock solution) was mixed with 300 µL of FRAP reagent and incubated in the dark for 30 min. The absorbance of the colored product (ferrous Tripyridyltriazine complex) was measured at 593 nm. Trolox taken as a positive control was prepared under the same conditions as the samples. A calibration curve was established with Trolox whose concentrations varied from 500 to 50 µg. mL^{-1}. The antioxidant capacity of the different extracts was expressed in mg of Trolox equivalent/g of dry matter (mg of ET/mg of extract).

Results analysis

The results of the pharmacological study are expressed as mean ± Mean Standard Error (SEM). The different figures were drawn using GraphPad Software Prism version 5.01. The series were considered significant when the probability of error (p) was lower than the agreed risk: 0.05 ($p<0.05$).

RESULTS AND DISCUSSION

Physico-chemical characteristics of plant matter

The leaf powder of Feretia apodanthera had a slightly bitter taste, an astringent odor and was green in color. The appearance of the powder was uniform to the naked eye with some rare debris of leaf petioles. The macroscopic and organoleptic characteristics observed in the powder can be useful for quality control of the raw material and to differentiate it from other neighboring powders [19]. This will make it possible to immediately recognize the plant drug, check its degree of purity according to the present or absence of foreign elements, mold, etc. and possibly detect adulteration or falsification [20].

Figure 2 : Image of powder of *Feretia apodanthera*

The RMC of the *Feretia apodanthera* powder used was 7.3±0.03%. Verification of the residual humidity level allows the estimation of the real weight of the plant material but above all to qualify the conservation conditions [21]. The content allowed in a drug for its good conservation must not exceed 10% [16]. Indeed, high residual water contents (≥10%) allow the development of microorganisms (bacteria, yeasts, molds) and certain enzymatic reactions, leading to harmful consequences on the appearance of drugs, their organoleptic characteristics, their therapeutic properties [22]. Thus, *Feretia apodanthera* powder could be stored without risk of contamination and/or alteration of these physicochemical compounds over a determined period.

The average pH of the Feretia apodanthera powder used for the work was 5.75± 0.061. pH is also a parameter for controlling the quality and stability of powders. Indeed, the pH of a powder could vary depending on the region of harvest due to the physico-chemical properties of the soil, which could influence the physico-chemical properties of the plant materials. It could vary over time of conservation under the influence of environmental factors [23].

The powder particle size results are shown in the figure below.

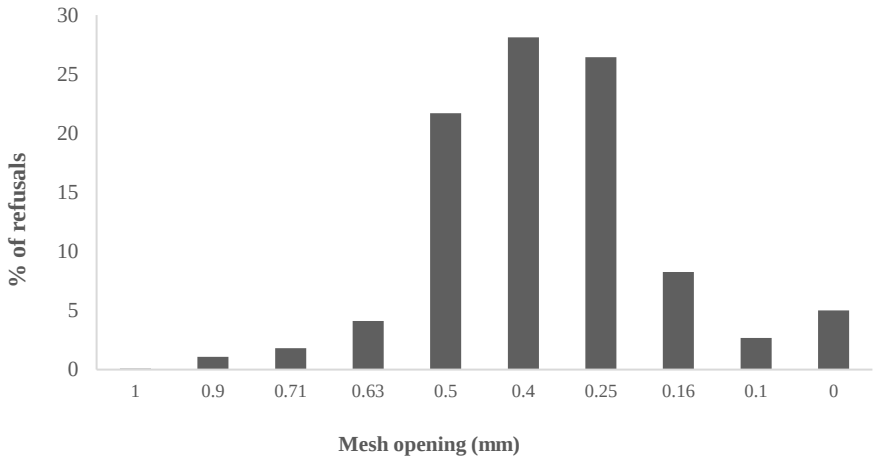


Figure 3: Frequency curve of the particle size of *Feretia apodanthera* leaf powder

The powders were all homogeneous and moderately fine. After shaking the sieves at 50 vibrations per minute for 15 minutes, more than 95% of the 100g of the powder passes through sieve No. 1400 and less than 40% passes through sieve No. 355, the powder is therefore classified as coarse. in terms of particle size and distribution more or less homogeneous according to the European Pharmacopoeia 10th Edition [11]. The average particle diameter of the powder is 0.461+/- mm. The particle size intervenes in the physical and functional properties of a powder (flow, density, solubility, wettability, etc.) [24]. This particle size is could be used during extraction, as it influences solubility and wettability [24].

Results of freeze-drying optimization

The results of the optimization of the freeze-drying conditions are shown in Table 3. The extracts were considered completely dry when their RMC was less than 10%. No extract contained in the 3 flasks filled at different proportions had a RMC lower than 10 after 24 hours of freeze-drying. After 48 hours, only the extracts contained in the 50 and 250 ml flasks filled 1/3 had RMCs lower than 10%. All the extracts contained in the flasks (50, 250 and 500 ml) filled 1/3 had their RMC less than 10% after 60 hours of freeze-drying. After 72 hours, all the extracts contained in the 50 ml flasks filled 3/4; 1/2 and 1/3 had RMCs less than 10% while only the extracts contained in the 250 and 500 Flask filled to 1/3 had RMCs less than 10%. The duration of freeze-drying was inversely proportional to the thickness of the raw material contained in the flask.

A reduction in the thickness of the raw material would reduce the resistance to heat transfer and internal materials by increasing the drying speed which would lead to a reduction in the freeze-drying time [25]. Indeed, during sublimation, the ice is converted into vapor which condenses in the freeze dryer chamber preventing its return to the flask [26, 27]. As this sublimation progresses, the sublimation front, which is the interface between the dry part and the frozen part, moves from the surface of the product towards the center, thus increasing the dry proportion of the material [28]. Also, for a given Flask volume, drying is faster in lightly filled Flask (third of the volume) [29]. In fact, the smaller and less filled the Flasks, the shorter the freeze-drying time. The duration of freeze-drying at 60 hours with a flask 1/3 filled was maintained as optimal condition. Furthermore, the hydroalcoholic extracts lyophilized

under these conditions (Flask 1/3 filled and 60 hours of lyophilization) also gave a dry lyophilisate with RMC less than 10%. The 500 ml flask filled 1/3 at 60 hours could be preferred over the 50 and 250 ml flasks filled 1/3 at the same time since its volume at 1/3 exceeds the sum of 1/3 volume of the other two Flasks. Optimal freeze-drying conditions could be obtained at 60 hours with a 500 ml flask filled 1/3.

Table 3 : Results of freeze-drying optimization

Flask Volume (ml)	Filling the Flask	Time (hours)			
		24	48	60	72
50	**3/4**	**-**	**-**	**-**	**+**
	1/2	**-**	**-**	**-**	**+**
	1/3	**-**	**+**	**+**	**+**
250	**3/4**	**-**	**-**	**-**	**-**
	1/2	**-**	**-**	**-**	**-**
	1/3	**-**	**+**	**+**	**+**
500	**3/4**	**-**	**-**	**-**	**-**
	1/2	**-**	**-**	**-**	**-**
	1/3	**-**	**-**	**+**	**+**

+ symbol: RMC is less than 10%, therefore the lyophilisate is dry

- symbol: RMC greater than 10% or presence of ice in the Flask

Extraction yield

The following table represents the extraction yields of the aqueous and hydro-ethanolic extracts following the optimization plan (Table 4).

Table 4 : Extraction yields of aqueous and hydro-ethanolic extracts

		Temps				
Extract	**m/v ratio (g/mL)**	5 min	30 mins	02 hours	12 hours	24 hours
Aqueous	**5/50**	12.8	14	14.4	14.7	21.7
	5/100	14.4	17.8	20.4	20.8	25*
	5/200	16.4	19.6	23.6*	24.8*	26.8*
Hydro ethanolic	**5/50**	20.2	24.6	25.7	21.4	22.8
	5/100	24.1	26.6	29.5	31.6	31.8
	5/200	29.8	32.4#	34.8#	36.8#	40.8

* No significant difference with the highest yield with the aqueous extract

No significant difference with the highest yield with the hydro-alcoolic extract

For each of the two types of solvent, the highest yields were obtained with extracts whose maceration was 24 hours with a mass/volume ratio of 1/40 (5/200) and the lowest yields obtained with macerations of 5 min with a mass/volume ratio of 1/10 (5/50). The hydroethanolic extracts had the highest yields compared to those of the aqueous extracts despite variations in the two parameters, namely the mass/volume ratio from 1/10 to 1/40 and the maceration time from 5 min to 24 hours.

The lowest yield, for aqueous maceration, was 12.8% for the proportion of 5/50 at time 5 min and the highest was 26.8% for the proportion 5/200 at time 24 hours. After a statistical analysis, there was no difference between the yield obtained with the proportion 5/200 at 24 hours and those obtained at 2 hours with the proportion 5/200 and 5/100. The best yield was obtained with the proportion 5/100 at time 02 hours.

The yields after hydro-ethanolic maceration were between 20.2% obtained at time 5 min with the mass/volume ratio of 5/50 and 40.8% obtained at time 24 hours with the m/v ratio of 5/200. After a statistical analysis, there is no difference between the yield obtained at 24 hours and that at 12 hours at the same mass/volume ratio of 5/200. There is also no statistical difference between the yields obtained at times 30 min, 02 and 12 hours with the same proportion 5/200. The best yield was obtained at time 30 min with the ratio 5/200.

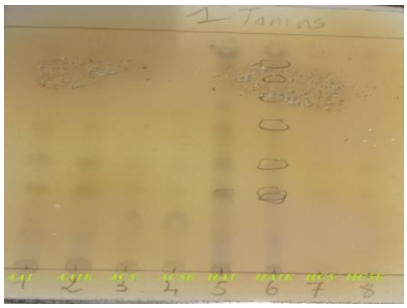
Extraction is a mass transfer process involving three steps, penetration of the solvent into the solid phase (internal transport), dissolution of the solute (solubility) and diffusion of the solute from the solid phase to the solvent (external transport) [30]. The choice of solvents (water and 40:60 water-ethanol mixture) was made taking into account the traditional form of use (saliva). Indeed, the choice of solvent is made according to several criteria including the solubility of specific components in the solvent [31]. The higher yield with hydro-ethanolic extracts than aqueous extracts could be explained by the fact that ethanol, an organic solvent (low toxicity, easy acquisition, etc.), would give a fairly high yield of the same

extract as 'being not very selective for phenols [32]. On the other hand, the mixture of alcohol and in particular ethanol and water was found to be more effective in extracting phenolic constituents than the corresponding single-component solvent system [33]. The highest yield was obtained with the lowest mass/volume ratio (5/200) and the longest time (24 hours). Increasing the volume of solvent relative to the sample would allow an increase in mass transfer between the solvent and the sample. Indeed, a high volume of solvent makes it possible to dissolve phytochemical compounds more efficiently, thus improving the extraction yield [34]. Optimization, whose common objectives are to minimize costs or maximize desired profits, product quality and operating efficiency, is the act of obtaining the best possible result or the effort to obtain 'an optimal solution in a given set of circumstances [31]. In this study, the highest extraction yields were obtained with the highest volumes of the two solvents at the longest times. In optimization testing and after statistical analysis, the best performance was obtained with the proportion 5/100 at time 02 hours. Indeed, after an aqueous maceration of 12 hours compared to 2 hours, there is only 1.2% on the highest yield which was added in 10 hours interval. Also, the yield obtained at the highest volume (200 ml) was 23.6% while with half of this volume, i.e. at 100 ml, the yield was 20.4% (3.2% less than the volume of 200 ml). Taking into account the solvent cost ratio and the extraction yield, the best yield obtained with the proportion 5/100 at time 02 hours could be considered as the optimal yield. For hydro-ethanolic maceration, the best yield was obtained at 30 min with the ratio 5/200. In order to have the solvent cost ratio and the extraction yield, the yield of the mass-volume ratio of 5/100 obtained at 30 min could be chosen as optimal yield with a percentage of 26.6% or 5.8 % less than that of the mass-volume ratio of 5/200 at the same time. These extraction methods chosen as optimal would meet the optimization conditions which would be to maximize yield, quality and reduce the extraction cost [31].

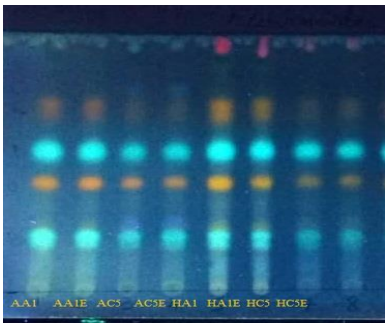
Results of phytochemical screening by TLC

The following figures 4 represent the chromatographic profiles of the aqueous and ethanolic extracts of *Feretia apodanthera* revealed by different reagents.

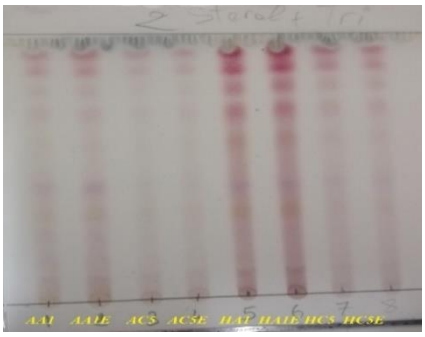
Tannins and phenolics compounds



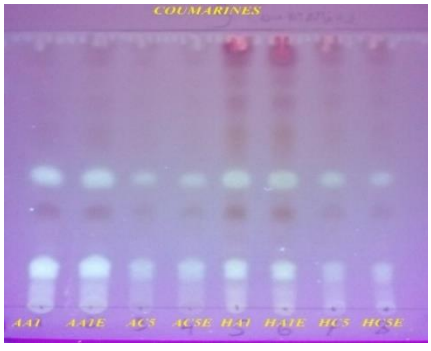
Flavonoids



Triterpenes and sterols



Coumarins



Saponosides

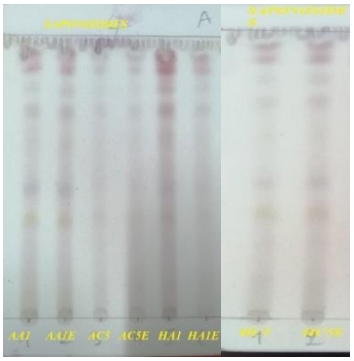


Figure 4: Chromatographic revelation of different groups of *Feretia apodanthera* extracts

* Solvent system : Ethyl acetate /Methanol/Water 10/1.7/1.3

Chemical groups such as flavonoids, tannins, saponosides, coumarins, triterpenes and sterols were demonstrated in the liquid extracts and lyophilisate of *Feretia apodanthera* leaves. Chromatographic profiles of crude liquid extracts are similar to those of extracts reconstituted from lyophilisate. Lyophilization appears not to denature the extracts and would be a good drying method in order to keep all the chemical groups present in the crude extracts intact. Also, it was noticed that the same chemical groups were found in the extracts from the different solvents (aqueous and hydroalcoholic). Finally, the extracts with the highest yields and those with the lowest yields had the same chromatographic profiles. The extraction parameters studied (nature of the solvent, duration, m/V ratio) seem not to influence the quality of the chemical constitution of the powder. Previous work carried out in Mali on leaves also highlighted the presence of tannins, saponosides and coumarins but not flavonoids, triterpenes and sterols [35]. This difference could be explained by the method of detection used which was the tube test in the other and the TLC in this present study. Researched phytochemicals are known to have therapeutic properties. The two extraction solvents used being polar could exclude the presence of certain non-polar phytochemicals. Indeed, phenolic compounds, most abundant in polar solvents, are the most common phytochemicals in all parts of a plant, including leaves, bulbs, flowers and stems [36]. They constitute one of the largest groups of secondary metabolites produced by all plants [6]. Phenolic compounds play a large role in protection against certain diseases due to their possible interaction with numerous enzymes and their antioxidant properties [37], hence their choice as compounds of interest in the remainder of the study.

Compounds of interest contents

The results of the determination of the compounds of interest are recorded in Table 5.

Table 5: Total phenolic and flavonoid contents of extracts

Extracts	Total phenolics in mg EAT/g DM	Flavonoids in mg EQ/g DM
A1	355.75 ±64.50	76.25± 0.60
A2	461.57 ± 68.52	78.18 ± 0.57
HA1	400.46± 26.16	76.95 ± 0.98
HA2	484.42± 40.72	81.62 ± 0.92

* A1 and A2: Aqueous extract with the lowest yield A1 and the highest A2

HA1 and HA2: hadro-alcoolic extract with the lowest yield HA1 and the highest HA2

The total phenolic content of the samples ranged from 355.75 ± 64.50 to 484.42 ± 40.72 mg EAT/g DM. The highest content of total phenolics was obtained with the hydroalcoholic lyophilisate having the highest yield with 484.42 ± 40.72 mg EAT/g DM while the aqueous lyophilisate having the lowest yield gave the lowest content with 355.75 ± 64.50 mg EAT/g DM. There was no statistical difference between the contents of the lyophilized aqueous and ethanolic extracts with the lowest yields. Under the same extraction condition, the hydroalcoholic lyophilisates had a high total phenolic content compared to that of the aqueous lyophilisates. The solubility of secondary metabolites like polar compounds could be increased in mixed solvents (co-solvents) compared to single solvents [17] which could explain the higher content of phenolic compounds in hydroalcoholic lyophilisates even if those were not very big. A small variation in total flavonoids was observed between the different lyophilisates. The highest flavonoid content was observed with the hydroalcoholic lyophilisate having the highest yield with 81.62 ± 0.92 mg EQ/g DM while the lowest flavonoid content of 76.25 ± 0.98 mg EQ/g DM was obtained with the lowest yield aqueous lyophilisate. After a statistical analysis, there was no difference between the content of aqueous lyophilisates and that of hydroalcoholic lyophilisates. The phenolic compound contents of Feretia apodanthera in this study were higher than those of the same plant in literature data [38]. This difference could be explained by the nature of the solvents used and also by the soil conditions. Flavonoids are the most commonly used because of their varied properties such as antioxidant, anti-inflammatory, antispasmodic, antiallergic, antitumor, antibacterial, anticancer, etc. [39]. Studies on medicinal plants have established a correlation between phenolic compounds and antioxidant power [40], hence the evaluation of the antioxidant activity of extracts by the DPPH and FRAP methods. In optimization studies, the activity of the extracts can be considered as one of the responses allowing a choice to be made on the most optimal extraction conditions [36].

Antioxidant activity

The results of the antioxidant activity of the extracts are shown in Table 6.

Table 6: Antioxidant activity of the extracts

Extracts	DPPH IC_{50} (µg/mL)	FRAP (mg TE/mg E)
A1	8,44±1,09	0,87± 0,02
A2	8,58±0.83	1,33±0,06
HA1	6,85±0.68	1,12 ± 0,06
HA2	7,78±0.11	1,32± 0,04
Trolox	7,96±0.6	--------
Ellagic acid	---------	0,73 ± 0,07

* A1 and A2: Aqueous extract with the lowest yield A1 and the highest A2

HA1 and HA2: hadro-alcoolic extract with the lowest yield HA1 and the highest HA2

By the DPPH method, the inhibitory concentrations 50 (IC_{50}) of the extracts varied from 6.85±0.68 µg/mL with the hydroalcoholic extract having the lowest yield to 8.58±0.83 µg/mL with the aqueous extract having the lowest yield. highest yield compared to 7.96±0.6 µg/mL corresponding to the IC_{50} of Trolox. The IC_{50} of the extracts are very close to that of Trolox, demonstrating an existing anti-radical activity comparable to that of Trolox, which is a reference compound. After statistical analysis, there is no difference between the activity of the aqueous and hydroethanolic lyophilisates and that of the reference compound, between the two lyophilisates (aqueous and ethanolic) both with the highest and lowest yields. The antioxidant activity of the lyophilisates by the FRAP method varied from 0.87± 0.02 mg ET/mgE with the aqueous lyophilisate having the lowest yield to 1.33± 0.06 mg ET/mgE with the aqueous lyophilisate having the highest yield. There was no statistical difference between the activities of the hydroethanolic lyophilisates (lowest and highest yields) and that of the most active aqueous lyophilisate. The activity of the three statistically identical lyophilisates was greater than that of the reference compound used in this study (ellagic acid). Only the activity of the aqueous extract with the lowest yield was similar to that of the reference compound.

Work carried out in previous studies on the aerial parts of the plant had also obtained higher antioxidant activities with the

acetone-water co-solvent than the aqueous solvent [41]. In this study, the freeze-dried aqueous and hydroethanolic extracts of *Feretia apodanthera* leaves had statistically the same activities. This difference could be explained by the fact that the antioxidant activity does not necessarily depend on the content of phenolic compounds, although they were linked, but on their chemical structure, their characteristic of donating hydrogen to free radicals, their iron binding capacity, etc. [42]. Indeed, the extracts with the lowest yields and that of the extracts with the highest yields had similar antioxidant activities despite the different contents of phenolic compounds. The antioxidant potential of freeze-dried extracts of Feretia apodanthera leaves can help manage diseases related to free radicals, aging, sleep disorders, etc.

CONCLUSION

This study on Feretia apodanthera leaf powder made it possible to obtain the best freeze-drying conditions (choice of flask, filling volume and freeze-drying time) which were a 500 ml flask filled to 1/3 with freeze-drying time 60 hours. Also, with the different variations of the two extracts (aqueous and freeze-dried hydro-ethanolic) namely the mass/volume ratio and the maceration time, the highest yields of 26.8% for the aqueous macerated and 40.8% for the hydro-ethanolic macerated were obtained after 24 hours with m/v ratios of 1/40. The yield of the aqueous extract, 20.4%, obtained at the ratio 5/100 at time T 02 hours and that of the hydro-ethanolic extract (32.4%) obtained at the ratio 5/200 at time T 30 min could be taken as the best returns. The phytochemical screening carried out on the different freeze-dried aqueous and hydro-ethanolic extracts indicated the presence of compounds of interest, such as polyphenols, tannins, flavonoids, sterols, coumarins and saponosides. The study of total phenolic and flavonoid contents showed that hydro-ethanolic extracts have a better total phenolic content. However, there were no significant differences between the flavonoid contents of the different extracts depending on their yield and the extraction solvents. The evaluation of the antioxidant power of the extracts shows that they act both by trapping free radicals and by reducing metal ions. The results obtained in this work could guide the choice of optimal conditions for experimental studies.

Conflicts of interest

The author(s) declare no conflict of interest.

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