



## Original Research Article

Effect of freeze-drying and spray-drying on total phenolics content and antioxidant activity from aqueous extract of *Justicia secunda* leavesEMMANUEL N'DRI KOFFI<sup>1\*</sup>, AMIAN BRISE BENJAMIN KASSI<sup>2</sup>, FELIX ANOH ADJE<sup>3</sup>, YVES FRANÇOIS LOZANO<sup>4</sup> AND YVES-ALAIN BEKRO<sup>5</sup><sup>1</sup>Ecole Normale Supérieure (ENS), Département des Sciences et Technologie, 08 BP 10 Abidjan 08, Côte d'Ivoire<sup>2</sup>Laboratoire de chimie organique et de substances naturelles (LCOSN), Université de Félix HOUPOUËT-BOIGNY, 22 BP 582 Abidjan 22, Côte d'Ivoire<sup>3</sup>INPHB, Laboratoire des Procédés Industriels, de Synthèse, de l'Environnement et des Energies Nouvelles BP 1093 Yamoussoukro, Côte d'Ivoire<sup>4</sup>CIRAD, UMR-110 INTREPID (INTensification Raisonnée et Ecologique pour une Pisciculture Durable), TA B 110-16, 73 rue J. F. Breton, 34098 Montpellier cedex 5, France<sup>5</sup>Université Nangui Abrogoua, Laboratoire de Chimie Bio Organique et de Substances Naturelles (LCBOSN), 02 BP 801 Abidjan 02, Côte d'Ivoire 02

## ABSTRACT

This study aims to evaluate the effect of freeze-drying and spray-drying on total phenolics contents and antioxidant capacity from aqueous extract of *Justicia secunda* leaves. Total phenolic compounds extraction procedure consists of using an extraction-concentration procedure performed at three-step process, including ultrasound-assisted water extraction followed by cross-flow microfiltration of the crude extract and its concentration by reverse osmosis. After final step of extraction procedure, a concentrated extract, namely RO extract was obtained. In order to convert RO extract to powder form, two drying methods are used; freeze-drying and spray-drying. The powder obtained by freeze drying, namely P1, presented the higher content of polyphenols ( $9542 \pm 38 \mu\text{mol.g}^{-1}$  GAE) and antioxidant capacity ( $4256 \pm 15 \mu\text{mol.g}^{-1}$  TE) than that obtained by spray drying and namely P2. The freeze-drying technology could be a good outcome to preserve polyphenols structure and consequently their antioxidant capacity in aqueous extract of *J. secunda* leaves. Moreover, seven minor phenolic compounds of RO extract were characterized by HPLC coupled to UV/Vis diode array detection and mass spectrometry with electrospray ionization (LC/DAD/ESI-MS). There were kaempferol-3-O-coumaroyl glucoside (**10**), justiflorinol (**14**), luteolin-7-O-rutinoside (**18**), apigenin 6-C-glucoside-7-O-rhamnosyl-hexoside (**19**), quercetin-7-O-glucoside-3-O-rutinoside (**20**), apigenin-7-O-neohesperidoside (**23**), and naringenin-7-O-rutinoside (**25**) among the characterized compounds.

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## 1. Introduction

Medicinal plants are potential sources of biologically active compounds with therapeutic properties (Egamberdieva et al., 2016; Mohammadhosseini et al., 2017). *Justicia secunda* Vahl (Acanthaceae) leaves are used in folk medicine for the treatment of anemia (Koné et al., 2012) and hypertension (N'Guessan et al., 2010). Antianemic and anti-hypertensive properties attributed to extracts of this plant were confirmed by Mpiana et al. (2010) and Abo et al. (2016), respectively. Other pharmacological studies showed anti-inflammatory, antinociceptive and antioxidant activities of these extracts (Oneja et al., 2017). A biological screening of the activity of *J. secunda* Vahl extracts and fractions against several Gram positive and Gram-negative bacterial strains of Enterobacteriaceae was reported by Herrera-Mata et al. (2002). Furthermore, the water and methanol extracts of *J. secunda* Vahl leaves showed glucosidase inhibiting effects.

These extracts contained the 2-caffeoyloxy-4-hydroxy-glutaric acid and three diastereomers of secundarellone (Theiler et al. 2017). Koffi et al. (2013) identified the two major phenolic compounds of *J. secunda* aqueous leaf extract as polyphenols (luteolin derivatives).

Polyphenols have been often associated with beneficial effects against the development of degenerative diseases, including atherosclerosis and disorders affecting the central nervous system (Potì et al., 2019). In fact, cardiovascular diseases, cancer, obesity, diabetes, and infectious diseases are the major treatment targets for polyphenols (Rasouli et al., 2017). Their protective activity was attributed to their antioxidant, free radical scavenger, and metal chelator properties along with to their ability to inhibit or reduce different enzymes (Li et al., 2014).

The beverage of *J. secunda* Vahl used by village communities in southern countries is usually obtained by boiling the aerial parts of this plant in water. The polyphenol contents of these extracts were unstable during

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storage (Malick and Bradford, 2008; Chedea et al., 2011). Therefore, for the preservation of these extracts freeze-drying and spray-drying could be used.

The present work deals with the evaluation of the effect of drying processes (freeze-drying and spray-drying) on total phenolics content and their antioxidant activity from *J. secunda* Vahl leaf aqueous concentrated extract obtained by reverse osmosis. Also, the minor phenolic compounds of this extract were identified.

## 2. Experimental

### 2.1. Plant material

Fresh leaves of *J. secunda* Vahl were harvested in Yamoussoukro area (Centre part of Cote d'Ivoire). Its botanical identification was authenticated by Dr. Yao Konan of the Botanic Institute Ake-Assi of Andokoi (Abidjan). A voucher specimen catalogued IBAAN 03836 was deposited at the herbarium of this institute for reference purposes.

The leaves were then dried at room temperature ( $30 \pm 2$  °C) during 7 days in LAPISEN laboratory (Yamoussoukro, Cote d'Ivoire) and kept away from direct sun exposure under an open-sided shed. The dried leaves were packed in plastic bags and shipped to CIRAD laboratory (Montpellier, France), where they were stored at 4 °C until processed and analysed.

### 2.2. Reagents and chemicals

All reagents were of analytical grade. Sodium hydroxide (NaOH), sodium carbonate salt ( $\text{Na}_2\text{CO}_3$ ), mono-hydrated citric acid, di-hydrated mono-sodium phosphate ( $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ ), di-sodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ), Folin-Ciocalteu's phenol reagent were purchased from Carlo Erba (Spain). Gallic acid, quercetin, trolox (6-hydroxy-2,5,7,8-tetramethylchroman-1-carboxylic acid), fluorescein, AAPH (2,2'-azobis(2-methyl-propanimidamide) dihydrochloride), were purchased from Sigma-Aldrich (Germany).

### 2.3. Extraction and stabilization process

The process includes several steps such as ultrasound-assisted water-maceration of dried leaves, membrane filtration and concentration of the extract, stabilisation of the concentrated extract by spray-drying or freeze-drying. The Fig. 1 summarizes extraction and stabilization processes used for this study.

#### 2.3.1. Ultrasound assisted extraction

Ultrasound-assisted extraction of dried leaves (1.25 kg) was performed with 100 L of acidified water ( $\text{H}_2\text{O}$ , 0.01 N citric acid) during 40 min using ultrasonic (US) pilot plant unit equipped with anchor-shape and slow-motion stirrer (40 kHz US frequency, 200-500 W US variable power, REUS, Contes, France). The water extract obtained was filtered using a nylon cloth to give a crude extract (CE).

#### 2.3.2. Pilot plant extract concentration

The crude extract obtained by ultrasound assisted extraction was then clarified by cross-flow microfiltration (CFM) at a constant trans-membrane pressure of 0.6 bar to give a CFM permeate volume (VCFM) of about 92 L, which was then concentrated by reverse osmosis (RO) at a constant trans-membrane pressure of 40 bar. The final volume of the RO concentrated extract obtained (VRO) was generally of 3 L, which was close to that of the dead volume of the RO pilot plant unit. The performance of this RO concentration step was characterized by calculating concentration factor (Eqn. 1):

$$CF = CRO/CCFM \quad (\text{Eqn. 1})$$

Where CF implies the concentration factor, while CRO accounts for concentration of total polyphenols, total flavonoids or antioxidants content in the RO concentrated and CCFM shows the concentration of total polyphenols or antioxidants content in the CFM permeate.

### 2.3.3. Drying processes

#### 2.3.3.1. Freeze drying process

A mass of 1 kg of reverse osmosis (RO) concentrated extract was frozen at -30 °C in cold room and dried using freeze dryer (type cryonext, France) for 48 h. The freeze dryer was set at -80 °C and the pressure was set less than 0.2 bar.

#### 2.3.3.2. Spray drying process

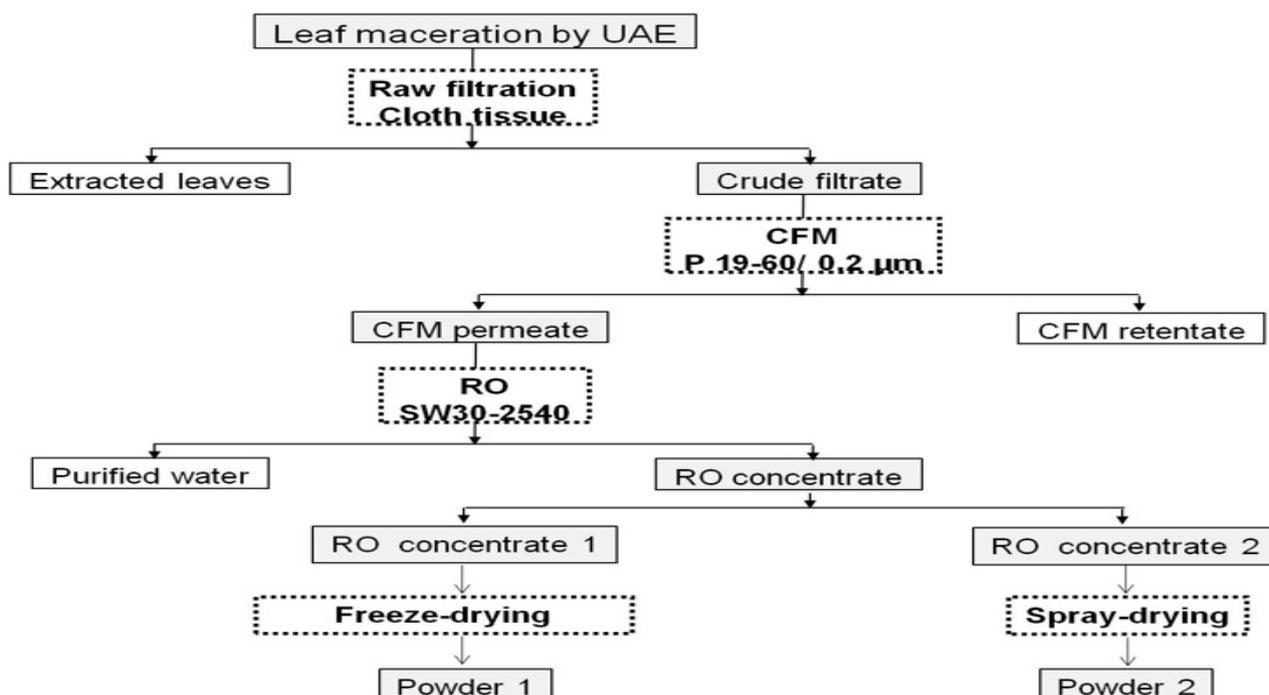
A mass of 1 kg of reverse osmosis (RO) concentrated extract was dried using spray dryer (Minispray Dryer, B-290 Minispray Dryer). The inlet air temperature of the spray dryer was operated at 120 °C and the outlet air at 60 °C.

## 2.4. Total phenolics content

Total polyphenols content was determined by colorimetry, using the Folin-Ciocalteu (F-C) method (Singleton and Rossi, 1965; Wood et al., 2002). To 30  $\mu\text{L}$  sample extract, 2.5 mL of diluted Folin-Ciocalteu's phenol reagent (1/10) were added. After 2 min of incubation in the dark at room temperature, 2 mL of aqueous sodium carbonate ( $75 \text{ g} \cdot \text{L}^{-1}$ ) were added. After slight stirring, the mixture was put in a water bath at 50 °C for 15 min then cooled down. The absorbance was measured at  $\lambda_{\text{max}} = 760 \text{ nm}$  using a UV/Vis spectrophotometer (Jenway 6705, Barloworld Scientific SAS, France). Total phenolics content was expressed as  $\mu\text{mol}$  gallic acid equivalent (GAE) per gram of dried leaves. Samples were analysed in triplicate.

## 2.5. Antioxidant capacity

Antioxidant capacity was carried by oxygen radical absorbance capacity (ORAC) assay. The ORAC method used was described by Ou et al. (2001). The automated ORAC assay was carried out on a VICTOR™ X3 Multilabel Plate Reader (Perkin-Elmer, USA) with fluorescence filters for an excitation wavelength at 485 nm and an emission wavelength at 535 nm (Zulueta et al., 2009).



**Fig. 1.** Extraction process of polyphenols from *Justicia secunda* leaves.

The reaction was started by thermal decomposition of AAPH in  $75 \text{ mmol.L}^{-1}$  phosphate buffer (pH 7.4) at  $37^\circ\text{C}$ . A stock solution of fluorescein (FL) was prepared by weighing 22 mg of FL, dissolving it in 100 mL of phosphate buffer (PBS) ( $75 \text{ mmol.L}^{-1}$ , pH 7.4), and then storing it in complete darkness under refrigeration conditions. The working solution ( $78 \text{ nmol.L}^{-1}$ ) was prepared daily by dilution of 0.334 mL of the stock solution in 25 mL of phosphate buffer. The AAPH radical ( $221 \text{ mmol.L}^{-1}$ ) was prepared daily by taking 0.6 g of AAPH and making it up to 10 mL with PBS. 100  $\mu\text{L}$  of FL and 100  $\mu\text{L}$  of diluted sample, PBS or standard (Trolox  $5\text{-}50 \mu\text{mol.L}^{-1}$ ) were placed in each well of a 96 well-plate and pre-incubated during 15min. After, 50  $\mu\text{L}$  of AAPH were added into the wells. The fluorescence was measured every minute during 60 min with emission and excitation wavelength of 485 and 535 nm, respectively, which was maintained at  $37^\circ\text{C}$ . The ORAC values were calculated as area under the curve (AUC) and were expressed as  $\mu\text{mol TE}$  (Trolox Equivalent) per gram of dried leaves water-extracted. Samples were analysed in triplicate.

## 2.6. HPLC-ESI-MS analyses

An ion trap mass spectrometer (Bruker Daltonics Amazon, Bremen, Germany) was connected via an electrospray ionization (ESI) interface for high performance liquid chromatography-tandem mass spectrometry (HPLC-MS) to UPLC-DAD (Waters Acquity, Milford, MA) equipped with a RP18 column (Acquity BEH column,  $10 \times 1 \text{ mm}$ ,  $1.7 \mu\text{m}$  particle size, Waters, Milford, MA) placed in a controlled temperature oven set at  $35^\circ\text{C}$ . The injection volume was 0.5  $\mu\text{L}$ . The mobile phase was a binary solvent system of A (water:formic acid, 99:1, v/v) and B (methanol:formic acid, 99:1, v/v). The multi-linear gradient profile was: 2% B from start to 1

min, 2 to 30% B, from 1 to 10 min, 30% B from 10 to 12 min, 30 to 75% B from 12 to 25 min, 75 to 90% B from 25 to 30 min, and 90% B from 30 to 35 min. The elution flow rate was set at  $0.08 \text{ mL.min}^{-1}$ . The mass spectrometer operated in negative ion mode (capillary voltage: 2.5 kV; end plate off set:  $-500 \text{ V}$ ; temperature:  $200^\circ\text{C}$ ; nebulizer gas: 10 psi and dry gas:  $5 \text{ L.min}^{-1}$ ; collision energy for fragmentation in MS/MS set at 30%). Polyphenols were detected at 280 nm. UV/Vis spectra were recorded from 210 nm to 600 nm.

## 2.7. Statistical analysis

Results were expressed as mean  $\pm$  standard deviation of three replicate. Data were evaluated by one-way analysis of variance (ANOVA) using Statistica 7.0 (StatSoft, Inc., USA) software. Newman-keuls test was performed to determine significant differences at  $p < 0.05$ .

## 3. Results and Discussion

### 3.1. Extraction and concentration process

Total phenolics content and total antioxidant capacity of aqueous extracts were determined at each stage of the process (Table 1). It was observed that total phenolics content and antioxidant activity of CFM extract was lower than those of crude extract. Indeed, the study of Nyamien et al. (2017) showed that certain polyphenols could be retained by microfiltration membranes. Also, total phenolics content and antioxidant activity were higher in RO concentrated extract than crude extract or CFM extract. The coupled-process applied, lead to concentrated (28 times)

aqueous extracts. The concentration factors of total phenolic content (CFTPC = 17), total flavonoids content (CFTFC = 15) and antioxidant capacity (CFAOC = 21) were lower than the volume reduction factor (VRF = 28), indicating that the same percentage of compound losses occurred with both phenolics content and antioxidant capacity during plant leaf processing. Only a slight loss was observed during cloth filtration of CE, obtained at the first step of the process. Similar results were obtained by Adjé et al. (2012) and Koffi et al. (2015) when using cross flow microfiltration coupled with reverse osmosis apparatus to prepare their extracts.

### 3.2. Effect of drying processes on reverse osmosis

#### concentrated extract

Reverse osmosis concentrated extracts were dried into powders, using comparatively freeze-drying (Powder 1) and spray-drying (powder 2). Table 2 presents the recovery of polyphenols content and antioxidant capacity of two obtained powders. Total polyphenols content and antioxidant capacity of powder 1 (freeze-dried extract) were higher than those of powder 2 (spray-dried extract). Consequently, powder 1 gave better recovery yields (> 90%) than did spray-drying (71-78%). This study showed that freeze-drying is a good process to stabilize phenolic antioxidant from *J. secunda* Vahl leaf aqueous extract. Similar results were obtained by Koffi et al. (2015) when they spray-dried and freeze-

**Table 1**

Total polyphenol content and antioxidant capacity of aqueous extracts in each stage of the process.

Process co-products	Total polyphenol	Total flavonoid	Antioxidant capacity
	( $\mu\text{mol.g}^{-1}$ GAE)	( $\mu\text{mol.g}^{-1}$ QE)	( $\mu\text{mol.g}^{-1}$ TE)
Crude extract	653 $\pm$ 6 <sup>a</sup>	229 $\pm$ 10 <sup>a</sup>	263 $\pm$ 12 <sup>a</sup>
Microfiltrated extract	618 $\pm$ 10 <sup>a</sup>	207 $\pm$ 7 <sup>a</sup>	247 $\pm$ 9 <sup>a</sup>
RO concentrated extract	10328 $\pm$ 61 <sup>b</sup>	3151 $\pm$ 48 <sup>b</sup>	4692 $\pm$ 20 <sup>b</sup>
Concentration factor	17	15	21
VRF	28		

**Table 2**

The recovery of polyphenols content from *Justicia secunda* leaves extract and their antioxidant capacity from two drying procedures; freeze-drying (powder 1) and spray drying (powder 2).

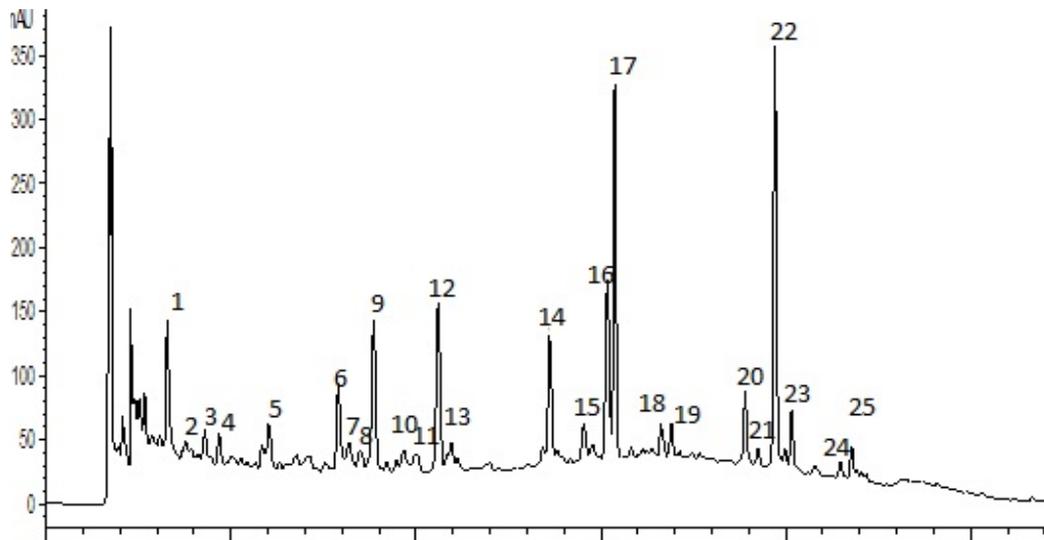
Co-products	Total phenolics content		Total flavonoids content		Antioxidant capacity	
	Content	Recovery (%)	Content	Recovery (%)	Content	Recovery (%)
RO concentrated	10328 $\pm$ 61 <sup>a</sup>	100	3151 $\pm$ 48 <sup>a</sup>	100	4692 $\pm$ 20 <sup>a</sup>	100
Powder 1	9542 $\pm$ 38 <sup>b</sup>	92.39	2896 $\pm$ 56 <sup>b</sup>	91.9	4256 $\pm$ 15 <sup>b</sup>	90.71
Powder 2	7312 $\pm$ 25 <sup>c</sup>	70.8	2469 $\pm$ 31 <sup>c</sup>	78.36	3668 $\pm$ 25 <sup>c</sup>	72.68

For each column, letters equals indicate that the means difference is not significant at  $p > 0.05$ ; RO: reverse osmosis; GAE: gallic acid equivalent; QE: quercetin equivalent; TE: trolox equivalent.

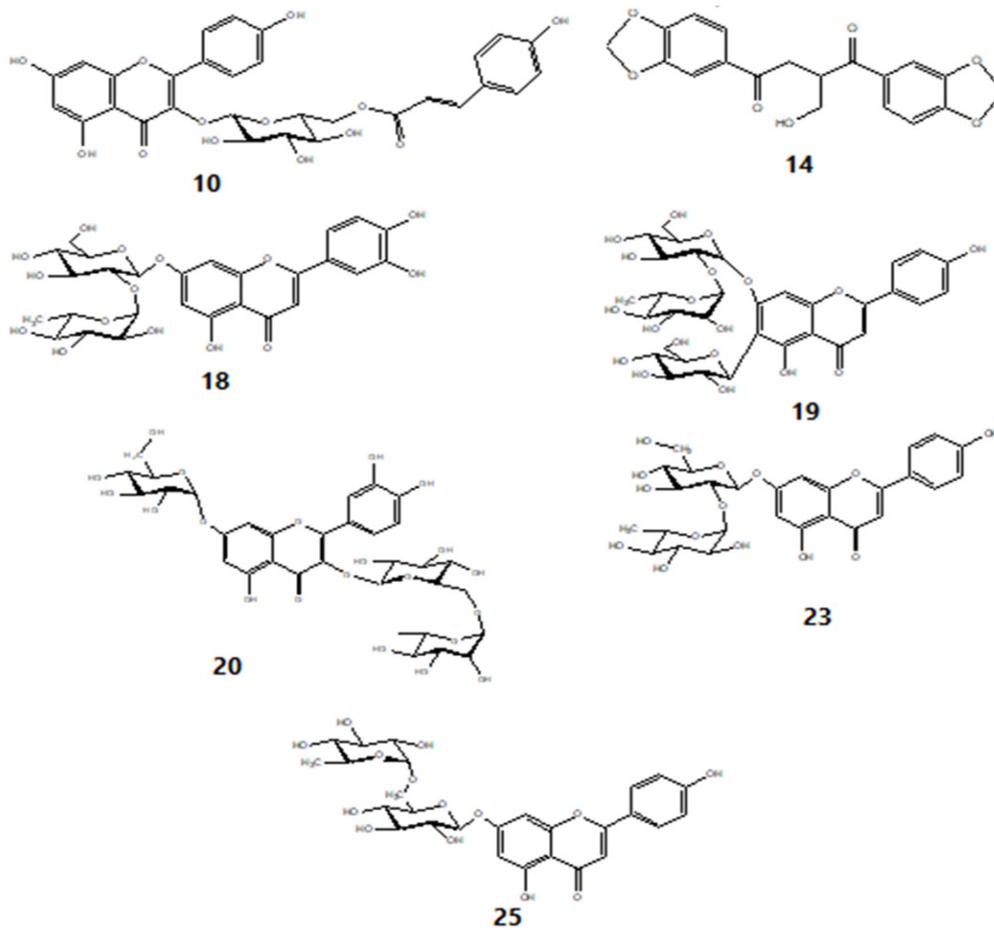
dried *Tectona grandis* leaf aqueous extract. Wesley et al. (2018) and Wilkowska et al. (2016) have also reported that freeze-dried extract showed better polyphenols content and antioxidant activity than for the spray-dried extract from papaya pulp and Blueberry (*Vaccinium myrtillus*) Juice, respectively. The excellent quality of freeze-dried extracts is based on the desiccation of a frozen product; due to the absence of liquid water and the low temperatures required for the process. Then, the most of deterioration and microbiological reactions are stopped which gives a final product of excellent quality (Ratti, 2001).

### 3.3. Identification of the minor phenolic compounds from concentrated RO extract

HPLC-DAD and UV/Vis analyses were shown the chromatogram (Fig. 2) profile of RO concentrated in our previous study (Koffi et al. 2013). In the same study, the two major compounds (**17** and **22**) were identified by NMR analysis as luteolin 7-O- $[\beta$ -glucopyranosyl-(1 $\rightarrow$ 2)- $\beta$ -rhamnosyl-(1 $\rightarrow$ 6)]  $\beta$ -glucopyranoside and luteolin 7-O- $[\beta$ -apiofuranosyl-(1 $\rightarrow$ 2)]  $\beta$ -xylopyranoside. In the present study, the minor compounds **10**, **14**, **18**, **19**, **20**, **23** and **25** (Fig. 3) were identified on the basis of their



**Fig. 2.** HPLC-DAD profile at 325 nm of polyphenols from RO extract of *Justicia secunda*.



**Fig. 3.** Phenolic compounds from *Justicia secunda* RO extract.

**Table 3**LC-SM data obtained from the analysis of *Justicia secunda* extract.

Peak	$\lambda_{\max}$	Positive ion mode			Negative ion mode		
		[M+H] <sup>+</sup>	Fragments	Neutral loss	[M-H] <sup>-</sup>	Fragments	Neutral loss
10	266-328	-	-	-	593	383-353	9210-240
14	230-314	-	-	-	355	337-317-209-191	18-38-146-164
18	266-345	595	449-287	146-308	593	285	308
19	266-340	741	595-433-271	146-308-470	739	269-(431-473-336)	470-(308-266-403)
20	240-266-347	771	463-301	308-470	-	-	-
23	266-349	579	433-301	146-278	577	299-285	278-292
25	255-260-349	581	419-287	162-294	-	-	-

LC-SM (Table 3) fragmentation MS and MS<sup>2</sup> and the shape of their UV/Vis spectra as follows. Compound **10** gave [M-H]<sup>-</sup> at  $m/z$  593 with UV/Vis  $\lambda_{\max}$  at 266 and 328 nm. In MS<sup>2</sup>, two fragments ions were obtained at  $m/z$  353 [M-H-240]<sup>-</sup>. These fragmentations indicate the characteristic of kaempferol-3-O-coumaroyl glucoside (Zhang et al., 2018).

The UV spectrum ( $\lambda_{\max}$  at 314 nm) and mass characteristics ([M-H]<sup>-</sup> at  $m/z$  355) of compound **14** were typical of justiflorinol, a nor-lignane identified in the family of Acanthaceae (Cabanillas et al., 2010).

Compound **18** with [M-H]<sup>-</sup> molecular ion at  $m/z$  593 and a fragment at  $m/z$  285 obtained after the loss of 308 amu (rutinose) was assigned to luteolin-7-O-rutinoside (Temraz, 2011).

Compound **19** gave a molecular peak of [M-H]<sup>-</sup> at  $m/z$  739. In MS<sup>2</sup>, we observed fragment ions at 431 amu and 269 amu (apigenin) after loss of 308 amu (rutinoside moiety) and 308+162 amu (rutinoside and hexoside moieties). The characteristics of this compound matched with those of apigenin 6-C-glucoside-7-O-rhamnosyl-hexoside described by Barros et al. (2011).

Compound **20** gave a molecular peak ion of [M+H]<sup>+</sup> at  $m/z$  771 with a fragment ions at 463 amu ([M+H-308]<sup>+</sup>) and 301 amu ([M+H-(308+162)]<sup>+</sup>) after loss of 308 amu (rutinoside moiety) and 308+162 amu (rutinoside and hexose moieties), typical fragments of quercetin-7-O-glucoside-3-O-rutinoside (Brito et al., 2014).

Compound **23** identified as apigenin rhamnosyl glucose gave a molecular peak ion of [M-H]<sup>+</sup> at  $m/z$  579. In MS<sup>2</sup>, we observed fragments at  $m/z$  433 amu [M+H-146]<sup>+</sup> and 301 amu [M-H-(146+132)]<sup>+</sup> after the loss of rhamnose (146 amu) and rhamnose + pentose (146+132 amu), respectively. The protonated aglycon with 301 amu was assigned to apigenin-7-O-neohesperidoside (Gattuso et al., 2006).

The molecular ion peak [M+H]<sup>+</sup> for the compound 25 was  $m/z$  581 with fragment ions at  $m/z$  419 [M+H-(162)]<sup>+</sup> and 287 [M+H-(162+132)]<sup>+</sup>, typical fragments of naringenin-7-O-rutinoside (Lin and Harnly, 2007).

#### 4. Concluding remarks

A pilot plant scale eco-friendly and multi-step process allows producing powders of natural polyphenol compounds with interesting antioxidant capacities. The optimized processing conditions, including reduced operation time and membrane concentration at room temperature of water-extracts of *J. secunda* Vahl leaves, lead to end-products as concentrated liquids or powders, with little antioxidant capacity losses of extracted polyphenol compounds. The freeze-drying method could be a potential advantage for preservation of its quality during storage of this traditional medicine plant extracts.

#### Conflict of interest

The authors declare that there is no conflict of interest.

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