ANTIOXIDANT CAPACITY OF POWDERED POLYPHENOL EXTRACTS OBTAINED AT PILOT PLANT SCALE FROM *JUSTICIA SECUNDA* LEAVES USED AS NATURAL MEDICINE

*EMMANUEL N. KOFFI [1], PAUL R. LOZANO [1], FELIX A. ADJE [2], YVES-ALAIN BEKRO [3], YVES F. LOZANO [1]**

INTRODUCTION

Justicia secunda Vahl., a native tropical herb originated from South America, is cultivated by village people in tropical countries to prepare medicinal beverages. In Côte d'Ivoire, water-extracts of plant leaves were traditionally homemade to cure various illnesses such as anaemia or hypertension.
A pilot plant coupled-process was developed, mimicking the traditional recipes using plant leaves, to prepare functional and active polyphenol concentrated extracts with antioxidant properties. For better stability and longer shelf-life, the end-product was turned into powder, using comparatively 2 drying technology.

EXTRACTION-STABILISATION PROCESSING

❖ The substrate

▪ **Fresh leaves** of *J. secunda* were **harvested** around Yamoussoukro area (centre area of Côte d'Ivoire). They were **dried** locally at 30 °C during day time, under an open-sided shed..

❖ The pilot plant scale process

▪ **Ultrasound-Assisted water-Extraction** - **UAE** (1.25kg dried leaves, 100 L acidified water 0.01N citric acid, 30min, 40 kHz), was used to extract water-soluble compounds within a reduced maceration time.
▪ **Membrane clarification and concentration** of the crude filtrate obtained was made at room temperature, using Cross Flow Microfiltration - **CFM** (industrial P19-60 ceramic membrane) coupled to Reverse Osmosis – **RO** (industrial SW30-2540 membrane).
▪ **End-product stabilisation** was obtained by drying the concentrated RO extract into powder, using **Spray-Drying** or **Freeze-Drying**.

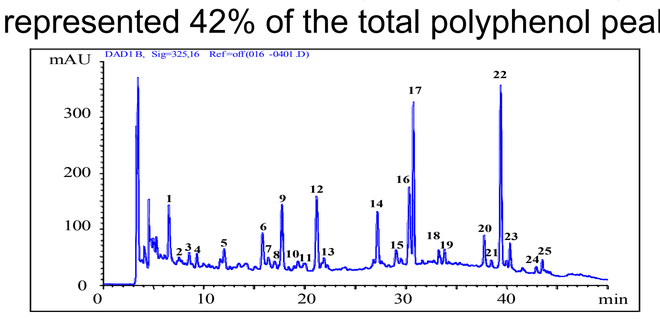
UAE pilot plant

CFM + RO pilot plants

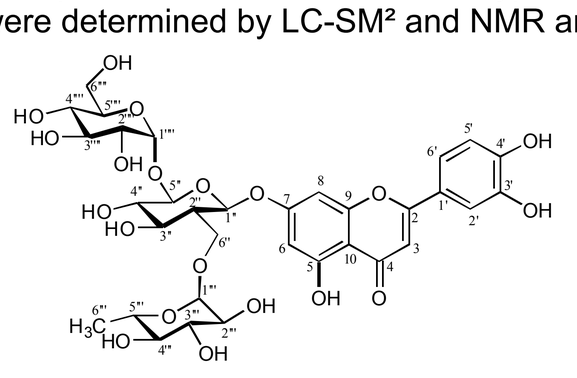
RESULTS and DISCUSSION

❖ Analysis

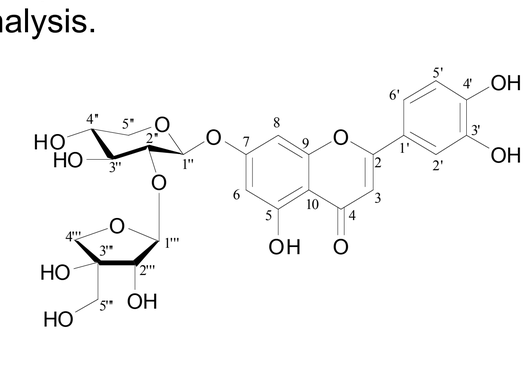
▪ **Total Polyphenol Contents** – **TPC** were determined at λ=760 nm using to the Folin-Ciocalteu method. Total Flavonoid Content - **TFC** was determined by HPLC-DAD peak surface ratio.
▪ **AntiOxidant Capacity** – **AOC** was measured using 2 methods i) the Oxygen Radical Absorbance Capacity assay (**ORAC**), using the AAPH radical to degrade fluorescein in the presence of the protective antioxidant leaf-extract sample. Fluorescence kinetic was followed at λ=485nm and 535nm (emission and excitation wavelengths), and ii) the Trolox Equivalent Antioxidant Capacity (**TEAC**) using the $ABTS^{\circ+}$ radical discoloration kinetic at λ= 734 nm.
▪ **HPLC-DAD polyphenol analysis** showed a 25-peak profile at λ=325 nm. Peaks 4, 10, 13, 17-20, and 22-25 displayed in their UV-vis spectra the 2 specific absorption bands for flavone-type compounds (300-400nm and 240-285nm). They represented 42% of the total polyphenol peak area recorded.



Two major flavonoid compounds (peaks 17 and 22) accounted for 64% of the total peak surface of the flavone-type compounds. Their chemical structures were determined by LC-SM² and NMR analysis.



Peak 17: luteolin 7-*O*-[β-glucopyranosyl-(1→2)-β-rhamnosyl-(1→6)] β-glucopyranoside



Peak 22: luteolin 7-*O*-[β-apiofuranosyl-(1→2)] β-xylopyranoside

❖ Extraction and concentration process

The coupled-process applied, lead to concentrated (28 times) water-extracts and dried end-products,. **Concentration factors** of TPC, TFC and AOC (15 - 21) were slightly lower than the **volume reduction factor** (28), showing that this process preserved the quality of the concentrated extracts obtained.

Processed co-products	TPC *, a	TFC **, a	AOC ***, a	
			ORAC	ABTS
UA crude extract	650	230	260	218
CFM permeate	620	207	250	200
RO concentrate	10330	3150	4690	3810
UA/RO Concentration Factor	17	15	21	19
UA/RO Vol. Reduction Factor		28		

•µmol.g^{-1} acid gallic equivalent, ** µmol.g^{-1} quercetin equivalent,
•*** µmol.g^{-1} trolox equivalent, a : average std deviation = ± 5%

❖ Drying effect on AOC of powders 1 and 2

RO concentrates were dried into powders, using comparatively **spray-drying** and **freeze-drying**. Recovery of polyphenol contents (TPC, TFC) and of AOC in powders, were generally better than 70% : freeze-drying gave better recovery yields (>90%) than did spray-drying (71-78%).

Extract		RO Concentrate	Powder 1	Powder 2
TPC	Content *	10330	9540	7310
	Recovery	-	93.3%	70.7%
TFC	Content **	3150	2895	2470
	Recovery	-	91.9%	78.4%
AOC (ORAC)	Content ***	4690	4255	3670
	Recovery	-	90.7%	78.2%

CONCLUSION

A pilot plant scale eco-friendly and multi-step process allows producing powders of natural polyphenol compounds with interesting antioxidant capacities. The optimised processing conditions, including reduced operation time and membrane concentration at room temperature of water-extracts of *J. secunda* leaves, lead to end-products as concentrated liquids or powders, with little AOC losses of extracted polyphenol compounds. The powder form of leaf water-extracts could be a potential advantage for preservation of its quality during storage and marketing of this traditional medicine at the village level in tropical countries.

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AFFILIATION

1 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. Email address : yves.lozano@cirad.fr
2 Institut National Polytechnique Houphouët-Boigny (**INP-HB**), Laboratoire de Procédés Industriels de Synthèse de l'Environnement et des Energies Nouvelles (LAPISEN), Equipe Polyphénols, BP 1093, Yamoussoukro, Côte d'Ivoire.
3 Université Nanguy Abrogoua (**UNA**), Laboratoire Environnement et Biologie Aquatique, BP 801, Abidjan 02, Côte d'Ivoire.

http: //umr-intrepid.cirad.fr/principaux-projets/alimentalg-microalgues-pour-la-pisciculture

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