



Optimized microwave-assisted extraction of antioxidant pterocarpan compounds from the bark of *Pittosporum viridiflorum* (Pittosporaceae)



Franky Rodrigue KAMGA TAGNE¹, Jean MOMENI², Martin Benoît NGASSOUM³ and Barthelemy NGAMENI¹

1. Department of Organic Chemistry, Faculty of Science, University of Yaounde I, P.O. BOX 812, Yaounde, Cameroon.
2. Department of Chemistry, Faculty of Science, University of Ngaoundere, P.O. Box 454 Ngaoundere, Cameroon
3. National Advanced School of Agro-Industrial Sciences, University of Ngaoundere, P.O. Box 455 Ngaoundere, Cameroon

ABSTRACT: The aim of this work was to optimize the extraction of total antioxidant phenolic compounds by using microwave-assisted extraction technique. The central composite design has been used to organize the experiments and to evaluate the contribution of four factors (extraction time, microwave output power, liquid/solid ratio and ethanol polarity) on the extraction of total phenolic compounds. The results indicated that the models generated fitted quadratic polynomial models. The optimal extraction condition is obtained at 40 s for extraction duration, 400 W for microwave output power; 10:1 for liquid/solid ratio and 60% for ethanol polarity. The separation of antioxidant compounds from the optimized extract by chromatographic column over silica gel led to the isolation for the first time from the genus *Pittosporum* of antioxidant pterocarpan compounds characterized using MS, UV, 1D and 2D NMR spectroscopic data.

Introduction

The growing interest granted to the phenolic compounds by food industries, pharmaceutical firms and scientific researches is focused on recognition of phenolic compounds as food preservations, dietetic supplements, anti-inflammatory, anti-viral, anti-carcinogenic; analgesic and antioxidant compounds [1, 2,]. The role these compounds play in the prevention of cancers, diabetes, cardiovascular and neurodegenerative diseases has also been attractive[3]. Fortunately, 90% of plants used in Africa are traditionally used in medicine but very few are phytochemically investigated [3]. *Pittosporum viridiflorum* Sims (Pittosporaceae) is a highland shrub traditionally used to treat fever, malaria, inflammation, stomach ache and as an antidote for snake bites or insect bites [4,]. To the best of our knowledge, no phytochemical investigation has isolated phenolic compounds from this plant whereas its phenolic compounds have demonstrated significant cytotoxic effects on HL-60 promyelocytic leukaemia cells [5]. The purpose of the present study was to determine the optimal conditions for the extraction of total phenolic compounds from *P. viridiflorum* and to isolate by chromatographic column antioxidant phenolic compounds on optimized extract.



P. viridiflorum leaves and fruits

Material and methods



Fresh bark were collected in June 2014 at Sissieu in the West region of Cameroon. They were air-dried and grounded to an average size of 0.2 mm and stored in sealed plastic bag at ambient temperature until use.. They were air dried and powdered. The four different parameters which influence the MAE of phenolic compounds were extraction time (10, 20, 30, 40, 60, 80, 100, 120, 140, 160, 180s). microwave power (0, 200, 400, 600 and 800 W) liquid/solid ratio (5:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1 and 40:1) and ethanol polarity (20, 40, 60, 80 and 100%). The responses followed during this optimization are total phenolic contents determined by Folin-Ciocalteu method and DPPH scavenging test. The isolation of phenolic compounds was carried out on 17 g of the yellowish optimized extract on silica gel column with AcOEt/MeOH as eluent with increasing polarity .



Results and discussion

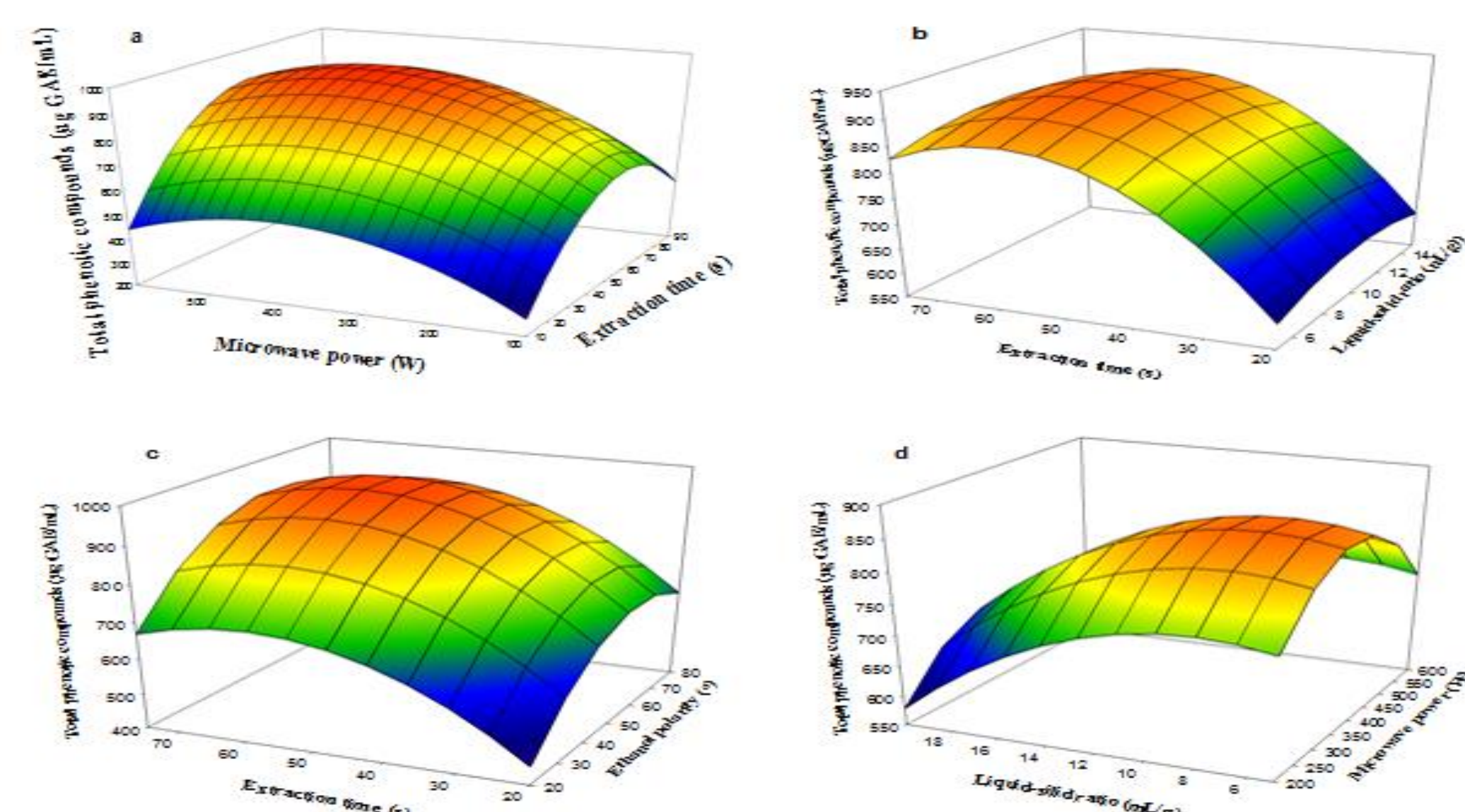


Figure 1: interaction of factors on total phenolic extraction

Table ¹ H-, ¹³ C- and 2D NMR data of compound KAF2 (600 MHz for ¹ H NMR and 2D NMR, 150 MHz for ¹³ C NMR in MeOH-d ₄ .				
Position	δ _H (J in Hz)	δ _C	COSY	HMBC (¹³ C→ ¹ H)
1	7.28 (1H, d, J=8.5)	133.1	2	11a
2	6.50 (1H, dd, J=8.5/2.4)	110.7	1, 4	4
3	-	160.1	-	1, 2, 4
4	6.32 (1H, d, J=2.4)	104.1	2	1, 2
4a	-	158.1	-	1, 4, 6α, 6β, 11a
6α	4,24(1H,dd, J=10,8/5,0)	67,5	6β, 6a	6a, 11a
6β	3,59(1H,t, J=10,8)	-	6α, 6a	-
6a	3.50 (1H, ddd, 10,8/7,0/5,0)	41.6	6α, 6β	6α, 6β, 7, 11a
6b	-	119.9	-	6α, 6β, 6a, 10, 11a
7	6.82 (1H, s)	106,0	-	6a, 10
8	-	143,1	-	7, 10, 13α, 13β
9	-	149,5	-	7, 10, 13α, 13β
10	6.39 (1H, s)	93,3	-	7
10a	-	155,9	-	6a, 7, 10
11a	-	80,1	6a	1, 4, 6α, 6β
11b	-	113,0	-	2, 4, 11a
13α	5.87 (1H, d, J=1.1)	102.5	13α	-
13β	5.89 (1H, d, J=1.1)	-	13β	-

References:

1. Chen, A.-H.; Liu, Q.-L.; Ma, Y.-L.; Jiang, Z.-H.; Tang, J.-Y.; Liu, Y.-P.; Chen, G.-Y.; Xu, W.; Fu, Y.-H. Pterocarpan compounds from the Stems and Leaves of Ochrosia elliptica. Chemistry of Natural Compounds 2018, 54 (3), 553-555.
2. Hamauzu, H. Yasui, T. Inno, C. Kume and M. Omanyuda, Phenolic profile, antioxidant property and anti-influenza viral activity of Chinese Quince (*Pseudocarya sinensis* Schneid.), Quince (*Cydonia oblonga* Mill.) and Apple (*Malus domestica* Mill.) fruits, Journal of Agricultural and Food Chemistry 53 (2005) 928-934.
3. J. M. Berger, Isolation, characterization, and synthesis of bioactive natural products from rainforest flora, PhD Thesis, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, United State, 210 p
4. J. Momeni, N. W. P. Djaleu, Fadimatou, M. T. Akam and M. B. Ngassoum, Antioxidant activities of some Cameroonian plants extracts used in the treatment of intestinal and infectious diseases, Indian Journal of Pharmaceutical Sciences 72 (2010) 140-144.
5. K. D. Nyongbela, A. M. Lannang, G. A. Ayimele, M. N. Ngemenya, Q. Bickle and S. Efangé Isolation and identification of an antiparasitic triterpenoid ester saponin from stem bark of *Pittosporum mannii*, Asian Pacific Journal Disease 3(5) (2013) 389-392.
6. Peng, T.; Zhao, F.; Chen, X.; Jiang, G.; Wang, S. Chemical study of the Chinese medicine Pi Han Yao. Biomedical Reports 2016, 4, 219-222.
7. Poschner S. (2013).Anti-cancer effects, cytotoxicity and metabolism of purified Cameroonian plant extracts. Master Thesis, Faculty of Life Sciences, University of Vienna, Austria, 62p.
8. Poschner S. (2013).Anti-cancer effects, cytotoxicity and metabolism of purified Cameroonian plant extracts. Master Thesis, Faculty of Life Sciences, University of Vienna, Austria, 62p.

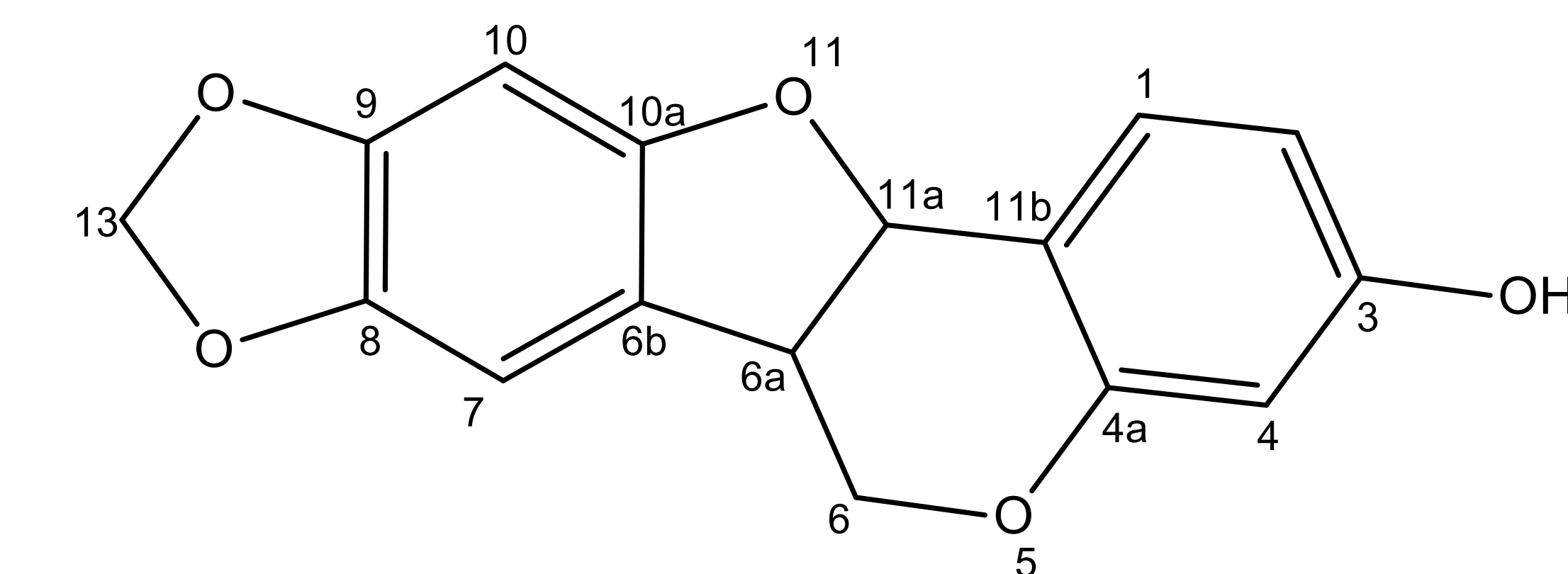


Figure 3: Structure of Maackiain, the first pterocarpan isolated from *Pittosporum viridiflorum*

Acknowledgement & Financial support:

International Foundation for Science (IFS): Grant N° 4895