Table 3
Adjusted coefficient of determination (R^2_{adj}) for the 3 modeled responses.

Compounds	R^2_{adj}			Compounds	R^2_{adj}		
	$\log(k_{tR})$	$\log(w_l)$	$\log(w_r)$		$\log(k_{tR})$	$\log(w_l)$	$\log(w_r)$
1	0.9995	0.9305	0.8374	8	0.9996	0.9016	0.9816
2	0.9995	0.9278	0.9754	9	0.9996	0.9901	0.9592
3	0.9995	0.8926	0.9763	10	0.9997	0.7972	0.7215
4	0.9996	0.9828	0.6205	11	0.9995	0.7923	0.6341
5	0.9997	0.8521	0.8258	12	0.9995	0.7876	0.8241
6	0.9993	0.8202	0.7595	13	0.9998	0.8588	0.7886
7	0.9995	0.9641	0.9497	***Mean***	***0.9995***	***0.8844***	***0.8349***

3.5. Determination of extraction efficiency

One gram of dried and powdered leaves was extracted as described in Fig. 2. The leaves were first extracted for 1 h. The residue was recuperated and extracted again with fresh solvent for 1 h. This was repeated until the exhaustion of leaves in dicentrine. Each extract obtained after each extraction was evaporated to dryness separately and fractionated. The alkaloidic fraction was injected separately onto HPLC column. The kinetics of the extraction was established by monitoring the peak area of dicentrine.

3.6. Method validation

Selectivity, response function, trueness, precision (repeatability and intermediate precision), accuracy, linearity, range, limits of detection (LOD) and quantification (LOQ) were the criteria determined to validate the method [20,24,25]. The validation of the method was done during three days to evaluate time-dependant intermediate precision. Data were analyzed with e-noval V3.0 software (Arlenda, Liège, Belgium).

3.6.1. Calibration standards

Stock solution of 1 mg/ml of dicentrine was prepared in methanol. This solution was diluted and four concentration levels (10, 25, 50 and 75 μg/ml) were used to establish the calibration curve. Each level of the calibration standards was analyzed in duplicate.

3.6.2. Validation standards

Validation standards were prepared within matrix. Dried extract of leaves was diluted in 40 ml of methanol. This solution was spiked to reach final validation standards at 10, 25, 50, and 75 μg/ml of dicentrine. Each level of the validation standards was analyzed independently in triplicates.

4. Results and discussion

4.1. Experimental design

The first step is to verify the validity of the model for each response. In total, 13 peaks were identified as aporphine alkaloids in the total alkaloid extract. As depicted in Fig. 3a, the adequacy between the experimental retention times and the predicted ones is good. The adjusted coefficients of determination (R^2_{adj}) are summarized in Table 3. Furthermore, the corresponding residuals were normally distributed for most compounds – for each response p-values were >0.05; (p-values for $\log(k_{tR})$ are presented in Table 4) – and mainly located into the interval [−2,2] minutes (Fig. 3b).

Despite the high number of interactions added in the model (see Eq. (1)), a curved tendency can be observed on the residuals in Fig. 3b. It suggests that some terms of higher orders (e.g. pH^3) could have been added in the model to avoid this slight lack of fit situation. Additionally, the heteroscedasticity of the residuals distribution in Fig. 3b highlights the need to perform the logarithmic transformation used to predict $\log(k_{tR})$. Indeed, the p-value computed on the modeled response ($\log(k_{tR})$) were acceptable (>0.05; see Table 4) for 10 of the 13 compounds suggesting that the models were suitable for the optimization of the separation.

Then, once the responses are correctly modeled, the error can be propagated from them to the separation criterion S. This step is carried out to estimate the prediction error affecting S and to give confidence in predicted optima. Thus, the predicted criterion was computed over the whole experimental domain using a grid search approach. Moreover, the probability to reach the criterion threshold $S > 0$ min was also computed from 2000 Monte Carlo simulations over each point of this grid [10–12]. Fig. 4 displays the probability surfaces to at least reach a complete separation for each studied parameters: $P(S > 0\,\text{min})$.

The optimal separation was predicted at pH 3.0 with a gradient starting at 32% of methanol and whose slope was 0.42%/min. In Fig. 4, the DS is hedged in by a red line. The DS corresponds to a probability of 14% to get a complete separation. This quality level may seem low, but in the light of the residuals range (between −2 and +2 min) all compounds should be separated at the optimal operating conditions. This low quality level and the small DS size also suggest that the method will not be very robust. However, as depicted in Fig. 5b, the chromatogram recorded at the above mentioned optimal operating condition allows the complete separation of all the investigated compounds and is in accordance to the predicted chromatogram (Fig. 5a).

The use of DoE and DS allowed separating of 13 compounds with UV and MS spectra indicating that they possess an aporphine skeleton, among them dicentrine, a positional isomer of dicentrine and neolitsine. Other compounds (isocorydine and glaucine) were identified by the comparison of their mass spectrum with literature and with our database [26].

4.2. Extraction efficiency

The kinetic of the extracted amount of dicentrine (Fig. 6) showed that total extraction was observed after four 1 h extractions (area = 0). An extraction efficiency of 99.61 ± 0.01% ($n = 3$) was then obtained. For all experiments, four 1 h extractions using each time fresh solvent were applied.

Table 4
Shapiro–Wilk normality test p-values for $\log(k_{tR})$.

Compounds	p-Value	Compounds	p-Value
1	0.243	8	0.524
2	0.028	9	0.446
3	0.028	10	0.057
4	0.091	11	0.433
5	0.075	12	0.127
6	0.004	13	0.502
7	0.622		

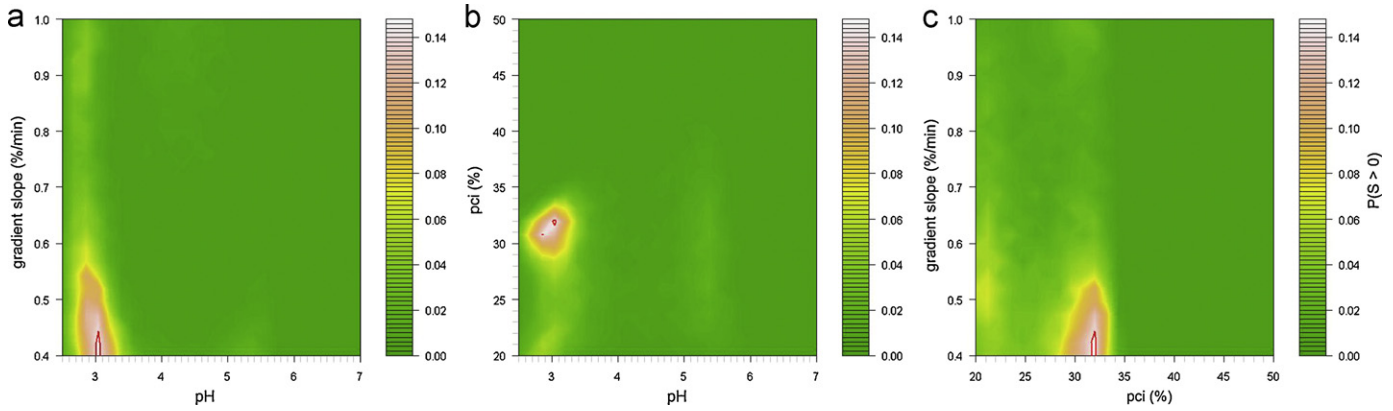

Fig. 4. Probability surfaces to reach at least $S > 0$ min. (a) pH vs gradient slope (%/min), (b) pH vs pci (%) and (c) pci (%) vs gradient slope (%/min). The DS is encircled by the red lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

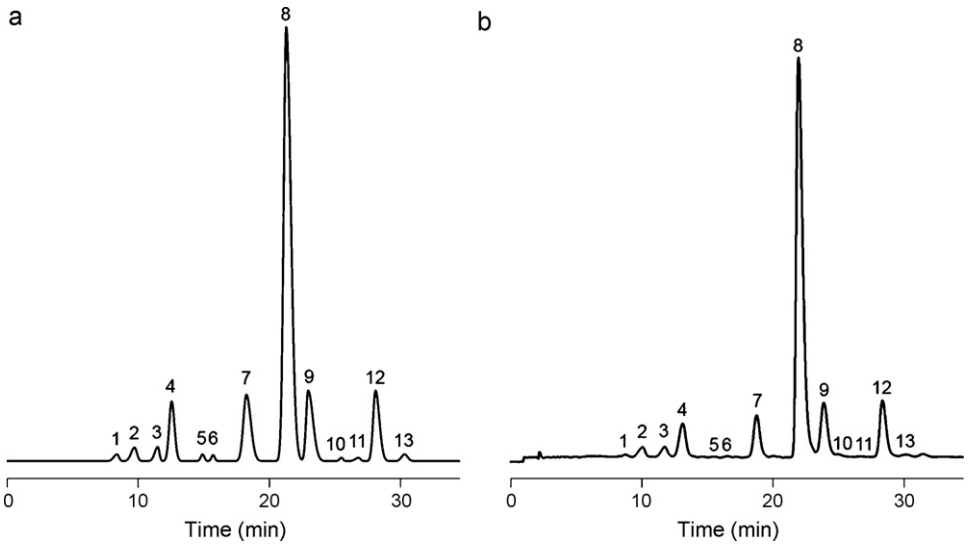

Fig. 5. (a) Predicted chromatogram at optimal conditions (see text for details), (b) experimental chromatogram recorded at optimal conditions of total alkaloids extract of *Spirospermum penduliflorum*. Peak numbering: 1 = unidentified peak, 2 = isocorydine, 3–6 = unidentified peaks, 7 = glaucine, 8 = dicentrine, 9–11 = unidentified peaks, 12 = neolitsine, 13 = unidentified peak.

4.3. Method validation

The method validation focused on the demonstration of the ability of the optimized method to accurately quantify dicentrine in extracts of *S. penduliflorum*. The assessed validation criteria were method selectivity, trueness, precision, linearity, range, LOQ and LOD as well as the accuracy of the generated results. The selection of the most adequate response function was also performed during the method validation phase [16,27]. To achieve this, the accuracy profile methodology was endorsed which allows to decide about the validity of the method. This accuracy profile is based on the predictive quality of future results that will be generated by the method under trial [28,29]. In this framework two values have to be defined a priori. The first one is the acceptance limits. They represent the maximum relative error that is acceptable for the future application of the method being validated. These acceptance limits were set at ±30% as commonly done for plant matrices [25,30,31]. The second one is the guarantee or minimum probability to have future results included within these limits. It was set at 90%.

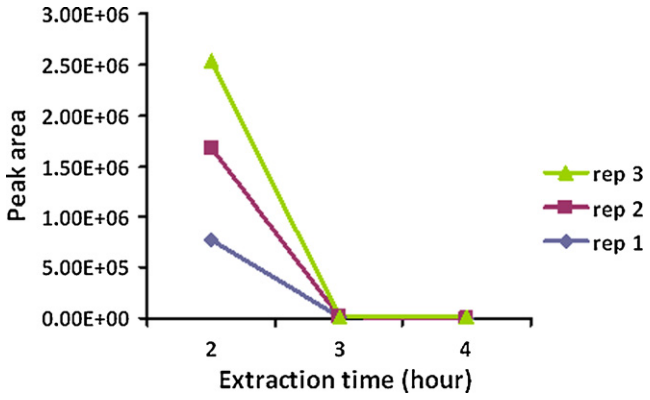

Fig. 6. Dicentrine peak area responses with increasing times of extraction obtained with the same powdered leaves of *Spirospermum penduliflorum* ($n = 3$).

4.3.1. Selectivity

The selectivity of the method was insured by the comparison of the retention time and mass spectra with the reference standard of dicentrine. Mass spectra at the beginning, the middle and the end of the chromatographic peak of dicentrine were recorded and found to be comparable (Fig. 7).

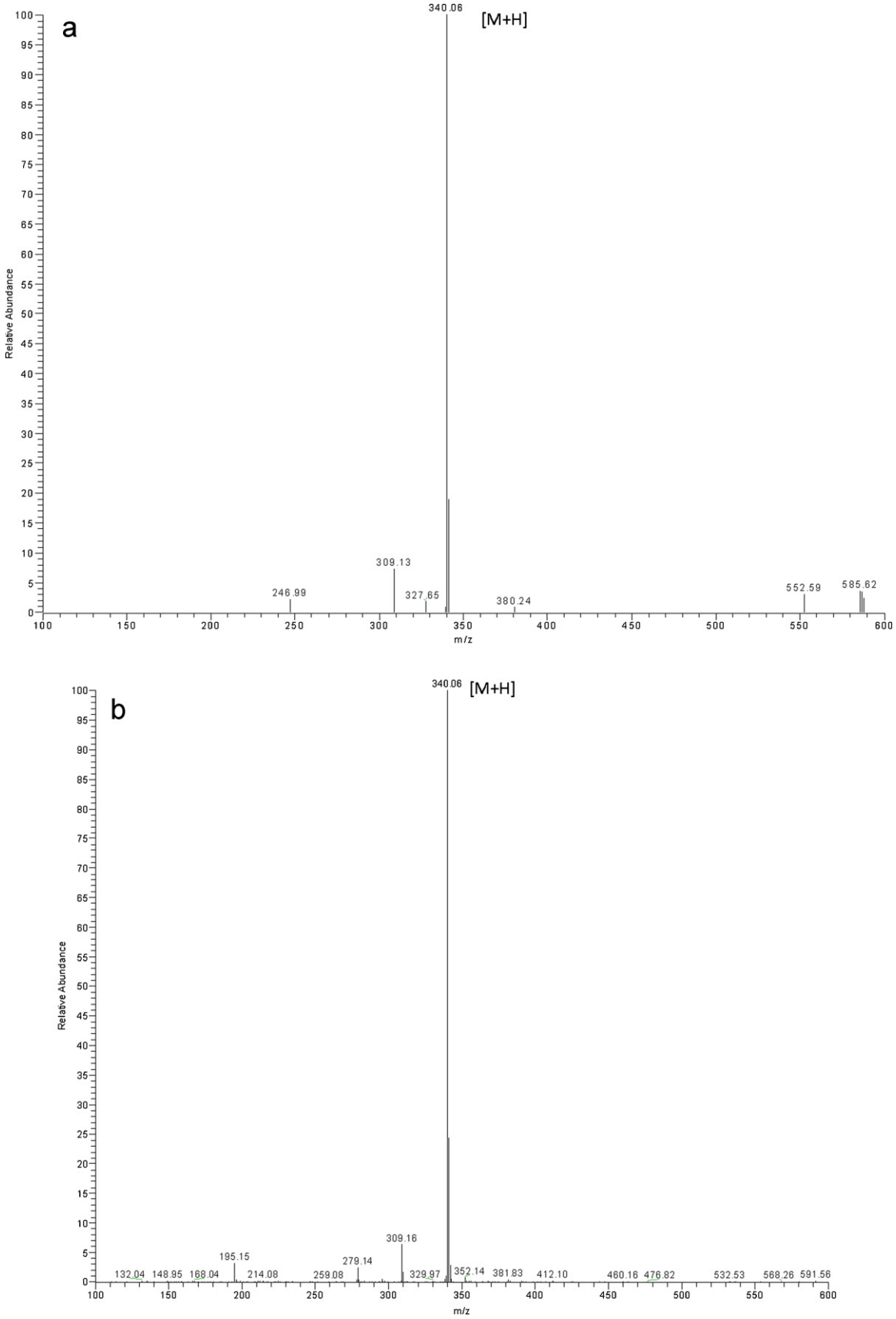

Fig. 7. Positive ion mode mass spectra of standard dicentrine (a), and in total alkaloid extract (b) of *Spirospermum penduliflorum* [M+H = 340].

Table 5
Validation results of the HPLC-UV method for the determination of dicentrine in leaves extract of *Spirospermum penduliflorum* (3 series, 3 repetitions).

Validation criteria	Dicentrine		
Response function	Weighted ($1/X^2$) linear regression Calibration range (4 levels) 10–75 μg/ml		
Trueness	Concentration (μg/ml)	Relative bias (%)	
	10	−9.4	
	25	−5.6	
	50	−2.8	
	75	−1.4	
Precision	Concentration (μg/ml)	Repeatability (RSD%)	Intermediate precision (RSD%)
	10	9.1	9.6
	25	5.6	5.9
	50	2.8	7.9
	75	1.7	2.8
Accuracy	Concentration (μg/ml)	90% β-expectation lower and upper tolerance limits of the relative error (%)	
	10	−28.6, 9.9	
	25	−17.5, 6.3	
	50	−26.5, 20.9	
	75	−8.3, 5.6	
Linearity			
Slope	0.9995		
Intercept	−1.176		
r^2	0.991		

4.3.2. Response function

Calibration standards without matrix were prepared at four concentration levels of dicentrine. Analyses were performed in duplicate. Validation standards were prepared independently with the matrix and also contained known concentrations of dicentrine at the same four concentration levels. They were analyzed in triplicate. These validation standard concentration levels were considered as conventionally true values. The concentration for both calibration and validation standards ranged from 10 to 75 μg/ml. Different regression models were fitted on the calibration standards such as: simple linear regression, weighted ($1/X$) linear regression, weighted ($1/X^2$) linear regression, linear regression through 0 fitted with the highest calibration level only, linear regression after logarithmic transformation, quadratic regression, weighted $1/X$ quadratic regression and weighted $1/X^2$ quadratic regression.

According to accuracy index [17], the weighted ($1/X^2$) linear regression model was the most adequate calibration model. Indeed, the 90% β-expectation tolerance intervals are included inside the ±30% acceptance limits within the range of 10–75 μg/ml of dicentrine [16,27,29]. These tolerance intervals are intervals one can claim to include each future result with 90% probability, thus giving a high level of guarantee about the suitability of the method for its intended purpose [27,29].

4.3.3. Trueness

The systematic error between the accepted true values of the validation standards and the mean experimental one represents

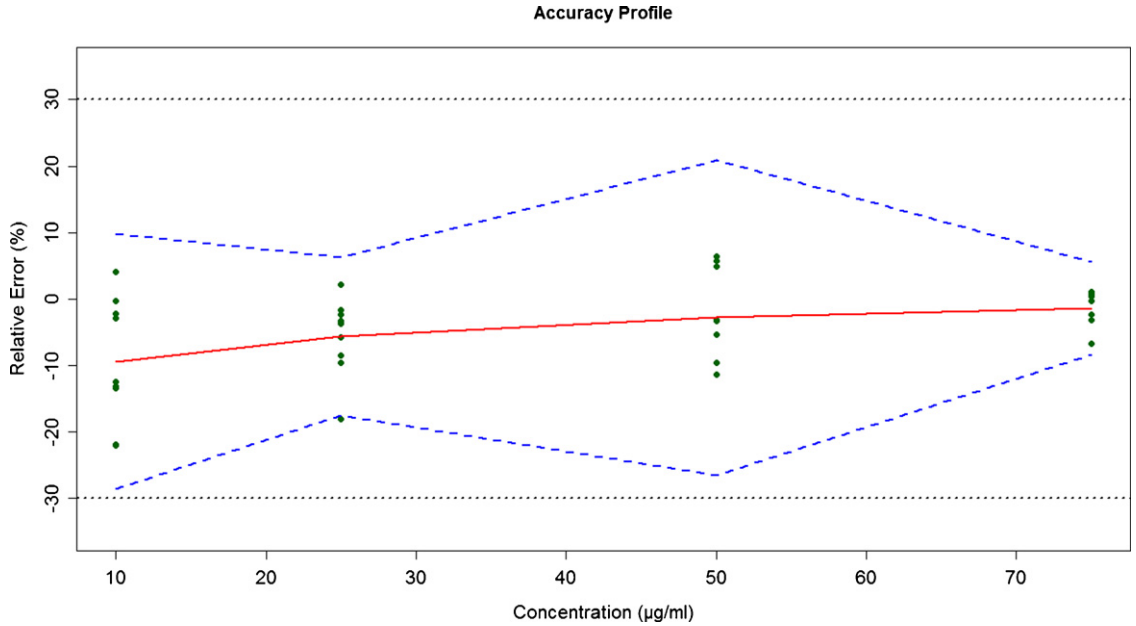

Fig. 8. Accuracy profile of dicentrine obtained with a weighted ($1/X^2$) linear regression model as calibration curve. The plain line is the relative bias (%), the dashed lines are the β-expectation tolerance limits (β = 90%) and the dotted lines represent the acceptance limits (±30%).

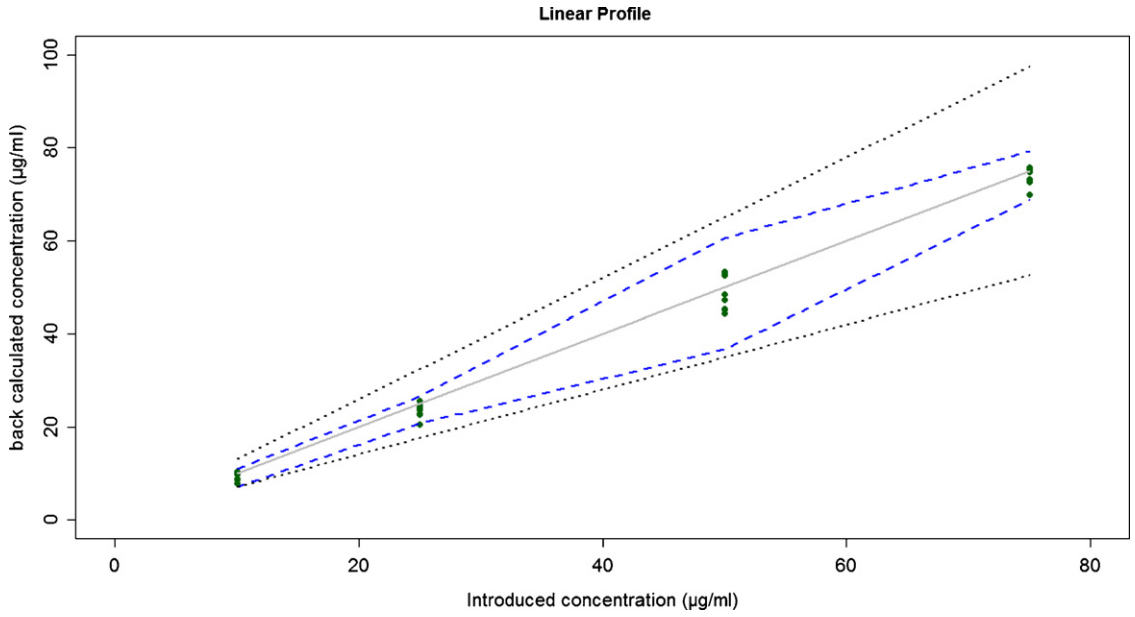

Fig. 9. Linearity profile of dicentrine. The continuous line is the identity line $y = x$, the dotted lines are the ±30% acceptance limits expressed in concentration units (μg/ml of dicentrine) and the dashed lines are the upper and lower β-expectation tolerance limits ($\beta = 90\%$) also expressed in μg/ml of dicentrine.

the trueness of the method [16,29]. It can be expressed in relative bias which did not exceed −10% (see Table 5) showing the adequate trueness of the method.

4.3.4. Precision

Precision represents the variation of the results of dicentrine in intraday (or repeatability) and interday conditions (intermediate precision) [20,24,29]. Precision was assessed during three days ($p = 3$) with three independent repetitions ($n = 3$) at each of the four concentration levels of the validation standards, leading to 9 results per level of dicentrine and a total amount of 36 results. It was evaluated in terms of relative standard deviation (RSD%) values. These values did not exceed 10% for repeatability as well as for intermediate precision.

4.3.5. Accuracy

Accuracy refers to the closeness of agreement between the test result and the accepted reference value, namely the conventionally true value [20,24,29]. Accuracy takes into account the sum of systematic and random errors giving the total error [29]. The acceptance limits have been set at ±30% according to the complexity of the matrix (plant extract) [25,30,31].

Fig. 8 shows that the 90% β-expectation upper and lower tolerance limits are totally included inside the acceptance limits for the whole concentration range of 10–75 μg/ml of dicentrine when a weighted ($1/X^2$) linear regression model is used as calibration curve. This demonstrates the accuracy of the results obtained by the developed method [16,27,28]. This dicentrine concentration interval is very small because of the closeness of the retention times of dicentrine and of its isomer. A high concentration level of dicentrine may cause a coelution with its isomer, impairing its accurate quantification.

4.3.6. LOD, LOQ and range

Limits of detection (LOD) and quantification (LOQ) were 3.0 μg/ml and 10.0 μg/ml respectively. The LOD was estimated using the mean intercept of the calibration model and the residual variance of the regression. For the LOQ it was obtained from the accuracy profile (Fig. 8) as the smallest concentration where the 90% β-expectation tolerance limits remain inside the ±30% acceptance limits [16,20,29]. Consequently, the range over which the method is valid extends from 10 to 75 μg/ml of dicentrine as shown in Fig. 8.

4.3.7. Linearity

The linearity of an analytical method is the ability within a definite range to obtain results directly proportional to the concentration of the analyte in the sample [16,20,29]. Good relationship was shown between introduced and back calculated concentration. A regression line fitted on the back-calculated results obtained with the selected calibration curve and the concentration levels of the validation standards led to the equation:

$$Y = -1.176 + 0.9995X.$$

The linearity of the results generated by the method is demonstrated in Fig. 9 showing that the 90% β-expectation tolerance limits expressed in concentration units are totally included between the ±30% acceptance limits.

4.4. Sample analyses

Two samples of leaves of *S. penduliflorum* collected in the same region but in different period were analyzed 3 times. Sample 1 was collected in March 2007 while the second one in December 2009. The amounts of dicentrine obtained using the newly developed and validated method were respectively 1.42 ± 0.06% (m/m) for S1 and 1.015 ± 0.007% for S2.

5. Conclusion

A novel analytical method for the quantification of dicentrine in leaves of *S. penduliflorum* was developed using DoE and DS methodology. This methodology allowed the separation of dicentrine and its positional isomer without using specific column or mobile phase. 11 other compounds were also separated and some of them were identified by MS detection, showing the advantage of hyphenated techniques for peak identification and to confirm the selectivity of the analytical method. The encountered optimal chromatographic method was not very robust but peaks were sufficiently separated. This method was then successfully

validated using the accuracy profile methodology within the range of 10–75 μg/ml of dicentrine.

This analytical method is suitable for routine analyses and was used for the quantification of dicentrine, the major vasorelaxing compound, in two plant samples, giving results of 1.42 and 1.015%. These preliminary results show the variability of the active principle content in plants according to their harvesting season. It also shows the necessity of a quantification of dicentrine to standardize the activity of the plant extract, and then of the development of such a validated method. Moreover, the developed method allowed the separation of several alkaloids and molecules whose structures were analyzed by MS. Nevertheless, MS did not allow to determine the stereochemistry and to distinguish between isomers, but the analytical method can also be the basis of preparative purifications of crude extract of *S. penduliflorum* to provide enough pure compounds for NMR analysis. The determination of the structures and stereochemistries of these compounds could be finally envisaged.

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References

[1] A. Zafera Rabesa, Pharmacopée de L'Alaotra, Antananarivo, 1986.
[2] G.H. Schmelzer, A. Gurib-Fakim Fondation, Ressources végétales de l'Afrique tropicale 11(1) Plantes médicinales 1, Pays-Bas, 2008.
[3] P. Rasoanaivo, S.U. Ratsimamanga, A. Rakoto-Ratsimamanga, Isoquinoline alkaloid constituents of *Spirospermum penduliflorum* and *Strychnopsis thouarsii* (Menispermaceae), Biochem. Syst. Ecol. 23 (1995) 679–680.
[4] C.-M. Teng, S.-M. Yu, F.-N. Ko, C.C. Chen, Y.-L. Huang, T.-F. Huang, Dicentrine, a natural vascular α1-adrenoreceptor antagonist isolated from *Lindera megaphylla*, Br. J. Pharmacol. 104 (1991) 651–656.
[5] S.-M. Yu, S.-Y. Hsu, F.-N. Ko, C.-C. Chen, Y.-L. Huang, T.-F. Huang, C.-M. Teng, Haemodynamic effects of dicentrine, a novel α_1-adrenoceptor antagonist: comparison with prazosin in spontaneously hypertensive and normotensive Wistar-Kyoto rats, Br. J. Pharmacol. 106 (1992) 797–801.
[6] M.R. Mustafa, F.I. Achike, Dicentrine is preferentially antagonistic to rat aortic than splenic alpha 1-adrenoceptor stimulation, Acta Pharmacol. Sin. 21 (2000) 1165–1168.
[7] M.-L. Young, M.-J. Su, M.-H. Wu, C.-C. Chen, The electrophysiological effects of dicentrine on the conduction system of rabbit heart, Br. J. Pharmacol. 113 (1994) 69–76.
[8] Y.-C. Lai, T.-F. Kuo, C.-K. Chen and S.-S. Lee, Dicentrine metabolites characterized in miniature pig urine by HPLC-SPE-NMR, http://gra103.aca.ntu.edu.tw/gdoc/97/D95423002a.pdf.
[9] T.-H. Tsai, T.-R. Tsai, C.-J. Chou, C.-F. Chen, Determination of dicentrine in rat plasma by high performance liquid chromatography and its application to pharmacokinetics, J. Chromatogr. B 681 (1996) 277–281.
[10] P. Lebrun, B. Govaerts, B. Debrus, A. Ceccato, G. Caliaro, P. Hubert, B. Boulanger, Development of a new predictive modelling technique to find with confidence equivalence zone and design space of chromatographic analytical methods, Chemom. Intell. Lab. Syst. 91 (2008) 4–16, http://hdl.handle.net/2268/1640.
[11] B. Debrus, P. Lebrun, A. Ceccato, G. Caliaro, E. Rozet, I. Nistor, R. Oprean, F.J. Rupérez, C. Barbas, B. Boulanger, P. Hubert, Application of new methodologies based on design of experiments, independent component analysis and design space for robust optimization in liquid chromatography, Anal. Chim. Acta 691 (2011) 33–42, http://hdl.handle.net/2268/88084.
[12] B. Debrus, P. Lebrun, A. Ceccato, G. Caliaro, B. Govaerts, B.A. Olsen, E. Rozet, B. Boulanger, P. Hubert, A new statistical method for the automated detection of peaks in UV-DAD chromatograms of a sample mixture, Talanta 79 (2009) 77–85, http://hdl.handle.net/2268/13236.
[13] B. Debrus, P. Lebrun, E. Rozet, I. Nistor, A. Ceccato, G. Caliaro, R. Oprean, B. Boulanger, P. Hubert, Nouvelle méthodologie pour le développement automatisé de méthodes analytiques en chromatographie liquide pour l'analyse de mélanges de composés inconnus, Spectra Analyse 268 (2009) 28–33, http://hdl.handle.net/2268/26406.
[14] I. Molnár, H.-J. Rieger, K.E. Monks, Aspects of the Design Space in high pressure liquid chromatography method development, J. Chromatogr. A 1217 (2010) 3193–3200.
[15] J.J. Peterson, A posterior predictive approach to multiple response surface optimization, J. Qual. Technol. 36 (2004) 139.
[16] P. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, N. Cohen, P.-A. Compagnon, W. Dewé, M. Feinberg, M. Laurentie, N. Mercier, G. Muzard, L. Valat, E. Rozet, Harmonization of strategies for the validation of quantitative analytical procedures A SFSTP proposal. Part IV, examples of application, J. Pharm. Biomed. Anal. 48 (2008) 760–771, http://hdl.handle.net/2268/6171.
[17] E. Rozet, V. Wascotte, N. Lecouturier, V. Préat, W. Dewé, B. Boulanger, P. Hubert, Improvement of the decision efficiency of the accuracy profile by means of a desirability function for analytical methods validation: application to a diacetyl-monoxime colorimetric assay used for the determination of urea in transdermal iontophoretic extracts, Anal. Chim. Acta 591 (2007) 239, http://hdl.handle.net/2268/6022.
[18] International Conference on Harmonization (ICH) of Guidance for Industry, Pharmaceutical Development, Topic Q8 (R2), 2009.
[19] W. Dewé, R.D. Marini, P. Chiap, P. Hubert, Guidance for robustness: ruggedness tests in method validation J. Crommen, B. Boulanger, Chemom. Intell. Lab. Syst. 74 (2004) 263–268, http://hdl.handle.net/2268/6036.
[20] International Conference on Harmonization (ICH) of Technical Requirements for registration of Pharmaceuticals for Human Use, Topic Q2 (R1): Validation of Analytical Procedures: Text and Methodology, Geneva, 2005.
[21] Y. Vander Heyden, A. Nijhuis, J. Smeyers-Verbeke, B.G.M. Vandeginste, D.L. Massart, Guidance for robustness: ruggedness tests in method validation, J. Pharm. Biomed. Anal. 24 (2001) 723–753.
[22] B. Dejaegher, Y. Vander Heyden, Ruggedness and robustness testing, J. Chromatogr. A 1158 (2007) 138–157.
[23] R.D. Marini, E. Rozet, Y. Vander Heyden, E. Ziemons, B. Boulanger, A. Bouklouze, A.-C. Servais, M. Fillet, J. Crommen, P. Hubert, Robustness testing of a chiral NACE method for *R*-timolol determination in *S*-timolol maleate and uncertainty assessment from quantitative data, J. Pharm. Biomed. Anal. 44 (2007) 640–651, http://hdl.handle.net/2268/21170.
[24] Guidance for industry: Bioanalytical Method Validation, US Department of Health and Human Services, US Food and Drug Administration (FDA), Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), Rockville, May 2001.
[25] C.T. Viswanathan, S. Bansal, B. Booth, A.J. DeStefano, M.J. Rose, J. Sailstad, V.P. Shah, J.P. Skelly, P.G. Swann, R. Weiner, Workshop/conference report—quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays, AAPS J. 9 (2007) E30–E42.
[26] C. Stevigny, J.-L. Habib Jiwan, R. Rozenberg, E. de Hoffmann, J.Q. Leclercq, Key fragmentation patterns of aporphine alkaloids by electrospray ionization with multistage mass spectrometry, Rapid Commun. Mass Spectrom. 18 (2004) 523–528.
[27] A. Bouabidi, E. Rozet, M. Fillet, E. Ziemons, E. Chapuzet, B. Mertens, R. Klinkenberg, A. Ceccato, M. Talbi, B. Streel, A. Bouklouze, B. Boulanger, P. Hubert, Critical analysis of several analytical method validation strategies in the framework of the fit for purpose concept, J. Chromatogr. A 1217 (2010) 3180–3192, http://hdl.handle.net/2268/29471.
[28] B. Boulanger, E. Rozet, F. Moonen, S. Rudaz, P. Hubert, A risk-based analysis of the AAPS conference report on quantitative bioanalytical methods validation and implementation, J. Chromatogr. B 877 (2009) 2235–2243, http://hdl.handle.net/2268/18777.
[29] E. Rozet, A. Ceccato, C. Hubert, E. Ziemons, R. Oprean, S. Rudaz, B. Boulanger, P. Hubert, Analysis of recent pharmaceutical regulatory documents on analytical method validation, J. Chromatogr. A 1158 (2007) 111–125, http://hdl.handle.net/2268/3646.
[30] M.H. Rafamantanana, E. Rozet, G.E. Raoelison, K. Cheuk, S.U. Ratsimamanga, P. Hubert, J. Quetin-Leclercq, An improved HPLC-UV method for the simultaneous quantification of triterpenic glycosides and aglycones in leaves of *Centella asiatica* (L.) Urb (APIACEAE), J. Chromatogr. B 877 (2009) 2396–2402, http://hdl.handle.net/2268/18776.
[31] D.S.S. Kpoviessi, F. Gbaguidi, J. Gbenou, G. Accrombessi, M. Moudachirou, E. Rozet, P. Hubert, J. Quetin-Leclercq, Validation of a method for the determination of sterols and triterpènes in the aerial part of *Justicia anselliana* (Nees) T. Anders by capillary gas chromatography, J. Pharm. Biomed. Anal. 48 (2008) 1127–1135, http://hdl.handle.net/2268/18778.

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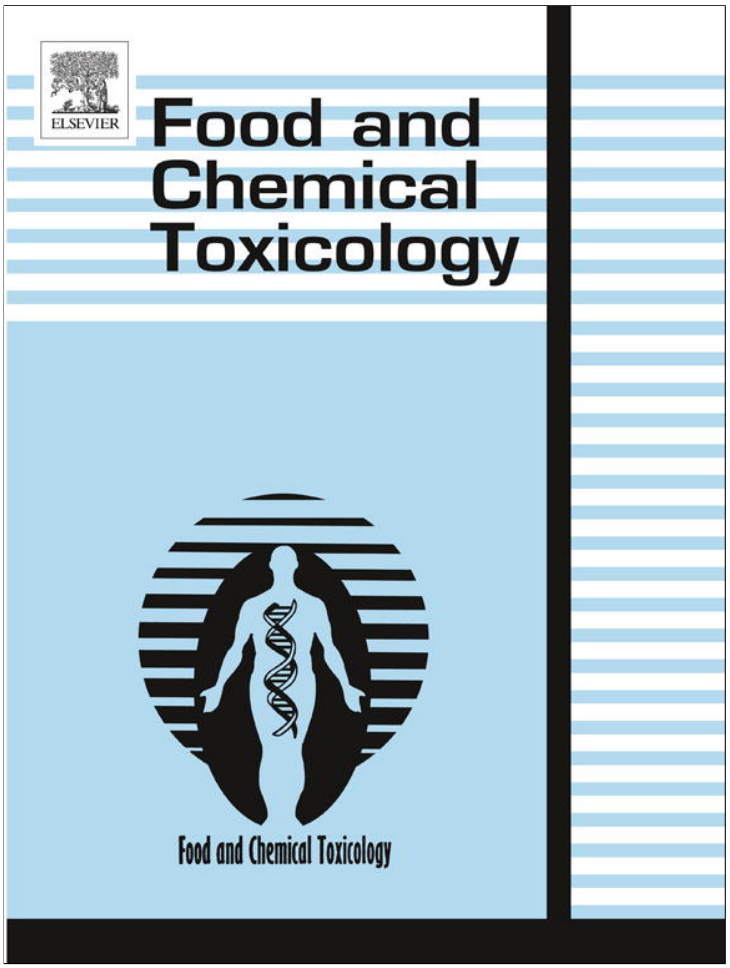

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Chemical composition and anticancer, antiinflammatory, antioxidant and antimalarial activities of leaves essential oil of *Cedrelopsis grevei*

Samia Afoulous [a], Hicham Ferhout [b], Emmanuel Guy Raoelison [c], Alexis Valentin [d], Béatrice Moukarzel [d], François Couderc [a], Jalloul Bouajila [a,*]

[a] Université de Toulouse, Laboratoire des Interactions Moléculaires et Réactivité Chimique et Photochimique, UMR CNRS 5623, Université Paul-Sabatier, 118 route de Narbonne, F-31062 Toulouse, France
[b] Nat'Ex Biotech. Bat 7, 55 avenue Louis Breguet, 31400 Toulouse, France
[c] Laboratoire de Phytochimie et Standardisation, IMRA, BP 3833, Antananarivo 101, Madagascar
[d] Université de Toulouse, UMR 152 IRD-UPS, Pharma-DEV, Université Paul Sabatier Toulouse 3, Faculté de Pharmacie, 35 chemin des maraîchers, 31062 Toulouse Cedex 9, France

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ABSTRACT

The essential oil from *Cedrelopsis grevei* leaves, an aromatic and medicinal plant from Madagascar, is widely used in folk medicine. Essential oil was characterized by GC–MS and quantified by GC–FID. Sixty-four components were identified. The major constituents were: (*E*)-*β*-farnesene (27.61%), *δ*-cadinene (14.48%), *α*-copaene (7.65%) and *β*-elemene (6.96%). The essential oil contained a complex mixture consisting mainly sesquiterpene hydrocarbons (83.42%) and generally sesquiterpenes (98.91%). The essential oil was tested cytotoxic (on human breast cancer cells *MCF-7*), antimalarial (*Plasmodium falciparum*), antiinflammatory and antioxidant (ABTS and DPPH assays) activities. *C. grevei* essential oil was active against *MCF-7* cell lines (IC_{50} = 21.5 mg/L), against *P. falciparum*, (IC_{50} = 17.5 - mg/L) and antiinflammatory (IC_{50} = 21.33 mg/L). The essential oil exhibited poor antioxidant activity against DPPH (IC_{50} > 1000 mg/L) and ABTS (IC_{50} = 110 mg/L) assays. A bibliographical review was carried out of all essential oils identified and tested with respect to antiplasmodial, anticancer and antiinflammatory activities. The aim was to establish correlations between the identified compounds and their biological activities (antiplasmodial, anticancer and antiinflammatory). According to the obtained correlations, 1,4-cadinadiene (R^2 = 0.61) presented a higher relationship with antimalarial activity. However, only (*Z*)-*β*-farnesene (R^2 = 0.73) showed a significant correlation for anticancer activity.

1. Introduction

Cedrelopsis grevei is endemic species to Madagascar. This tree of the vernacular, named 'katafa' or 'katrafay' (katra-bitter, fay-juice), may reach 15 m in height, it has branches in gray bark and fragrant (Smell of cedar) (Cabanis et al., 1969; Courchet, 1906). Flowering is from September to December (after the first rains) and followed by fruiting from October to December (Rasoanaivo and De La Gorce, 1998). The *panniculus* is the highly branched inflorescence bearing a numerous small yellow flowers polygamous giving each a brown capsule containing five valves fruit dry. This species is mainly present in dense forests or dry bush (South and West Island), at 900 m above sea level (Rasoanaivo and De La Gorce, 1998), on different soil types: silica, limestone, sand, etc. The wood is rot resistant insect and it is used in the production of royal tombs (Sakalava). In addition, *Cedrelopsis* produces hardwood and sought for the construction of houses and traditional cabinetry.

Cedrelopsis was used in traditional medicine to treat malaria, fever and fatigue (Mulholland et al., 1999). *C. grevei* trunk bark has wide-ranging empirical uses such as to relieve muscle fatigue and reduce capillary fragility, as an ingredient of a cough syrup against persistent catarrh and also as febrifuge and antihypertensive beverages (Um et al., 2003). The bark essential oil is also used to cure rheumatism and muscular pains and is known to exert antifungal and antibiotic activities. All these popular uses may be explained by the presence of biologically active volatile constituents. The widespread use of *C. grevei* in traditional medicine stimulated us to explore its potential biological activity.

To the best of our knowledge, no previous study of the anticancer, antimalarial, antiinflammatory and antioxidant activities of the essential oil of *C. grevei* have been reported. We report here the chemical composition of the leaves essential oil of *C. grevei* and its anticancer, antimalarial antiinflammatory and antioxidant activities. Moreover, we reviewed bibliographical of all essential oils having an activity against *P. falciparum*, *MCF-7* cell line and 5-Lipoxygenase in order to identify, by correlation, the main active compounds.

* Corresponding author. Tel.: +33 562256825; fax: +33 562256826.
E-mail address: jalloul.bouajila@univ-tlse3.fr (J. Bouajila).

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2. Experimental part

2.1. Extraction of the essential oil

The leaves of *C. grevei* were collected in Antananarivo, Madagascar (June 2008). The leaves are dried outdoors in the shade. Steam-distillation was used to extract the essential oil according to the protocol of the European Pharmacopeia (European Pharmacopoeia, 1983). The essential oil was dried by anhydrous sodium sulfate, filtered and stored in sealed vials at 4 °C, prior to analyses.

2.2. Chemicals used

All chemicals used were of analytical reagent grade. All reagents were purchased from Sigma–Aldrich–Fluka (Saint-Quentin France).

2.3. Gas chromatography and gas chromatography–mass spectrometry

Quantitative and qualitative analysis of the essential oil was carried out by gas chromatography–flame ionization detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS), respectively. Gas chromatography analyses were carried out on a Varian Star 3400 Cx chromatograph (Les Ulis, France) fitted with a fused silica capillary DB-5MS column (5% phenylmethylpolysyloxane, 30 m × 0.25 mm, film thickness 0.25 μm). Chromatographic conditions were 60–260 °C temperature rise with a gradient of 5 °C/min and 15 min isotherm at 260 °C. A second gradient was applied to 340 °C at 40 °C/min. Total analysis time was 57 min. For analysis purposes, the essential oil was dissolved in petroleum ether. One microliter of sample was injected in the split mode ratio of 1:10. Helium (purity 99.999%) was used as carrier gas at 1 mL/min. The injector was operated at 200 °C. The GC–MS system (Varian Saturn 2000 ion trap GC/MS with CP-3800 GC) was used with the same chromatographic conditions for GC–FID. Mass spectrometer was adjusted for an emission current of 10 μA and electron multiplier voltage between 1400 and 1500 V. Trap temperature was 250 °C and that of the transfer line was 270 °C. Mass scanning was from 40 to 650 amu.

Compounds were identified by (i) comparison of their retention indices (RI) relative to C5-C24 *n*-alkanes obtained on a nonpolar DB-5MS column, with those provided in the literature and (ii) by comparison of their mass spectra with those recorded in NIST 08 (National Institute of Standards and Technology), or reported in published articles or by co-injection of available reference compounds. The samples were analyzed in duplicate.

The percentage composition of the essential oil was computed by the normalization method from the GC peak areas, assuming identical mass response factor for all compounds. Results were calculated as mean values of two injections from essential oil, without using correction factors. All determinations were performed in triplicate and averaged.

2.4. Antioxidant activity

2.4.1. Free radical scavenging activity: DPPH test

Antioxidant activity was studied using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) as described by Blois (1958) with some modifications. 1.5 mL of various dilutions of essential oil (EO) was mixed with 1.5 mL of a 0.2 mmol/L methanolic DPPH solution. After an incubation period of 30 min at 25 °C, the absorbances at 520 nm (the wavelength of maximum absorbance of DPPH) were recorded as $A_{(sample)}$, using a Helios spectrophotometer (Unicam, Cambridge, UK). A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as $A_{(blank)}$. The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\% \text{ inhibition} = 100(A_{(blank)} - A_{(sample)})/A_{(blank)}$$

Antioxidant activity of the essential oil was expressed as IC_{50}, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH concentration. Ascorbic acid was used as a standard. All measurements were performed in triplicate.

2.4.2. ABTS radical-scavenging assay

The radical scavenging capacity of the samples for the ABTS (2,2′-azinobis-3-ethylbenzothiazoline-6-sulphonate) radical cation was determined as described by Re et al. (1999) with some modifications. ABTS radical cation was generated by mixing a 7 mmol/L of ABTS at pH 7.4 (5 mmol/L NaH_2PO_4, 5 mmol/L Na_2HPO_4 and 154 mmol/L NaCl) with 2.5 mmol/L potassium persulfate (final concentration) followed by storage in the dark at room temperature for 16 h before use. The mixture was diluted with water to give an absorbance of 0.70 ± 0.02 units at 734 nm using spectrophotometer (Helios). For sample, solutions of the essential oil in methanol (100 μL) were allowed to react with fresh ABTS solution (900 μL), and then the absorbance was measured 6 min after initial mixing. Ascorbic acid was used as a standard and the capacity of free radical scavenging was expressed by IC_{50} (mg/L). The capacity of free radical scavenging (IC_{50}) was determined using the same previously used equation for the DPPH method. All measurements were performed in triplicate.

All data of antioxidant activity were expressed as means ± standard deviations (SD) of triplicate measurements. The confidence limits were set at $p < 0.05$. SD did not exceed 5% for the majority of the values obtained.

2.5. Antiplasmodial activity

The chloroquine-resistant FcB1-Columbia strain of *P. falciparum* (IC_{50} for chloroquine: 186 nM) was cultured continuously according to Trager and Jensen (1976) with modifications (Desoubzdanne et al., 2008). The IC_{50} values for chloroquine

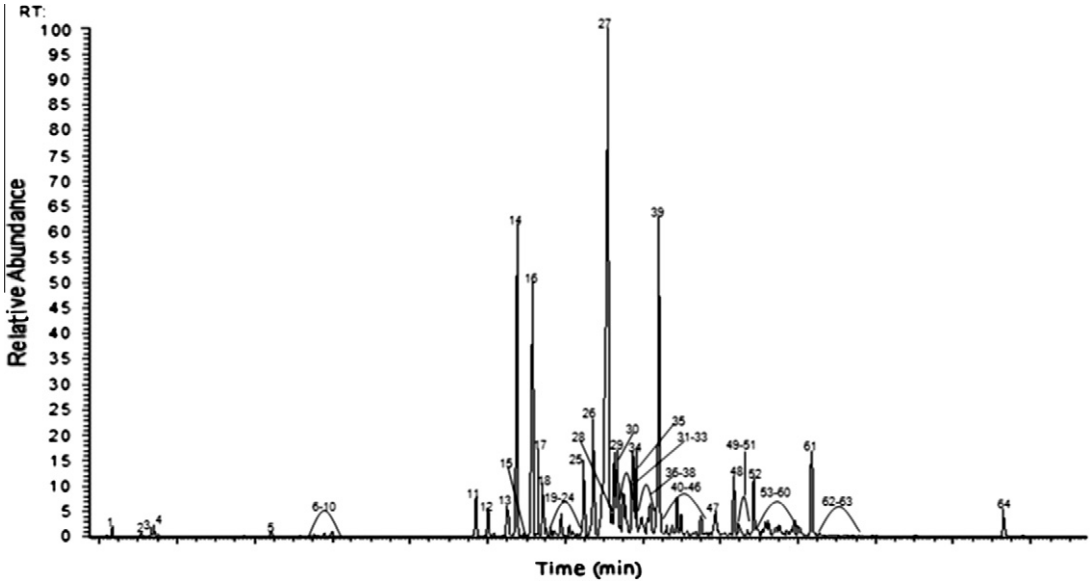

Fig. 1. Chromatograms of leaves essential oil of *Cedrelopsis grevei* (1: α-pinene; 2: β-pinene; 3: *o*-cymene; 4: *m*-cymene; 5: sylvestrene; 6: (*E*)-pinocarveol; 7: isopinocampheol; 8: *p*-cymen-8-ol; 9: α-terpineol; 10: myrtenal; 11: δ-elemene; 12: α-cubebene; 13: α-longipinene; 14: isoledene; 15: α-copaene; 16: β-bourbonene; 17: β-elemene; 18: isoitalicene; 19: cyperene; 20: 9,10-dehydro-isolongifolene; 21: α-gurjunene; 22: α-cedrene; 23: β-caryophyllene; 24: β-gurjunene; 25: (*Z*)-β-farnesene; 26: (*Z*)-β-farnesene; 27: neoclovene; 28: (*E*)-β-farnesene; 29: α-amorphene; 30: γ-muurolene; 31: α-curcumene; 32: ar-curcumene; 33: β-selinene; 34: β-guaiene; 35: viridiflorene; 36: α-muurolene; 37: α-bulnesene; 38: β-dihydroagarofuran; 39: γ-cadinene; 40: δ-cadinene; 41: 1,4-cadinadiene; 42: 4,5,9,10-dihydro-isolongifolene; 43: α-calacorene; 44: elemol; 45: spathulenol; 46: lauric acid; 47: germacrene D-4-ol; 48: viridiflorol; 49: epicedrol; 50: (*E*)-isolongifolanone; 51: 2-*p*-tolyl-6-methyl-5-hepten-2-ol; 52: hinesol; 53: daucol; 54: β-eudesmol; 55: α-bisabobloxide B; 56: bulnesol; 57: valeranone; 58: 5-allyl-4,7-dimethoxy-1,3-benzodioxole; 59: α-bisabolol; 60: γ-dodecalactone; 61: juniper camphor; 62: (*Z*)-β-santalol; 63: alloevodionol; 64: verticiol).

were checked every 2 months, and we observed no significant variations. The parasites were maintained *in vitro* in human red blood cells (O^{+}; EFS; Toulouse, France), diluted to 4% hematocrit in RPMI 1640 medium (Lonza; Emerainville, France) supplemented with 25 mM Hepes and 30 M $NaHCO_3$ and complemented with 7% human AB^{+} serum (EFS).

Parasites cultures were synchronized by combination of magnetic enrichment followed by D-sorbitol lysis (5% of D-sorbitol in sterile water) as described by Lambros and Vanderberg (1979). The antimalarial activity of essential oil was evaluated by a radioactive micromethod described elsewhere (Benoit-Vical et al., 1999). Tests were performed in triplicate in 96-well culture plates (TPP) with cultures mostly at ring stages (synchronization interval, 16 h) at 0.5–1% parasitemia (hematocrit, 1.5%). Parasite culture was incubated with each sample for 48 h. Parasite growth was estimated by [^{3}H]-hypoxanthine (Perkin–Elmer; Courtaboeuf, France) incorporation, which was added to the plates 24 h before freezing. After 48 h incubation, plates were frozen-defrosted and each well was harvested on a glass fiber filter. Incorporated (^{3}H)-hypoxanthine was then determined with a β-counter (1450-Microbeta Trilux; Wallac-Perkin Elmer). The control parasite cultures, free from any sample, was referred to 100% growth. IC_{50} were determined graphically in concentration versus percent inhibition curves. Chloroquine diphosphate was used as positive control.

The antimalarial activity of essential oil was expressed by IC_{50}, representing the concentration of drug that induced a 50% parasitaemia decrease compared to the positive control culture referred to as 100% parasitaemia (Muñoz et al., 1999). According to the literature concerning plant antiplasmodial activities a sample is very active if $IC_{50} < 5$ mg/L, active if IC_{50} between 5 and 50 mg/L, weakly active if IC_{50} between 50 and 100 mg/L and inactive if $IC_{50} > 100$ mg/L (Ouattara et al., 2006).

2.6. Cytotoxicity evaluation

Cytotoxicity of essential oil was estimated on human breast cancer cells (*MCF-7*). The cells were cultured in the same conditions as those used for *P. falciparum*, except for the 10% human serum, which was replaced by 10% foetal calf serum (Lonza). For the determination of essential oil activity, cells were distributed in 96-well plates at 3×10^4 cells/ well in 100 μL, and then 100 μL of culture medium containing sample at various concentrations were added. Cell growth was estimated by (^{3}H)-hypoxanthine incorporation after 48 h incubation exactly as for the *P. falciparum* assay. The (^{3}H)-hypoxanthine incorporation in the presence of essential oil was compared with that of control cultures without sample (positive control being doxorubicin) (Cachet et al., 2009).

2.7. Antiinflammatory activity

5-Lipoxygenase is known to catalyze the oxidation of unsaturated fatty acids containing 1,4-pentadiene structures. In the body, arachidonic acid (biological substrate) is oxidized to hydroperoxyeicosatetraenoic acid (HPETE's) by the 5-Lipoxygenase. Linoleic acid (substrate) is oxidized *in vitro* to a conjugate diene by 5-Lipoxygenase, the activity is evaluated by the spectrophotometric measurement of the conjugated diene at 234 nm. 20 μL of various concentrations (0.23–3.16 g/L) of essential oil was mixed individually with sodium phosphate buffer (pH 7.4) containing 5-LOX (500 U) and 60 μL of linoleic acid (3.5 mM), yielding a final volume of 1 mL. However, the blank does not contain the substrate, but will be added 30 μL of buffer solution. The mixture was incubated at 25 °C for 10 min, and the absorbance was determined at 234 nm. The percentage of enzyme activity was plotted against concentration of the essential oil. The IC_{50} value is the concentration of essential oil that caused 50% enzyme inhibition (Bylac and Racine, 2003).

2.8. Statistical analysis

All data were expressed as mean ± standard deviation of triplicate measurements. The confidence limits were set at $p < 0.05$. Standard deviations (SD) did not exceed 5% for the majority of the values obtained.

3. Results and discussion

3.1. Chemical composition of the essential oil

The essential oil yield of *C. grevei* obtained from hydrodistillation of leaves was 0.73%. Gauvin et al. (2004) have quantified the yield of leaves essential oils, which were between 0.1% and 0.3%. The yield of our work was far superior to that of Gauvin et al. (2004).

Sixty-seven components of leaves essential oil were identified (Fig. 1). This essential oil contained a complex mixture consisting mainly sesquiterpene hydrocarbons (82.80%) and oxygenated sesquiterpenes (13.19%) (Table 1). We had a little portion of oxygenated monoterpenes (0.41%) and monoterpene hydrocarbons (0.70%). The major essential oil constituents characterized were: (*E*)-β-farnesene (27.67%), δ-cadinene (14.5%), α-copaene (7.67%) and β-elemene (6.98%).

Table 1
Chemical composition of leaves essential oil of *Cedrelopsis grevei*.

No.	RI	Compounds	% Area
1	927	α-Pinene	0.14
2	971	β-Pinene	0.20
3	1015	*o*-Cymene	0.13
4	1018	*m*-Cymene	0.18
5	1022	Sylvestrene	0.06
6	1137	*E*-pinocarveol	0.11
7	1175	Isopinocampheol	0.03
8	1178	*p*-Cymen-8-ol	0.03
9	1187	α-Terpineol	0.08
10	1196	Myrtenal	0.16
11	1334	δ-Elemene	0.96
12	1346	α-Cubebene	0.72
13	1351	α-Longipinene	0.09
14	1365	Isoledene	1.01
15	1375	α-Copaene	7.67
16	1382	β-Bourbonene	0.17
17	1390	β-Elemene	6.98
18	1396	Isoitalicene	0.15
19	1400	Cyperene	1.27
20	1404	9,10-Dehydro-isolongifolene[a]	0.21
21	1409	α-Gurjunene	0.36
22	1413	α-Cedrene	0.19
23	1419	β-Caryophyllene	0.59
24	1428	β-Gurjunene	0.34
25	1432	(*E*)-α-bergamotene	0.15
26	1443	(*Z*)-β-farnesene	2.31
27	1453	Neoclovene	3.83
28	1469	(*E*)-β-farnesene	27.67
29	1471	α-Amorphene	0.56
30	1475	γ-Muurolene	1.61
31	1479	α-Curcumene	1.69
32	1481	ar-Curcumene	0.10
33	1484	β-selinene	0.77
34	1487	β-guaiene	0.17
35	1494	Viridiflorene	2.80
36	1498	α-Muurolene	2.58
37	1504	α-Bulnesene	0.62
38	1510	β-Dihydroagarofuran[a]	0.27
39	1513	γ-Cadinene	0.87
40	1523	δ-Cadinene	14.52
41	1532	1,4-Cadinadiene	0.31
42	1539	4,5,9,10-Dihydro-isolongifolene	0.53
43	1544	α-Calacorene	0.99
44	1549	Elemol	0.79
45	1556	Spathulenol[a]	0.25
46	1565	Lauric acid	0.18
47	1571	Germacrene D-4-ol	0.57
48	1588	Viridiflorol	1.23
49	1610	Epicedrol	2.50
50	1616	*E*-isolongifolanone	0.44
51	1627	2-*p*-Tolyl-6-methyl-5-hepten-2-ol[a]	0.25
52	1636	Hinesol	1.26
53	1639	Daucol	0.33
54	1651	β-Eudesmol	0.27
55	1654	α-Cadinol	1.62
56	1664	Bulnesol	0.14
57	1668	Valeranone	0.18
58	1677	5-Allyl-4,7-dimethoxy-1,3-benzodioxole[a]	0.15
59	1683	α-Bisabolol	0.05
60	1688	γ-Dodecalactone	2.05
61	1693	Juniper camphor	0.09
62	1709	(*Z*)-β-santalol	2.14
63	1874	Alloevodionol[a]	1.32
64	1886	Verticol[a]	0.03
		Total	100
		Monoterpenes hydrocarbons	0.7
		Monoterpenes oxygenated	0.41
		Sesquiterpenes hydrocarbons	82.8
		Sesquiterpenes oxygenated	13.19
		Others	2.90

[a] Tentatively identified supported by good match of MS.

One study in the literature has cited the chemical composition of essential oil leaves of *C. grevei*. Gauvin et al. (2004) have shown that the chemical composition of leaves essential oil of *C. grevei* was dominated by (*E*)-*β*-farnesene (35.6%), *β*-pinene (12.8%), (*Z*)-sesquisabinene-hydrate (9.8%) and ar-curcumene (8.6%). Their essential oil contained monoterpene (hydrocarbons (16.5%) and oxygenated (0.5%)), who were very poor in our work. On the other hand, we found that our oil contained more *δ*-cadinene (14.52%), *α*-copaene (7.67%), *β*-elemene (6.98%), (*Z*)-*β*-farnesene (2.31%) compared to Gauvin et al. (2004) respectively 0.4%, trace, 1.1% and 1%. Also, *β*-pinene (0.2%), *β*-caryophyllene (0.59%), and ar-curcumene (0.1%) were obtained in lowest concentration compared to study of Gauvin et al. (2004): 12.8%, 3.5% and 0.1% respectively.

We identified new compounds not reported in the previous study Gauvin et al. (2004). The total content of these new compounds (Fig. 2) varied between 1.32% and 3.83% like neoclovene (3.83%), viridiflorene (2.80%), *α*-muurolene (2.58%), epicedrol (2.50%), (*Z*)-*β*-santalol (2.14%), *γ*-dodecalactone (2.05%) and alloevodionol (1.32%).

We compared the composition of the essential oil of our leaves of *C. grevei* with those of bark from the same species. Cavalli et al. (2003) have obtained (*E*)-*β*-farnesene (9.3%), *α*-copaene (7.7%), *α*-selinene (5.8%), *β*-selinene (4.5%) and *δ*-cadinene (4.9%) as major compounds, the contents completely different from those of our essential oil, respectively, 27.67%, 7.67%, 0%, 0.77% and 14.58%. The bark contained 114 compounds identified but our essential oil consists of 64 compounds. Another study was conducted by Rakotobe et al. (2008), dealing the essential oils of the bark of *C. grevei* from several parts of Madagascar. The compounds have varied with sampling sites but the major compounds (>20%) were *α*-pinene, ishwarane, (*γ*,*δ*)-cadinene, *α*-copaborneol and eudesmol, knowing that the total percentage of essential oil compounds identified are between 23.9% and 71.2%. This composition was markedly different from our essential oil from the leaves.

It is supposed that this difference of chemical composition between our study and Gauvin et al. (2004) work quoted with the top is due to the several factors like the process extraction to use, the genetic factors and environmental (climatic, seasonal, geographical, and geological).

3.2. Antioxidant activity

Antioxidant activity of essential oil of *C. grevei* leaves has been determined by DPPH and ABTS assays. To our knowledge there is no published literature discussing the antioxidant activity of *C. grevei* essential oil and no other *Cedrelopsis*' species. All results were presented in Table 2. The low reactivity by DPPH assay (IC_{50} > 1000 - mg/L) was explained by the high concentration of sesquiterpene hydrocarbons in essential oil (83.43%). The ABTS assay showed a moderate antioxidant activity of essential oil (IC_{50} = 110 ± 3.6 mg/L). Although, the DPPH test is widely applied in the literature, it is based on the use of a very crowded radical. However, the use of a low crowded radical such as ABTS can be more suitable. In addition, the DPPH test is performed in methanol while the ABTS test is carried out mainly in water, conditions closer to the physiological conditions. The differences between the two results can be explained by the mechanism of the involved reaction. The ABTS radical reactions involve electron transfer and take place at a much faster rate compared to DPPH radicals (Ennajar et al., 2009) whose degree of discoloration is attributed to hydrogen donating ability of tested compounds.

3.3. Cytotoxic activity

The essential oil showed a pronounced effect against the cell line *MCF-7* with the IC_{50} values of 21.5 ± 2 mg/L (Table 2). A cytotoxic activity of *C. grevei* essential oil was showed for the first time. To our knowledge, the abundant compounds ((*E*)-*β*-farnesene, *δ*-

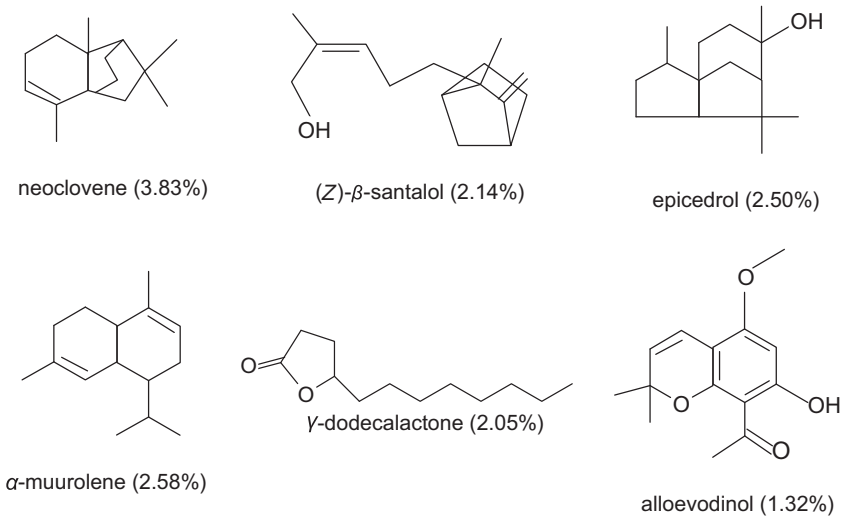

Fig. 2. Structures of new abundant compounds identified in leaves essential oil of *Cedrelopsis grevei*.

Table 2
Antioxidant, anticancer, antimalarial and antiinflammatory activities (IC_{50} (mg/L)) of leaves essential oil of *Cedrelopsis grevei*.

Sample	Antioxidant activity (DPPH assay)	Antioxidant activity (ABTS assay)	Anticancer activity	Antimalarial activity	Antiinflammatory activity
Essential oil	>1000	110.0 ± 3.6	21.5 ± 2.0	17.5 ± 1.0	21.3 ± 0.5
Control	3.75 ± 0.01[a]	1.84 ± 0.03[a]	0.22 ± 0.04[b]	0.14 ± 0.09[c]	1.23 ± 0.14[d]

[a] Ascorbic acid.
[b] Doxorubicin.
[c] Chloroquine.
[d] Nordihydroguaiaretic acid (NDGA).

Table 3
Cytotoxic activity (IC_{50} (mg/L)) and chemical composition of essential oils. Our work, I: *Cedrelopsis grevei*. El Babili et al. (2011), II: *Origanum campactum*. Imelouane et al. (2010), III: *Lavandula dentata* (flower); IV: *Lavandula dentata* (leaf). Li et al. (2009), V: *Schefflera heptaphylla*. Al-Kalaldeh et al. (2010), VI: *Laurus nobilis*; VII: *Origanum syriacum*; VIII: *Origanum vulgare*; IX: *Salvia triloba*. Bendaoud et al. (2010), X: *Schinus molle*; XI: *Schinus terebinthifolius*. Sibanda et al. (2004), XII: *Heteropyxis dehniae*. El Hadri et al. (2010), XIII: *Salvia of ficinalis*. Hussain et al. (2010a), XIV: *Rosmarinus officinalis*. Liu et al. (2009), XV: *Melaleuca alternifolia*. Monajemi et al. (2005), XVI: *Citrus sinensis*; XVII: *Citrus medica*; XVIII: *Citrus limon*. Haber et al. (2008), XIX: *Talouma gloriensis*. Hussain et al. (2010b), XX: *Mentha arvensis* (Summer); XXI: *Mentha arvensis* (Winter); XXII: *Mentha piperita* (Summer); XXIII: *Mentha piperita* (Winter); XXIV: *Mentha longifolia* (Summer); XXV: *Mentha longifolia* (Winter); XXVI: *Mentha spicata* (Summer); XXVII: *Mentha spicata* (Winter). Chabir et al. (2011), XXVIII: *Melaleuca armillaris*. Loizzo et al. (2007), XXIX: *Satureia thymbra*; XXX: *Sideritis perfoliata*; XXXI: *Laurus nobilis* (leaves); XXXII: *Laurus nobilis* (fruits); XXXIII: *Pistacia palestina*; XXXIV: *Salvia officinalis*. Sharopov and Setzer (2012), XXXV: *Artemsia scoparia*. Sonboli et al. (2010), XXXVI: *Dracocephalum surmandinum*. Cole et al. (2007), XXXVII: pure compounds).

Essential oil	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX
Cytotoxic activity	21.5	>100	98.5 ± 1.02	101 ± 4.4	7.3	101.7 ± 7.9	130 ± 52.2	30.1 ± 1.14	174.3 ± 73.04	54 ± 10	47 ± 9	35.95 ± 8.11[a]	554.4 ± 1.5	190.1 ± 6.0	310[a]	0.5	1	10	14.1
Compounds																			
α-Pinene	0.14	0.62	8.38	7.78		5.82	0.38	0.64	3.35	4.34	6.49		3.18	12.3	2.06	0.2	1.5	0,2	1
β-Pinene	0.20		30,06	27.8	22.24	4.55	0.6	1.3	8.98	4.96	3.09		2.57	0.2	0.91		16.3		3.7
E-pinocarveol	0.11		8.59	14.77															
p-Cymen-8-ol	0.03	0.14								0.21	0.11	0.2							
α-Terpineol	0.08		0,5						0.62	8.38	5.6	3.6	0.27	2.3			11.3		
Myrtenal	0.16		6.81	8.18						0.1									
α-Cubebene	0.72				0.25					0.39	0.21								
α-Copaene	7.67	0.02								0.11	0.19	0.6							0.1
β-Bourbonene	0.17										0.06								
β-Elemene	6.98									0.09	0.28								
α-Gurjunene	0.36	0.02									0.08								
α-Cedrene	0.19									0.04	0.29								
β-Caryophyllene	0.59	0.78			5.61	0.37	2.5	1.79	2.23	0.04	0.29	1.6	1.68	1.12	7.56				0.9
β-Gurjunene	0.34									0.25	2.21								
(*E*)-α-bergamotene	0.15		0.38																
(*Z*)-β-farnesene	2.31																1.3		
(*E*)-β-farnesene	27.67		0.27						2.37	0.49	0.2			1.13			3.4		
γ-Muurolene	1.61	0.02								0.05	0.07	0.9		0.2					
β-Selinene	0.77		0.5	0.59						1.1		0.3							
α-Muurolene	2.58	0.02									0.1	0.7							1.7
γ-Cadinene	0.87	0.07								0.07	18.04	1.1							1.6
δ-Cadinene	14.52	0.1								0.27	0.69	2.8							3.3
1,4-Cadinadiene	0.31																		0.8
α-Calacorene	0.99	0.01																	
Elemol	0.79								0.03	0.11									
Spathulenol[a]	0.25	0.03						0.76		0.29		0.2							
Viridiflorol	1.23							0.6	0.79										
Epicedrol	2.50			0.42						0.33	0.82	3.1	0.46	0.2					
β-Eudesmol	0.27			0.68															
α-Cadinol	1.62	0.01				0.61						0.64							
Bulnesol	0.14									0.03	0.64								

	XX	XXI	XXII	XXIII	XXIV	XXV	XXVI	XXVII	XXVIII	XXIX	XXX	XXXI	XXXII	XXXIII	XXXIV	XXXV	XXXVI	XXXVII		
	55.3 ± 1.9	59.7 ± 2.2	75.2 ± 2.9	80.8 ± 3.2	45.2 ± 2.0	50.6 ± 2.0	80.0 ± 2.4	80.6 ± 2.0	12 ± 1	>400	>400	>400	>400	>400	>400	1000	14	20.6	19.7	69.6
α-Pinene			3.53	1.31					1.95	10.15	8.66	5.72	3.67	6.81	4.72	5.4	2.4	100		
β-Pinene			5.7	4.3	2.01	2.42			0.33	2.9	8.9	3.46	2.14	6.48	3.01	21.3	0.1			
E-pinocarveol	0.08																			
p-Cymen-8-ol					0.2	0.21														
α-Terpineol	0.71	0.67		6.13	0.19	0.07	1.32	0.49	0.16	1.53	0.27	2.42	0.4	2.43	3.18	0.2	0.2			
Myrtenal					0.11	0.1														
α-Cubebene																				
α-Copaene			0.41	0.22	0.81	0.41				1.67	0.12		0.17	0.2			0.3			100
β-Bourbonene	0.23	0.17	1.82	1.12	0.61	0.31	0.93	0.56		0.24	0.92									
β-Elemene			0.84	0.99						0.21		0,1	1	0.1						
α-Gurjunene					0.34	0.24				0.51										

Table 3 (*continued*)

Essential oil	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX
Cytotoxic activity	21.5	>100	98.5 ± 1.02	101 ± 4.4	7.3	101.7 ± 7.9	130 ± 52.2	30.1 ± 1.14	174.3 ± 73.04	54 ± 10	47 ± 9	35.95 ± 8.11[a]	554.4 ± 1.5	190.1 ± 6.0	310[a]	0.5	1	10	14.1
β-Gurjunene								1.35			0.67		0.1						
(*E*)-α-bergamotene											0.3								
(*Z*)-β-farnesene													0.13			0.1			
(*E*)-β-farnesene																			
γ-Muurolene			0.95	0.99	0.57	0.94	0.29	0.59	0.02							2			
β-Selinene									0.12	0.11					0.11				
α-Muurolene			0.73	0.77						0.37									
γ-Cadinene						0.16	0.21	0.75	0.12				0.36						
δ-Cadinene			1.21	1.02					0.36	3.11	0.57		0.14	1.51	0.1				
1,4-Cadinadiene																			
α-Calacorene			0.08																
Elemol									0.09										
Spathulenol[a]			0.47	0.78	0.09	0.46		0.37	0.22	0.61	0.88	0.31							
Viridiflorol										0.78	1.17				0.11				
Epicedrol																			
β-Eudesmol	0.32	0.22	0.13	0.17															
α-Cadinol	0.16								0.02										
Bulnesol																			

[a] IC_{50} calculated from percentage.

c- adinene and α-copaene) of this oil are not assessed against cancer. Li et al. (2010) showed that β-elemene has an anticancer activity against the cell line *MCF-7* (IC_{50} = 93.0 ± 7.8 mg/L). This compound was identified to 6.96% in our essential oil. In our results, β-elemene has an IC_{50} higher than that of the essential oil. So, β-elemene is not the responsible for anticancer activity in the essential oil. We have the same trends for these three compounds α-pinene, β-pinene and α-bisabolol as β-elemene, which have respectively IC_{50} = 172.2, 164.1 and 41.8 mg/L against the cell line *MCF-7* (Van Zyl et al., 2006).

The anticancer activity of the essential oil might be due to the synergic effects of all the terpenes in the oil, or perhaps there are some other active compounds responsible for the anticancer activity of the essential oil, which deserves attention in future study.

3.4. Antimalarial activity

The *in vitro* inhibitory effect of the *C. grevei* essential oil against the chloroquine-resistant *P. falciparum* (FCR-3) was showed in Table 2. The antimalarial activity (IC_{50} values) of the essential oil was 17.5 ± 1 mg/L. Since the value of IC_{50} was found between 5 and 50 mg/L, we can be considered that *C. grevei* essential oil has a good activity against *P. falciparum* (Ouattara et al., 2006). This high value of IC_{50} compared to that of chloroquine (IC_{50} = 0.1 ± 0.09 mg/L) can be explained by the low concentration of the active compound(s) since the essential oil is a multi-components mixture. To our knowledge and according to the literature, none of majority compounds in the essential oil is known for antimalarial activity. Van Zyl et al. (2006) showed that α-pinene has an antimalarial activity (IC_{50} = 1.20 ± 0.2 mg/L). This compound was identified to 0.14% in our essential oil. In our results, IC_{50} of the antimalarial activity for the essential oil was higher than those for β-pinene (IC_{50} = 294.7 mg/L) and α-bisabolol (IC_{50} = 307.3 mg/L), reported by Van Zyl et al. (2006).

3.5. Antiinflammatory activity

The essential oil displayed *in vitro* 5-Lipoxygenase inhibitory activity with an IC_{50} value of 21.33 ± 0.5 mg/L (Table 2). An essential oil with a $10 \leqslant IC_{50} \leqslant 30$ mg/L is defined as a good 5-Lipoxygenase inhibitor (Bylac and Racine, 2003). Therefore, we can conclude that the essential oil of *C. grevei* is a good 5-Lipoxygenase inhibitor.

It has been reported that terpenes such as α-pinene and sesquiterpenes such as β-caryophyllene and α-bisabolol exhibited activity in the *in vitro* 5-Lipoxygenase assay (Bylac and Racine, 2003), but no IC_{50} reported for these three compounds. The presence of these components may contribute to the activity of this essential oil as observed in the 5-Lipoxygenase assay.

3.6. Correlation

To study the role of the various compounds of an essential oil for the biological activities obtained, we performed an assessment

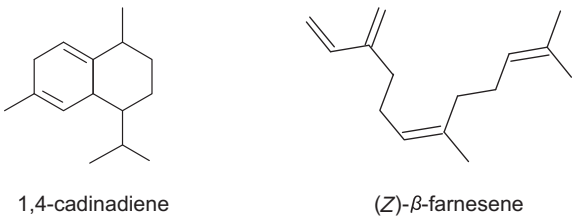

Fig. 3. Compounds with good correlations for anticancer ((*Z*)-β-farnesene) and antimalarial (1,4-cadinadiene) activities.

Table 4
Antimalarial activity (IC_{50} (mg/L)) and chemical composition of essential oils. Our work, I: *Cedrelopsis grevei*. El Babili et al. (2011), II: *Origanum camactum*. Boyom et al. (2003), III: *Xylopia phloiodora*; IV: *Pachypodanthium confine*; V: *Antidesma laciniatum*; VI: *Xylopia aethiopica*; VII: *Hexalobus crispiflorus*. Ortet et al. (2010), VIII: *Artemisia gorgonum*. Tabanca et al. (2005), IX: *Arnica longifolia*; X: *Aster hesparius*; XI: *Chrysothamnus nauseosus*. Kamatou et al., 2005, XII: *Salvia stenophylla*; XIII: *Salvia runcinata*; XIV: *Salvia repens*. Lopes et al. (1999), XV: *Virola surinamensis*. Kamatou et al. (2007), XVI: *Salvia albicaulis*; XVII: *Salvia dolomitica*. Liu et al. (2009), XVIII: *Melaleuca armillaris*. Lanfranchi et al. (2010), XIX: *Daucus crinitus* Desf. Van Zyl et al. (2006), XX: pure compounds (α-pinene, β-pinene and α-bisabolol).

Essential oil	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX		
Antimalarial activity	21.5	34	17.9	16.6	29.4	17.8	2	5.2 ± 0.7	No activity	No activity	No activity	4.38 ± 1.07	1.23 ± 0.31	1.68 ± 0.26	9[a]	6.4 ± 2.0	4.8 ± 0.7	27.0 ± 2.0	26.7	1.2 ± 0.20	294.77	307.30
Compounds																						
α-Pinene	0.14	0.62	0.58			4.05		1.6			1.1	2.7	1.8	6.6	49.7	1.9		1.95	9.9	95.48		
β-Pinene	0.20		0.68			10.07	0.12	0.7			19.8	0.7	0.8	3	1.5			0.33	0.2		96.32	
E-pinocarveol	0.11								0.4	0.3	0.5											
Isopinocampheol	0.03		1.23	0.31		5.42																
p-Cymen-8-ol	0.03	0.14	1.58							0.1		0.2										
α-Terpineol	0.08					4.99		0.2	0.2	0.3	0.1	1	0.2			2.7	6.2	0.16				
Myrtenal	0.16		0.28			2.85			0.1	0.1	0.2											
α-Cubebene	0.72			0.52		1.04	0.36				0.1								0.1			
α-Copaene	7.67	0.02	0.53	7.06	2.2	4.07	13.27				0.2		0.1		4.6				0.2			
β-Bourbonene	0.17				0.5						0.2								0.4			
β-Elemene	6.98		0.58	0.73		1.34	1.92												0.3			
Isoitalicene	0.15									0.1												
Cyperene	1.27		0.34	15.54		3.95	11.53															
α-Gurjunene	0.36	0.02		1.86		0.64								0.2								
β-Caryophyllene	0.59	0.78		0.28	5.2	1.67	1.33	0.5	0.5	0.1	0.1	7.3	11.4		1.6	2.1	0.3	0.05	5.4			
(*E*)-α-bergamotene	0.15		0.46	0.18							0.1											
(*E*)-β-farnesene	27.67							0.2					0.2			0.2			3.4			
γ-Muurolene	1.61	0.02		0.35	0.7	2.64	1.93		0.2	0.1	0.3							0.02	0.6			
ar-Curcumene	0.10							0.7		0.6	1.1											
β-Selinene	0.77			0.28				0.3										0.12				
Viridiflorene	2.80											0.3		3.5	1.4							
α-Muurolene	2.58	0.02			1.5	1.84	1.29		0.2	0.5	0.5								0.1			
γ-Cadinene	0.87	0.07	11.27	3.51	0.3		2.53		0.1	0.2	0.2							0.12				
δ-Cadinene	14.52	0.1							0.4		0.8	0.5		0.3	2.2			0.36				
1,4-Cadinadiene	0.31					0.67					0.1											
α-Calacorene	0.99	0.01	0.89			0.84	7.82		0.1	0.1	0.2											
Elemol	0.79		2.04	1.24		1.09					1.3							0.09				
Spathulenol[a]	0.25	0.03	1.02	2.16	1.4	6.33	1.97		3.3	2	0.2	0.3		1.0		2.0	1.5	0.22				
Viridiflorol	1.23										0.3					24.5						
Epicedrol	2.50		5.07	7.24	8.5	1.99	2.54	0.3	4	3.9	0.2					5.6						
β-Eudesmol	0.27					1.15	1.08	0.1	0.2	2.5	7.7											
α-Cadinol	1.62	0.01	0.5	1.16	3		1.41		0.1					0.3	0.8			0.02				
α-Bisabolol	0.05							1.1	8.2	0.8												90.32

[a] IC_{50} calculated from percentage.

Table 5
Antiinflammatory activity (IC_{50} (mg/L)) and chemical composition of essential oils. Our work, I: *Cedrelopsis grevei*. Lourens et al. (2004), II: *Helichrysum dasyanthum*; III: *Helichrysum excisum*; IV: *Helichrysum felinum*; V: *Helichrysum petiolare*. Benites et al., 2009, VI: *Lantana Camara*. Ashour et al. (2009), VII: *Bupleurum marginatum*. Albano et al. (2012), VIII: *Dittrichia viscosa*; IX: *Foeniculum vulgare*; X: *Origanum vulgare*; XI: *Salvia officinalis*; XII: *Thymbra capitata*; XIII: *Thymus camphoratus*; XIV: *Thymus carnosus*; XV: *Thymus mastichina*. Mulyaningsih et al. (2010), XVI: *Kadsura longipedunculata*. Dongmo et al. (2010), XVII: *Canarium schweinfurthii* (Lolodorf); XVIII: *Aucoumea klaineana* (Lolodorf); XIX: *Canarium schweinfurthii* (Mbouda). Hamdan et al. (2010), XX: *Citrus jambhiri*; XXI: *Citrus pyriformis*. Nyiligira et al. (2004), XXII: *Vitex pooara*; XXIII: *Vitex rehmannii*; XXIV: *Vitex obovata* ssp. *Abovata*; XXV: *Vitex obovata* ssp. *Wilmsii*; XXVI: *Vitex zeyheri*.

Essential oil	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Antiinflammatory activity	21.33 ± 0.5	31.24 ± 1.31	27.62 ± 0.43	22.89 ± 7.59	25.03 ± 0.57	81.5	63.64	291.2 ± 2 2.	67.7 ± 2.3	264.2 ± 20.7	827.9 ± 60.6	93.3 ± 1 0.5	334.3 ± 43.6
Compounds													
α-Pinene	0.14	16.6	2.8		6.8	0.1		0.2	25.8	1.1	8.4	1.6	11.9
β-Pinene	0.20	6.2	1.1		0.4			0.3	6.8		3.2	0.1	0.9
(*E*)-pinocarveol	0.11						0.17						0.8
p-Cymen-8-ol	0.03	0.1	0.2		0.3								
α-Terpineol	0.08	0.5	0.8		5.1		1.08	0.4	1.3		1.5	0.1	0.5
α-Cubebene	0.72						1.96	0.1					
α-Copaene	7.67	0.1	2.5	4	1.3		0.52	0.7			0.1		
β-Elemene	6.98					0.2	0.34						
α-Gurjunene	0.36		0.1	1	0.1								
β-Caryophyllene	0.59	13.3	5.7	27.6	14	0.1	5.53			1.7	0.9	1.1	
(*E*)-α-bergamotene	0.15		0.1	0.9						0.1			
(*E*)-β-farnesene	27.67			0.1			0.44						
γ-Muurolene	1.61							1.2					
β-Selinene	0.77												
Viridiflorene	2.80									0.1			
α-Muurolene	2.58				2.2		0.18						
γ-Cadinene	0.87		0.1	2			1.69	3.7					0.7
δ-Cadinene	14.52							5.7		0.2	0.1		
1,4-Cadinadiene	0.31			0.1									
α-Calacorene	0.99						1.33	0.4					
Elemol	0.79												
Spathulenol[a]	0.25			0.6	0.2		4.4						
Viridiflorol	1.23		18.2	0.7	0.9		0.78						0.5
α-Cadinol	1.62												
α-Bisabolol	0.05											0.1	

	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	XXIV	XXV	XXVI
	544.3 ± 64.5	1084.5 ± 146.1	38.58 ± 3.8	62.6 ± 4.2	No activity	No activity	40 ± 1.36	38 ± 0.82	25	40.5	42	48	64
α-Pinene	4.9	7	1.55	1.7	29.3	2.6	0.01	0.26	1.69	0.02	2.22	0.02	2.53
β-Pinene	2.8	5.3	0.81	0.4	0.8	1.2	0.44	0.92	0.16		1.57	3.01	0.23
(*E*)-pinocarveol	0.2	0.1											
p-Cymen-8-ol													
α-Terpineol	0.8	3.4	0.21	34.4	3.1	18	0.15	0.51					3.43
α-Cubebene			1.2								0.06		
α-Copaene			2.83								7.39	11.79	0.3
β-Elemene			1.5						0.28				
α-Gurjunene			1.16										
β-Caryophyllene	0.4	0.5	0.19							3.82		3.64	
(*E*)-α-bergamotene							0.32	0.28					
(*E*)-β-farnesene							0.01						
γ-Muurolene			1.41					0.02	0.42	3.19	4.9	5.08	
β-Selinene			1.74				0.04	0.88	14.41				1.47
Viridiflorene			0.48										
α-Muurolene										2.55	1.36	1.69	
γ-Cadinene									0.03	1.58	1.71	2.28	
δ-Cadinene										0.67		0.43	0.25
1,4-Cadinadiene			0.94										
α-Calacorene			1.37							0.11		1.07	
Elemol	1.1	0.5											

(*continued on next page*)

Table 5 (*continued*)

Essential oil	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Antiinflammatory activity	21.33 ± 0.5	31.24 ± 1.31	27.62 ± 0.43	22.89 ± 7.59	25.03 ± 0.57	81.5	63.64	291.2 ± 2 2.	67.7 ± 2.3	264.2 ± 20.7	827.9 ± 60.6	93.3 ± 1 0.5	334.3 ± 43.6
Spathulenol[a]		0.1	3.07										
Viridiflorol	0.2		0.75						0.34	1.58	0.38		1.52
α-Cadinol										2.85	3.27	3.57	
α-Bisabolol			0.3										

[a] IC_{50} calculated from percentage.

of the literature of all essential oils from various plants tested for biological activities (anticancer, antimalarial and antiinflammatory). Based on these results, we established correlations between biological activities and concentrations of compounds. The aim of the study was to find correlation(s) between compound(s) quantity and biological activity.

3.6.1. Correlation for cytotoxic activity

Data in Table 3 showed the cytotoxic activity against the *MCF-7* cells (IC_{50} mg/L) of all essential oils in literature and their compounds. Essential oils were extracted from *Cedrelopsis grevei*, *Origanum campactum*, *Lavandula dentata* (flower and leaf), *Schefflera heptaphylla*, *Laurus nobilis*, *Origanum syriacum*, *Origanum vulgare*, *Salvia triloba*, *Schinus molle*, *Schinus terebinthifolius*, *Heteropyxis dehniae*, *Salvia officinalis*, *Rosmarinus officinalis*, *Melaleuca alternifolia*, *Citrus sinensis*, *Citrus medica*, *Citrus limon*, *Talouma gloriensis*, *Mentha arvensis* (Summer and Winter), *Mentha piperita* (Summer and Winter), *Mentha longifolia* (Summer and Winter), *Mentha spicata* (Summer and Winter), *Melaleuca armillaris*, *Satureia thymbra*, *Sideritis perfoliata*, *Laurus nobilis* (leaves and fruits), *Pistacia palestina*, *Salvia officinalis*, *Artemsia scoparia*, *Dracocephalum surmandinum* and pure compounds (α-pinene, α-copaene and β-caryophyllene).

We established correlations between compound contents and anticancer activity against the *MCF-7* cells. (*Z*)-β-farnesene showed good correlation, $R^2 = 0.73$. To our knowledge, no literature cited this compound ((*Z*)-β-farnesene) for any anticancer activity (Fig. 3).

3.6.2. Correlations for antimalarial activity

Data in Table 4 summarize chemical composition and the antimalarial activity (IC_{50} (mg/L)) of all essential oils cited in literature. These essential oils have been obtained from *Cedrelopsis grevei*, *Origanum camactum*, *Xylopia phloiodora*, *Pachypodanthium confine*, *Antidesma laciniatum*, *Xylopia aethiopica*, *Hexalobus crispiflorus*, *Artemisia gorgonum*, *Arnica longifolia*, *Aster hesparius*, *Chrysothamnus nauseosus*, *Salvia stenophylla*, *Salvia runcinata*, *Salvia repens*, *Virola surinamensis*, *Salvia albicaulis*, *Salvia dolomitica*, *Melaleuca armillaris*, *Daucus crinitus* and pure compounds (α-pinene, β-pinene and α-bisabolol).

We established correlations between compounds contents and IC_{50} of antimalarial activity. 1,4-cadinadiene showed good correlation ($R^2 = 0.61$). To our knowledge, no literature cited this compound (1,4-cadinadiene) for any antimalarial activity.

3.6.3. Correlations for antiinflammatory activity

Data in Table 5 showed the antiinflammatory activity (IC_{50} (mg/L)) of all essential oils in literature and their chemical composition. Essential oils were extracted from *Cedrelopsis grevei*, *Helichrysum dasyanthum*, *Helichrysum excisum*, *Helichrysum felinum*, *Helichrysum petiolare*, *Lantana Camara*, *Bupleurum marginatum*, *Dittrichia viscose*, *Foeniculum vulgare*, *Origanum vulgare*, *Salvia officinalis*, *Thymbra capitata*, *Thymus camphorates*, *Thymus carnosus*, *Thymus mastichina*, *Kadsura longipedunculata*, *Canarium schweinfurthii* (Lolodorf and Mbouda), *Aucoumea klaineana* (Lolodorf), *Citrus jambhiri*, *Citrus pyriformis*, *Vitex pooara*, *Vitex rehmannii*, *Vitex obovata* ssp. *Abovata*, *Vitex obovata* ssp. *Wilmsii* and *Vitex zeyheri*.

We established correlations between compounds contents and IC_{50} of antiinflammatory activity. This study showed that there is no correlation between constituents and the IC_{50} of the antiinflammatory activity.

4. Conclusion

In conclusion, we have identified all volatile constituents of *C. grevei* leaves essential oil and evaluated the antioxidant, anticancer, antimalarial and antiinflammatory activities. Our results clearly show that this essential oil is active against cells line tested (tumor *MCF-7*), parasite (*FCR-3*), and inhibitory 5-Lipoxugenase. This anticancer activity against *MCF-7* could be explained, in part, by the presence of β-elemene. However other compounds may have anticancer and antimalarial activity like the majority compounds which are never evaluated. Based on established correlations for antimalarial and anticancer activity, compounds such as 1,4-cadinadiene and (*Z*)-β-farnesene, could be the best candidates for further analysis.

The results presented here can be considered as the first information on the anticancer, antimalarial and antiinflammatory properties of *C. grevei*. In a future study, we will identify and investigate the components responsible for the anticancer, antimalarial and antiinflammatory activities of *C. grevei*. Consequently, those results can be considered as a preliminary study in order to show the importance and the originality of this endemic species.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

Albano, S.M., Lima, A.S., Miguel, M.G., Pedro, L.G., Barroso, J.G., Figueiredo, A.C., 2012. Antioxidant, anti-5-lipoxygenase and antiacetylcholinesterase activities of essential oils and decoction waters of some aromatic plants. Rec. Nat. Prod. 6, 35–48.

Al-Kalaldeh, J.Z., Abu-Dahab, R., Afifi, F.U., 2010. Volatile oil composition and antiproliferative activity of *Laurus nobilis*, *Origanum syriacum*, *Origanum vulgare*, and *Salvia triloba* against human breast adenocarcinoma cells. Nutr. Res. 30, 271–278.

Ashour, M.L., El-Readi, M., Youns, M., Mulyaningsih, S., Sporer, F., Efferth, T., Wink, M., 2009. Chemical composition and biological activity of the essential oil obtained from *Bupleurum marginatum* (Apiaceae). J. Pharm. Pharmacol. 61, 1079–1087.

Bendaoud, H., Romdhane, M., Souchard, J.P., Cazaux, S., Bouajila, J., 2010. Chemical composition and anticancer and antioxidant activities of *Schinus molle* L. and *Schinus terebinthifolius* Raddi Berries Essential Oils. J. Food Sci. 75, 466–472.

Benoit-Vical, F., Valentin, A., Mallié, M., Bastide, J.M., Bessière, J.M., 1999. *In vitro* antimalarial activity and cytotoxicity of *Cochlospermum tinctorium* and *C. planchonii* leaf extracts and essential oils. Planta Med. 65, 378–381.

Benites, J., Moiteiro, C., Miguel, G., Rojo, L., Lopez, J., Venância, F., Ramalho, L., Feio, S., Dandlen, S., Casanova, H., Torres, I., 2009. Composition and biological activity of the essential oil of peruvian *Lantana camara*. J. Chil. Chem. Soc. 54, 379–384.

Blois, M.S., 1958. Antioxidant determination by use of free radical stable. Nature 181, 1199–1200.

Boyom, F.F., Ngouana, V., Amvam Zollo, P.H., Menut, C., Bessiere, J.M., Gut, J., Rosenthal, P.J., 2003. Composition and anti-plasmodial activities of essential oils from some Cameroonian medicinal plants. Phytochemistry 64, 1269–1275.

Bylac, S., Racine, P., 2003. Inhibition of 5-lipoxygenase by essential oils and other natural fragrant extracts. Int. J. Aromather. 13, 138–142.

Cabanis, Y., Chabouis, L., Chabouis, F., 1969. Végétaux et groupements végétaux de Madagascar et des Mascareignes. BDPA 70, Tananarive.

Cachet, N., Hoakwie, F., Bertani, S., Bourdy, G., Deharo, E., Stien, D., Houel, E., Gornitzka, H., Fillaux, J., Chevalley, S., Valentin, A., Jullian, V., 2009. Antimalarial activity of simalikalactone E, a new quassinoid from *Quassia amara* L. (Simaroubaceae). Antimicrob. Agents Chemother. 53, 4393–4398.

Cavalli, J.F., Tomi, F., Bernardini, A.F., Casanova, J., 2003. Composition and chemical variability of the bark oil of *Cedrelopsis grevei* H. Baillon from Madagascar. Flav. Fragr. J. 18 (6), 532–538.

Chabir, N., Romdhane, M., Valentin, A., Moukarzel, B., Ben Marzoug, H.N., Ben Brahim, N., Mars, M., Bouajila, J., 2011. Chemical study and antimalarial, antioxidant, and anticancer activities of *Melaleuca armillaris* (Sol Ex Gateau) Sm essential oil. J. Med. Food 14, 1383–1388.

Cole, R.A., Bansal, A., Moriarity, D.M., Haber, W.A., Setzer, W.N., 2007. Chemical composition and cytotoxic activity of the leaf essential oil of *Eugenia zuchowskiae* from Monteverde, Cota Rica. J. Nat. Med. 61, 414–417.

Courchet, L., 1906. Recherches morphologiques et anatomiques sur le Katafa ou Katrafay de Madagascar (*Cedrelopsis grevei* H. Baillon). Ann. Inst. Colon. Marseille 4, 29–118.

Desoubzdanne, D., Marcourt, L., Raux, R., Chevalley, S., Dorin, D., Doerig, C., Valentin, A., Ausseil, F., Debitus, C., 2008. Alisiaquinones and alisiaquinol, dual inhibitors of *Plasmodium falciparum* enzyme targets from a New Caledonian deep water sponge. J. Nat. Prod. 71, 1189–1192.

Dongmo, P.M.J., Tchoumbougnang, F., Ndongson, B., Agwanande, W., Sandjon, B., Zollo, P.H.A., Menut, C., 2010. Chemical characterization, antiradical, antioxidant and antiinflammatory potential of the essential oils of *Canarium schweinfurthii* and *Aucoumea klaineana* (Burseraceae) growing in Cameroon. Agric. Biol. J. North Am. 1, 606–611.

El Babili, F., Bouajila, J., Souchard, J.P., Bertrand, C., Bellvert, F., Fouraste, I., Moulis, C., Valentin, A., 2011. Oregano: chemical analysis and evaluation of its antimalarial, antioxidant, and cytotoxic activities. J. Food Sci. 76, 512–518.

El Hadri, A., Gómez del Río, M.A., Sanz, J., González Coloma, A., Idaomar, M., Ozonas, B.R., González, J.B., Sánchez Reus, M.I., 2010. Cytotoxic activity of α-humulene and *trans*-caryophyllene from *Salvia officinalis* in animal and human tumor cells. An. Real Acad. Farmacia. 76, 343–356.

Ennajar, M., Bouajila, J., Lebrihi, A., Mathieu, F., Abderraba, M., Raies, A., Romdhane, M., 2009. Chemical composition, antimicrobial and antioxidant activities of essential oils and various extracts of *Juniperus phoenicea* L. (cupressacees). J. Food Sci. 74 (7), M364–371.

European Pharmacopoeia, 1983. Maissoneuve SA, Sainte Ruffine.

Gauvin, A., Ravaomanarivo, H., Smadja, J., 2004. Comparative analysis by gas chromatography–mass spectrometry of the essential oils from bark and leaves of *Cedrelopsis grevei* Baill, an aromatic and medicinal plant from Madagascar. J. Chromatogr. A 1029, 279–282.

Haber, W.A., Agius, B.R., Stokes, S.L., Setzer, W.N., 2008. Bioactivity and chemical composition of the leaf essential oil of *Talauma gloriensis* Pittier (Magnoliaceae) from Monteverde, Costa Rica Rec. Nat. Prod. 2, 1–5.

Hamdan, D., El-Readi, M.Z., Nibret, E., Sporer, F., Farrag, N., El-Shazyl, A., Wink, M., 2010. Chemical composition of the essential oils of two *Citrus* species and their biological activities. Pharmazie 65, 141–147.

Hussain, A.I., Anwar, F., Chatha, S.A.S., Jabbar, A., Mahboob, S., Nigam, P.S., 2010a. *Rosmarinus officinalis* essential oil: antiproliferative, antioxidant and antibacterial activities. Braz. J. Microbiol. 41, 1070–1078.

Hussain, A.I., Anwar, F., Nigam, P.S., Ashraf, M., Gilani, A.H., 2010b. Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four *Mentha* species. J. Sci. Food Agric. 90, 36–1827.

Imelouane, B., Tahr, I.M., Ankit, M., Khdid, K., Amhamdi, H., Dubois, J., Elbachiri, A., 2010. The essential oil of eastern moroccan *Rosmarinus officinalis*: chemical composition, *in-vitro* antimicrobial and antioxidant activities. Rev. Microbiol. Indig. Sant. Environ. 4, 120–141.

Kamatou, G.P.P., Viljoen, A.M., Figueiredo, A.C., Tilney, P.M., Van Zyl, R.L., Barroso, J.G., Pedro, L.G., Van Vuuren, S.F., 2007. Trichomes, essential oil composition and biological activities of *Salvia albicaulis* Benth and *S. dolomitica* Codd, two species from the Cape region of South Africa. S. Afr. J. Bot. 73, 102–108.

Kamatou, G.P.P., Viljoen, A.M., Gono-Bwalya, A.B., Van Zyl, R.L., Van Vuuren, S.F., Lourens, A.C.U., Baser, K.H.C., Demirci, B., Lindsey, K.L., Van Staden, J., Steenkamp, P., 2005. The *in vitro* pharmacological activities and a chemical investigation of three South African *Salvia* species. J. Ethnopharmacol. 102, 382–390.

Lambros, C., Vanderberg, J.P., 1979. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. J. Parasitol. 65, 418–420.

Lanfranchi, D.A., Laouer, H., El Kolli, M., Prado, S., Maulay-Bailly, C., Baldovini, N., 2010. Bioactive phenylpropanoids from *Daucus crinitus* Desf. from Algeria. J. Agric. Food Chem. 58, 2174–2179.

Li, Q.Q., Wang, G., Huang, F., Banda, M., Reed, E., 2010. Antineoplastic effect of beta-elemene on prostate cancer cells and other types of solid tumour cells. J. Pharm. Pharmacol. 62, 1018–1027.

Li, Y.L., Yeung, C.M., Chiu, L.C.M., Cen, Y.Z., Ooi, V.E.C., 2009. Chemical composition and antiproliferative activity of essential oil from the leaves of a medicinal herb, *Schefflera heptaphylla*. Phytother. Res. 23, 140–142.

Liu, X., Zu, Y., Fu, Y., Yao, L., Gu, C., Wang, W., Efferth, T., 2009. Antimicrobial activity and cytotoxicity towards cancer cells of *Melaleuca alternifolia* (tea tree) oil. Eur. Food Res. Technol. 229, 247–253.

Loizzo, M.R., Tundis, R., Menichini, F., Saab, A.M., Statti, G.A., Menichini, F., 2007. Cytotoxic activity of essential oils from labiatae and lauraceae families against *in vitro* human tumor models. Anticancer Res. 27, 3293.

Lopes, N.P., Kato, M.J., Andrade, E.H.de A., Maiaa, J.G.S., Yoshida, M., Planchart, A.R., Katzin, A.M., 1999. Antimalarial use of volatile oil from leaves of *Virola surinamensis* (Rol.) Warb. by Waiãpi Amazon Indians. J. Ethnopharmacol. 67, 313–319.

Lourens, A.C.U., Reddy, D., Başer, K.H., Viljoen, A.M., Van Vuuren, S.F., 2004. *In vitro* biological activity and essential oil composition of four indigenous South African *Helichrysum* species. J. Ethnopharmacol. 95, 253–258.

Monajemi, R., Oryan, S., Haeri-Roohani, A., Ghannadi, A., Jafarian, A., 2005. Cytotoxic effects of essential oils of some Iranian *Citrus* peels. Iran. J. Pharm. Res. 3, 183–187.

Mulholland, D.A., Kotsos, M., Mahomed, H.A., Randrianarivelojosia, M., 1999. The chemistry of the Ptaeroxylaceae. Nig. J. Nat. Prod. Med. 3, 13–16.

Mulyaningsih, S., Youns, M., El-Readi, M.Z., Ashour, M.L., Nibret, E., Sporer, F., Herrmann, F., Reichling, J., Wink, M., 2010. Biological activity of the essential oil of *Kadsura longipedunculata* (Schisandraceae) and its major components. J. Pharm. Pharmacol. 62, 1037–1044.

Muñoz, V., Sauvain, M., Mollinedo, P., Callapa, J., Rojas, I., Gimenez, A., Valentin, A., Mallié, M., 1999. Antimalarial activity and cytotoxicity of (−) roemrefidine isolated from the stem bark of *Sparattanthelium amazonum*. Planta Med. 65, 448–449.

Nyiligira, E., Viljoen, A.M., Ozek, T., Baser, K.H.G., Vuuren, V.S., 2004. Essential oil composition and *in vitro* antimicrobial and antiinflammatory activity of South African *Vitex* species. S. Afr. J. Bot. 70, 611–617.

Ortet, R., Thomas, O.P., Regalado, E.L., Pino, J.A., Filippi, J.J., Fernandez, M.D., 2010. Composition and biological properties of the volatile oil of *Artemisia gorgonum* Webb. Chem. Biodivers. 7, 1325–1333.

Ouattara, Y., Sanon, S., Traoré, Y., Mahiou, V., Azas, N., Sawadogo, L., 2006. Antimalarial activity of *Swartzia madagascarensis* desv. (leguminosae), *Combretum glutinosum* Guill & Perr. (combretaceae) and *Tinospora bakis* miers (menispermaceae) Burkina fasso medicinal plants. Afr. J. Trad. Compl. Alter. Med. 3, 75–81.

Rakotobe, M., Menut, C., Andrianoelisoa, H.S., Rahajanirina, V., Collas de Chatelperron, P., Roger, E., Danthu, P., 2008. The bark essential oil composition and chemotaxonomical appraisal of *Cedrelopsis grevei* H. *Baillon* from Madagascar. Nat. Prod. Commun. 3 (7), 1145–1150.

Rasoanaivo, P., De La Gorce, P., 1998. Essential oils of economic value in Madagascar: present state of knowledge. HerbalGram 43, 31–59.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Rad. Biol. Med. 26, 1231–1237.

Sharopov, S.F., Setzer, W.N., 2012. The essential oil of *Salvia sclarea* L. from Tajikistan. Nat. Prod. Commun. 6, 75–79.

Sibanda, S., Chigwada, G., Poole, M., Gwebu, E.T., Noletto, J.A., Schmidt, J.M., Rea, A.I., Setzer, W.N., 2004. Composition and bioactivity of the leaf essential oil of *Heteropyxis dehniae* from Zimbabwe. J. Ethnopharmacol. 92, 107–111.

Sonboli, A., Esmaeili, M.A., Gholipour, A., Kanani, M.R., 2010. Composition, cytotoxicity and antioxidant activity of the essential oil of *Dracocephalum surmandinum* from Iran. Nat. Prod. Commun. 5, 341–344.

Tabanca, N., Demirci, B., Crockett, S.L., Baser, K.H.C., Wedge, D.E., 2005. Chemical composition and antifungal activity of *Arnica longifolia*, *Aster hesperius*, and *Chrysothamnus nauseosus* essential oils. J. Agric. Food Chem. 55, 8430–8435.

Trager, W., Jensen, J.B., 1976. Human malarial parasites in continuous culture. Science 193, 673–675.

Um, B.H., Lobstein, A., Weniger, B., Spiegel, C., Yice, F., Rakotoarison, O., Andriantsitohaina, R., Anton, R., 2003. New coumarins from *Cedrelopsis grevei*. Fitoterapia 74, 638–642.

Van Zyl, R.L., Seatlholo, S.T., Van Vuuren, S.F., Viljoen, A.M., 2006. The biological activity of 20 nature identical essential oil constituents. J. Essent. Oil Res. 18, 129–133.

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