

Comparison of Ultrasound-assisted Extraction and Dynamic Maceration Over Content of Tagitinin C obtained from *Tithonia diversifolia* (Hemsl.) A. Gray Leaves Using Factorial Design

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ABSTRACT

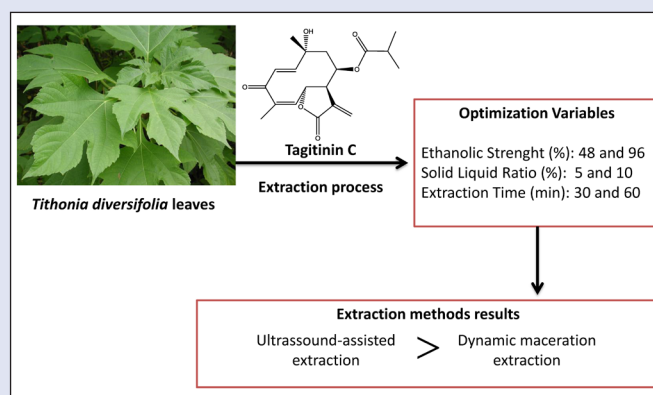
Background: *Tithonia diversifolia* belongs to the Asteraceae family. The leaves of *T. diversifolia* have been studied lately because of the presence of tagitinin C. **Objective:** Looking for an easy and inexpensive method to extract tagitinin C from *T. diversifolia* leaves, this work aims to conduct a screening to evaluate the influence of different experimental factors using the dynamic maceration and ultrasound-assisted extraction methods with 2³ factorial design based on response surface methodology in enhancing this chemical marker extraction. **Materials and Methods:** The experimental factors were: extraction time (ET) of 30 and 60 minutes, solid: liquid ratio (SLR) of 5 and 10 grams/grams and ethanolic strength (ES) 48 and 96% (w/w). The experiments were done tripled. The content of tagitinin C in each produced extract was quantified by HPLC method. **Results:** The highest concentrations of tagitinin C obtained under the experimental design were 0.53 mg/mL and 0.71 mg/mL, respectively for dynamic maceration (DM) and ultrasound-assisted extraction (UAE) from *Tithonia diversifolia* powdered leaves. For the UAE method, the main parameter for higher contents of tagitinin C was the solid: liquid ratio, followed by the ethanolic strength, and the extraction time was not significant for this method. As for the DM method, all the parameters (SLR, ES, and ET) were significant for a higher content of tagitinin C. **Conclusion:** Based on the obtained results, it was revealed that the ultrasound-assisted extraction was more effective than dynamic maceration for tagitinin C extraction from *T. diversifolia* powdered leaves.

Key words: Dynamic maceration, factorial design, response surface methodology, tagitinin C, ultrasound-assisted extraction

SUMMARY

- Tithonia diversifolia* leaves possess tagitinin C, a sesquiterpene lactone, as an important secondary metabolite with several biological activities, such as antimarial, gastroprotective, chemotherapeutic adjuvants, and toxic activities.

- Ultrasound-assisted extraction was more effective to obtain higher levels of tagitinin C when compared with dynamic maceration extraction.
- Factorial design can be employed as a screening tool to find the effects of factors investigated in the extraction processes.



Abbreviation used: DME: dynamic maceration extraction, UAE: ultrasound-assisted extraction, ET: extraction time, ES: ethanolic strength, SLR: solid:liquid ratio, Tag C: tagitinin C, HPLC: high-performance liquid chromatography.

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INTRODUCTION

The genus *Tithonia* belongs to Asteraceae family and is distributed in 11 species.^[1] Inside that, *Tithonia diversifolia* is the species most widely studied. Also known as "Mexican Sunflower," this species is a shrubby herb, abundant in tropical and subtropical regions.^[2,3] Its stem and leaves have been commonly used by traditional medicine in topical use for snake bites and abscesses and in oral form against malaria and as a snakebite antidote.^[4-6]

This plant has as secondary metabolites classes of sesquiterpenoids, diterpenoids, as well as flavonoids.^[7] Thereby, many classes of secondary metabolites have been isolated and studied to verify the biological applicability and the chemotaxonomic applications.^[8]

Among these metabolites, there are the sesquiterpene lactones as prominent group. These are located inside the glandular trichomes of

leaves and have as major substance the tagitinin C [Figure 1], which is a sesquiterpene lactone of heliangolido's type, responsible for many works looking for verification of pharmacologic activities.^[9]

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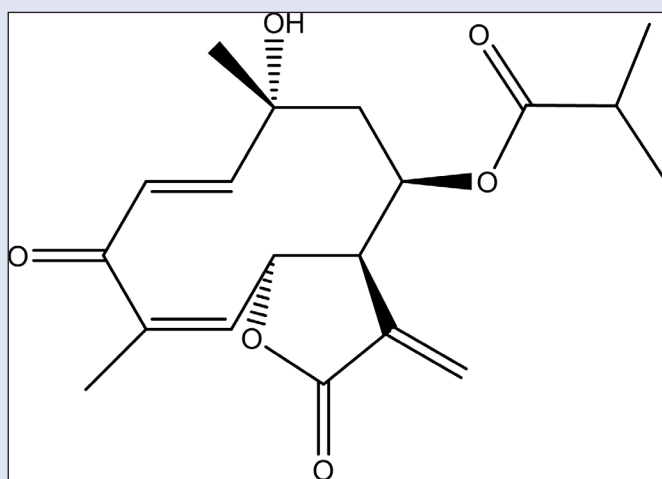


Figure 1: Chemical structure of tagitinin C

Lee *et al.*^[10] and Liao *et al.*^[11] indicated that *T. diversifolia* extract and the isolated tagitinin C act as chemotherapeutic adjuvants on the glioblastoma cells. The activity against *Plasmodium falciparum*^[12] was also reported by the presence of tagitinin C as well as the gastroprotective activity.^[13] In addition to these performances, the toxic activities are also reported. Elufioye *et al.*^[14] demonstrated the reduction of the parasitemia in mice with consequent renal and hepatic toxicity. Passoni *et al.*^[15] also demonstrated that the ingestion of extracts rich in STLs can cause kidney damage as shown by Fankule and Abatan.^[16] The tagitinin C between the others sesquiterpene lactones has also been reported in studies for agricultural benefits for control to cowpea seed bruchid, *Callosobruchus maculatus*;^[17] ants, *Atta cephalotes*;^[18] as well as caterpillar *Philosamia ricini*.^[19]

The tagitinin C has solubility in apolar solvents such as acetone,^[8] ether,^[20] and dichloromethane.^[9] However, as related before, the use of these chemicals is hampered by many attendant problems such as toxic residues in foods and humans, workers, safety and high cost of procurement.^[17,21] Besides, the hydroethanolic solutions are commonly used in extractive processes precisely because of their extraction efficiency and low toxicity compared with other organics solvents.^[22]

This chemical marker has been extracted from *T. diversifolia* leaves by different extraction methods using a range of solvents such as infusion method using water as a solvent,^[15] supercritical fluid extraction using methanol and CO₂ as a solvent,^[23] and also Soxhlet extraction using dichloromethane as a solvent.^[24]

The ultrasound-assisted extraction (UAE) is a simple, efficient, and inexpensive process that utilizes the energy of the sound waves to transmit frequencies higher than the human hearing capacity, causing a pressure variation in the liquid extractor, generating cavitation, which creates shear forces that break cell walls and facilitates metabolites transfer to solvent.^[25,26] This effect works in extraction process by increasing the mass transfer, breaking plant cells, increasing penetration of the solvent, and reducing capillaries effects.^[27] The method can be used as an alternative extraction technique and has been applied to the extraction effectively for various organic compounds derived from vegetable drugs.^[28,29] This technique has benefits such as high reproducibility, the possibility of using large sample size range, the fast processing, and low economic cost.^[30]

On the other hand, the dynamic maceration is a technique in which the extraction occurs by diffusion, where the organic solvents are used according to their polarity to extract the interest compounds. In general, there is the saturation of the extraction solvent or a diffusional

equilibrium between the solvent and the vegetable cell.^[31] This technique is one of the most used extraction method because of its suitability to different scales, simplicity, and inexpensive compared with other methods such as UAE.^[32-34]

The factorial design based on response surface methodology is a functional method to determine the effects of multiple factors and their interactions with the different variables.^[35] This method has been used especially in plant extraction experiments to find the optimum response between the different factor levels.^[36,37]

Given the various applications of the sesquiterpene lactones and its different methods of extraction, this paper aims to conduct a screening to evaluate the influence of different experimental factors for tagitinin C extraction from *T. diversifolia* leaves in dynamic maceration and UAE methods with a 2³ factorial design based on response surface methodology.

MATERIAL AND METHODS

Plant material

Leaves of native specimens of *T. diversifolia* were collected in Goiânia, Goiás, Brazil (16°39'08.78"S 49°15'47.66" W 710 m). Voucher specimens were deposited in the herbarium of Federal University of Goiás (record number UFG-48591). The leaves were dried at 40°C, grinded by the comminution method, and stored sheltered from light and moisture for subsequent use in the extractions.

Apparatus

The UAEs were performed with an ultrasonic device (USC 2800A, 40 kHz, Uniques) equipped with a digital timer and temperature controller. The dynamic maceration were performed with a shaker table orbital (Model NT155, 60 Hz, Novatecnica) with digital timer and speed shaker controlled at 120 rpm.

Chemicals

Samples of tagitinin C used for the quantification study were isolated according to the methodology used by Abe *et al.*^[38] The structural elucidation of this compound was carried out by ¹H and ¹³C NMR, and the spectral data were compared with those from authentic material and data from the literature.^[39,40]

Acetonitrile was analytical grade (Merck KGaA, Darmstadt, Germany). Additionally, acetic acid (Scharlau) and ultrapure water from a Milli-Q system (Millipore®, Bedford, MA, USA) were used.

Chromatographic conditions

In the HPLC method, tagitinin C was identified and quantified using a HPLC Waters® e2695 (Milford, MA, USA), comprising a quaternary pump, an online degasser, an autosampler, and a photodiode array detector model 2998. The treatment of data and control of HPLC equipment were performed in Empower® 2.0 software. An isocratic elution was performed column Zorbax Eclipse XDB-Agilent®, C18, 250 mm × 4.6 mm × 5 µm. The presence of tagitinin C was confirmed by comparison of their retention time, spiking the extract with standard and overlaying UV spectra with standard compound at a 254 nm.

The mobile phase was composed by acetonitrile and 1% (v/v) acetic acid in water (70:30) at a flow rate of 1 mL/min at 30°C. All solutions were degassed and filtered through a 0.45 µm pore size filter (Millipore, USA).

Experimental design and statistical analysis

To evaluate the influence of factors, main effects and interactions effects on the extractive process of tagitinin C by dynamic maceration (DME) and ultrasound (UAE) were used an experimental design 2³. The factors studied were extraction time (ET) of 30 and 60 min, solid:liquid ratio

(SLR) of 5 and 10 g/g, and ethanolic strength (ES) 48 and 96% (w/w). The experiments were done tripled. All the 24 trials were randomized to satisfy the statistical requirement of independence of observations [Table 1].

RESULTS AND DISCUSSION

The extraction results values are summarized in [Table 1]. The highest concentrations obtained under the experimental design were 0.53 and 0.71 mg/mL, respectively, for dynamic maceration (DME) and UAE from *T. diversifolia* leaves powder. In the most part of experiments, it can be seen that UAE values were higher than DME, which showed the positive effect of the ultrasound on the contents of tagitinin C and their kinetics extraction. These findings were also observed in the extraction of rutin from *Calendula officinalis* L. (Asteraceae).^[41]

Ultrasound-assisted extraction

The results showed that tagitinin C is affected most significantly by SLR, followed by ES in UAE, as can be seen in Figure 2a. The positive effect of SLR suggests that the increasing amount of powdered leaves could provide the highest levels of this chemical marker. Besides, in the UAE, the ethanol strength exerted positive effect over tagitinin C content. Also, in Figure 2a, it can be observed that there are no interactions between the investigated factors at a significance level of 5%.

In this study, the extraction time was not significant at a level of 5%, probably because the fast extraction of tagitinin C by the solvent. This feature could be useful in further applications of this species, resulting in

savings of time and energy.

Figure 2b shows the surface response plot for tagitinin C (mg/mL) as a function of ES and SLR. The plot clearly shows the increasing tendency of tagitinin C values with the increasing of ES and SLR. This can be attributed to the nonpolar feature of the chemical marker. The adjusted equation obtained for the tagitinin C response is shown in equation 1:

$$\text{Tagitinin C (mg/mL)} = 0.41 + 0.20\text{ES} + 0.28\text{SLR} \quad (1)$$

$$R_{\text{adj}}^2 = 0.8409$$

The analysis of variance (ANOVA) [Table 2] results in a good model performance with the determination coefficient adjusted value of 0.8409. The calculated model was able to explain 84.09% of the result in the tagitinin C extraction. So, the results indicated that the model could work well, providing information for further experimental designs with quadratic models, with the aim of finding the optimum conditions to extract tagitinin C using UAE. It can also be observed in Table 2 that variables ES and SLR showed significant difference individually, with *P* less than 0.05.

From the data obtained, it can be interpreted that, for tagitinin C extraction by UAE, the solvent was not saturated by the SLR, concluding that more powdered leaves could be used in higher ratios, and also that the use of ethanol with higher alcoholic graduation (96%) was the best condition. The extraction time in the conditions described did not interfere with the results and the factors act independently.

Table 1: Experiment design and observed responses of dynamic maceration and ultrasound extractions of *T. diversifolia* leaves powder

Runs	ES	SLR	ET	Tag C (mg/mL) by MDE	Tag C (mg/mL) by UAE
1	96	5	30	0.25 ± 0.02	0.35 ± 0.01
2	96	10	30	0.47 ± 0.06	0.65 ± 0.15
3	96	5	60	0.28 ± 0.03	0.35 ± 0.01
4	96	10	60	0.53 ± 0.05	0.71 ± 0.01
5	48	5	30	0.18 ± 0.01	0.17 ± 0.05
6	48	10	30	0.35 ± 0.01	0.42 ± 0.01
7	48	5	60	0.21 ± 0.02	0.22 ± 0.01
8	48	10	60	0.43 ± 0.03	0.44 ± 0.05

ES: Ethanolic strength; SLR: Solid-to-liquid ratio; ET: Extraction time

Table 2: Variance analysis (ANOVA) for UAE method

Factor	Sum of squares	df	Mean squares	F value	P
(1) ES	0.250167	1	0.250167	42.86412	0.000005
(2) SLR	0.476147	1	0.476147	81.58412	0.000000
(3) ET	0.005277	1	0.005277	0.90421	0.354981
(1) by (2)	0.012458	1	0.012458	2.13461	0.162245
(1) by (3)	0.000006	1	0.000006	0.00109	0.974017
(2) by (3)	0.000464	1	0.000464	0.005836	
Error	0.099217	17	0.005836		
Total SS	0.843736	23			

ES: Ethanolic strength; SLR: Solid-to-liquid ratio; ET: Extraction time; SS: Sum of squares

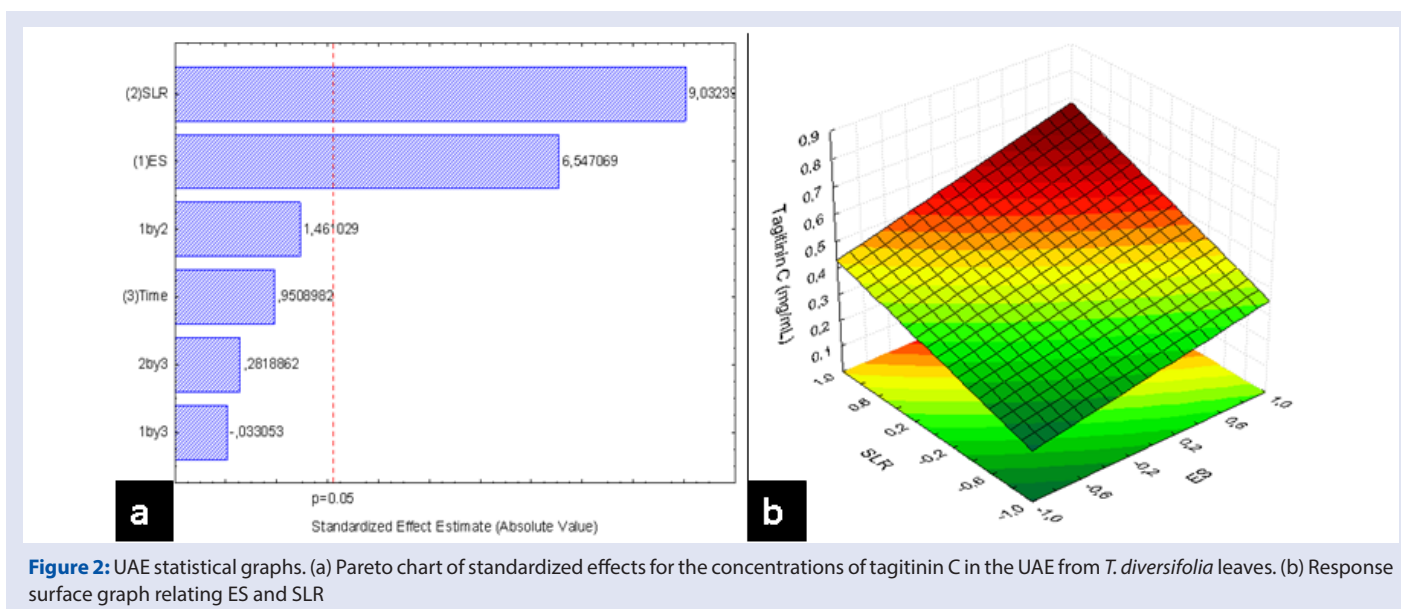


Figure 2: UAE statistical graphs. (a) Pareto chart of standardized effects for the concentrations of tagitinin C in the UAE from *T. diversifolia* leaves. (b) Response surface graph relating ES and SLR

Dynamic maceration

As shown in the Pareto chart [Figure 3a] for the dynamic maceration method, the factors ES, SLR, and ET exerted important influence for the concentration of tagitinin C in the extract obtained with estimated effects of approximately 11, 4.9, and 2.7, respectively. The response surface graphs demonstrate the interaction between the significant variables. Figure 3b show the interaction between SLR and ES. Higher values of SLR and ES imply in higher levels of tagitinin C. This relation can also

Table 3: Variance analysis (ANOVA) for dynamic maceration extraction

Factor	Sum of squares	df	Mean squares	F value	P
(1) ES	0.047945	1	0.04795	24.2670	0.000128
(2) SLR	0.278698	1	0.278698	141.0449	0.000000
(3) ET	0.014720	1	0.014720	7.4497	0.014270
(1) by (2)	0.002087	1	0.002087	1.0564	0.318449
(1) by (3)	0.000180	1	0.000180	0.0910	0.766554
(2) by (3)	0.003078	1	0.003078	1.5576	0.228933
Error	0.033591	17	0.001976		
Total SS	0.380300	23			

ES: Ethanollic strength; SLR: Solid-to-liquid ratio; ET: Extraction time; SS: Sum of squares

be seen in Figure 3c, which shows the positive effect between ES and ET, and in Figure 3d, which shows the positive effect between SLR and ET.

[Table 3] shows the variance analysis (ANOVA) for dynamic maceration. It can be seen that all the three factors (ES, SLR and ET) were significant ($P < 0.05$) on the tagitinin C extraction. It can also be seen that the interactions of the factors investigated were not significant at a level of 5%, showing no synergism between them in this extraction process.

CONCLUSIONS

In this work, the UAE and dynamic maceration to obtain tagitinin C from *T. diversifolia* were investigated with 2^3 factorial design based on response surface methodology in enhancing this chemical marker extraction. Thus, the design employed in this paper could be useful in further studies to optimize the extraction of tagitinin C. Based on the results, it was revealed that the UAE was more effective than dynamic maceration for *T. diversifolia*. The information found in this paper will be important for other exploitation and application of the resource.

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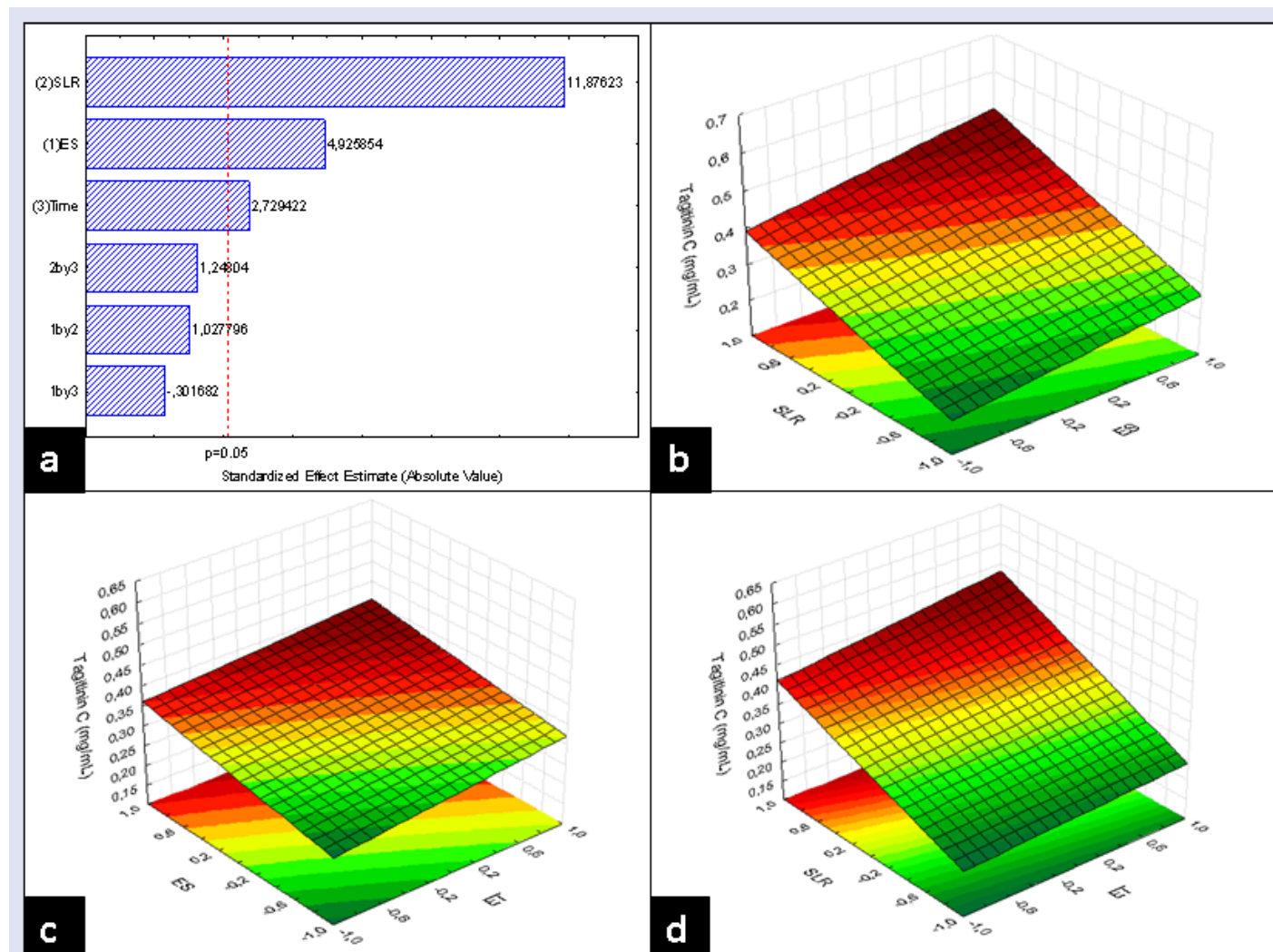


Figure 3: Dynamic maceration statistical graphs. (a) Pareto diagram to method of dynamic maceration. (b) Response surface graph relating ES and SLR. (c) Response surface graph relating ES and ET. (d) Response surface graph relating SLR and ET

Conflicts of interest

There are no conflicts of interest

REFERENCES

- Blake SF. Revision of the genus *Tithonia*. Department of Botany, Smithsonian Institution 1921;
- Pereira PS, Dias DA, Vichnewski W, Tucci Nasi AMT, Herz W. Sesquiterpene lactones from Brazilian *Tithonia diversifolia*. *Phytochemistry* 1997;45:1445-8.
- Lorenzi H, Souza HM. Plantas Ornamentais no Brasil-Arbustivas, herbáceas e trepadeiras. 2nd ed. Nova Odessa: Instituto Plantarum 1999.
- Heinrich M, Robles M, West JE, Ortiz de Montellano BR, Rodriguez E. Ethnopharmacology of Mexican Asteraceae (Compositae). *Annu Rev Pharmacol Toxicol* 1998;38:539-65.
- Owuor BO, Kisangau DP. Kenyan medicinal plants used as antivenin: a comparison of plant usage. *J Ethnobiol Ethnomed* 2006;27:2-7.
- Njoroge GN, Bussmann RW. Herbal usage and informant consensus in ethnoveterinary management of cattle diseases among the Kikuyus (Central Kenya). *J Ethnopharmacol* 2006;108:332-9.
- Zhao G-J, Xi Z-X, Chen W-S, Li X, Sun L, Sun L-N. Chemical constituents from *Tithonia diversifolia* and their chemotaxonomic significance. *Biochem System Ecol* 2012;44:250-4.
- Chagas-Paula DA, Oliveira RB, Rocha BA, Da Costa FB. Ethnobotany, chemistry, and biological activities of the genus *Tithonia* (Asteraceae). *Chem Biodivers* 2012;9:210-35.
- Ambrósio SR, Oki Y, Heleno VCG, Chaves JS, Nascimento PGBD, Lichston JE, *et al.* Constituents of glandular trichomes of *Tithonia diversifolia*: relationships to herbivory and antifeedant activity. *Phytochemistry* 2008;69:2052-60.
- Lee MY, Liao MH, Tsai YN, Chiu KH, Wen HC. Identification and anti-human glioblastoma activity of tagitinin C from *Tithonia diversifolia* methanolic extract. *J Agr Food Chem* 2011;59:2347-55.
- Liao M-H, Lin W-C, Wen H-C, Pu H-F. *Tithonia diversifolia* and its main active component tagitinin C induce survivin inhibition and G2/M arrest in human malignant glioblastoma cells. *Fitoterapia* 2011;82:331-41.
- Goffin E, Ziemons E, De Mol P, Madureira M, Martins AP, Cunha AP, *et al.* *In vitro* antiplasmodial activity of *Tithonia diversifolia* and identification of its main active constituent: tagitinin C. *Planta Med* 2002;68:543-5.
- Sánchez-Mendoza ME, Reyes-Ramírez A, Antônio LC, Jiménez LM, Rodríguez-Silverio J, Arrieta J. Bioassay-guided isolation of an anti-ulcer compound, tagitinin C, from *Tithonia diversifolia*: role of nitric oxide, prostaglandins and sulfhydryls. *Molecules* 2011;16:665-74.
- Elufioye TO, Alatise OI, Fakoya FA, Agbedahunsi JM, Houghton PJ. Toxicity studies of *Tithonia diversifolia* A Gray (Asteraceae) in rats. *J Ethnopharmacol* 2009;122:410-5.
- Passoni FD, Oliveira RB, Chagas-Paula DA, Gobbo-Neto L, Da Costa FB. Repeated-dose toxicological studies of *Tithonia diversifolia* (Hemsl.) A gray and identification of the toxic compounds. *J Ethnopharmacol* 2013;147:389-94.
- Fankule JO, Abatan MO. The toxicological effects of aqueous leaf extract of *Tithonia diversifolia* gray in rats. *J Anim Vet Adv* 2007;6:1223-6.
- Aderire CO, Akinneye JO. Biological activity of tree marigold, *Tithonia diversifolia*, on cowpea seed bruchid, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Ann Appl Biol* 2004;144:185-9.
- Castaño-Quintana K, Montoya-Lerma J, Giraldo-Echeverri C. Toxicity of foliage extracts of *Tithonia diversifolia* (Asteraceae) on *Atta cephalotes* (Hymenoptera: Myrmicinae) workers. *Ind Crops Prod* 2013;44:391-5.
- Dutta P, Bhattacharyya PR, Rabha LC, Bordoloi DN, Barua NC, Chowdhury PK, *et al.* Feeding deterrents for *Philosamia ricini* (*Samia cynthia* subsp. *ricini*) from *Tithonia diversifolia*. *Phytoparasitica* 1986;14:77-80.
- Ziémons E, Goffin E, Lejeune R, Angenot L, Thunus L. FT-IR measurement of tagitinin C after solvent extraction from *Tithonia diversifolia*. *Talanta* 2004;62:383-7.
- Sighamony S, Anees I, Chandrakala T, Oman Z. Efficacy of certain grain protectants against *Sitophilus orpae* (L.) and *Rhizopertha dominica* (F.). *J Stored Prod Res* 1986;22:21-2.
- Paula JAM, Brito LF, Caetano KLFN, de Moraes Rodrigues MC, Borges LL, da Conceição EC. Ultrasound-assisted extraction of azadirachtin from dried entire fruits of *Azadirachta indica* A Juss (Meliaceae) and its determination by a validated HPLC-PDA method. *Talanta* 2016;149:77-84.
- Ziemons E, Mbakop NW, Rozet E, Lejeune R, Angenot L, Thunus L, *et al.* Optimisation of SFE method on-line coupled to FT-IR spectroscopy for the real-time monitoring of the extraction of tagitinin C in *T. diversifolia*. *J Supercrit Fluids* 2007;40:368-75.
- Ziémons E, Goffin E, Lejeune R, Proença da Cunha A, Angenot L, Thunus L. Supercritical carbon dioxide extraction of tagitinin C from *Tithonia diversifolia*. *J Supercrit Fluids* 2005;33:53-9.
- Luque-García JL, Luque de Castro MD. Ultrasound: a powerful tool for leaching. *Trends Anal Chem* 2003;22:41-7.
- Yang B, Liu X, Gao YX. Extraction optimization of bioactive compounds (crocin, geniposide, and total phenolic compounds) from *Gardenia* (*Gardenia jasminoides* Ellis) fruits with response surface methodology. *Innovat Food Sci Emerg Technol* 2009;10:610-5.
- Toma M, Vinatoru M, Paniwnyk L, Mason TJ. Investigation of the effects of ultrasound on vegetal tissues during solvent extraction. *Ultrason Sonochem* 2001;8:137-42.
- Sousa JN, Pedrosa NB, Borges LL, Paula JR, Conceição EC. Optimization of ultrasound-assisted extraction of polyphenols, tannins and epigallocatechin gallate from barks of *Stryphnodendron adstringens* (Mart.) Coville bark extracts. *Pharmacog Mag* 2014;10:318-23.
- Rodrigues MC, Borges LL, Martins FS, Mourão RHV, Conceição EC. Optimization of ultrasound-assisted extraction of phenolic compounds from *Myrcia amazonica* DC (Myrtaceae) leaves. *Pharmacog Mag* 2016;12:9-12.
- Escriba C, Viana E, Molto JC, Pico Y, Manes JJ. Comparison of four methods for the determination of polycyclic aromatic hydrocarbons in airborne particulates. *J Chromatogr A* 1994;676:375-88.
- Ferrim PH. In *Qui mica de produtos naturais: métodos gerais*. Plantas Medicinais: Arte e Ciências (UNESP) 1996;
- Silva IDD, Aragão CFS. Avaliação de parâmetros de extração da *Cinchona* Vahl por métodos farmacopéicos e não farmacopéicos. *Rev Brasil Farmacog* 2009;19:776-80.
- Moura TFAL, Raffi FN, Santos ALR. Evaluation of a preservative system in a gel containing hydroalcoholic extract of *Schinus terebinthifolius*. *Rev Brasil Farmacog* 2011;21:532-6.
- Tour N, Talele GS. Gastric antiulcer and anti-inflammatory activities of *Calotropis procera* stem bark. *Rev Brasil Farmacog* 2011;21:1118-26.
- Box GEP, Wilson KG. On the experimental attainment of optimum conditions. *J R Stat Soc* 1951;13:1-45.
- Marín ML, Jiménez A, Vilaplana J, López J, Berenguer V. Determination of phenol in polymeric materials by supercritical fluid extraction combined with gas chromatography-mass spectrometry. *J Chromatogr A* 1998;819:289-96.
- Karvela E, Makris DP, Kalogeropoulos N, Karathanos VT, Kefalas P. Factorial design optimisation of grape (*Vitis vinifera*) seed polyphenol extraction. *Eur Food Res Technol* 2009;229:731-42.
- Abe AE, Oliveira CE, Dalboni TM, Chagas-Paula DA, Rocha BA, Oliveira RJ, *et al.* Anti-inflammatory sesquiterpene lactones from *Tithonia diversifolia* trigger different effects on human neutrophils. *Rev Brasil Farmacog* 2015;25:111-6.
- Scott R, Zdero C, Bohlmann F. Germacranolides, guaianolides and eudesmanolides from *Greenmaniella resinosa*. *Phytochemistry* 1987;26:1999-2006.
- Baruah NC, Sharma RP, Madhusudan K, Thyagarajan G, Herz W, Murari R. Sesquiterpene lactones of *Tithonia diversifolia* Stereochemistry of the Tags and related compounds. *J Org Chem* 1979;44:1831-5.
- Martins FS, Conceição EC, Bandeira ES, Silva JC, Costa RMR. The effects of extraction method on recovery rutin from *Calendula officinalis* L. (Asteraceae). *Pharmacog Mag* 2014;10:569-73.