Optimization in the Aqueous Two Phase Extraction of a Toxic Metabolite, Solasodine, From *S. Mauritianum* and Analysis via UHPLC-qTOF-MS

Tebogo Mokgehle (✉ tebogo.mokgehle24@yahoo.com)

University of Venda School of Mathematical and Natural Sciences <https://orcid.org/0000-0002-1960-4045>

Ntakadzeni Madala

University of Venda School of Mathematical and Natural Sciences

Wilson Gitari

University of Venda School of Environmental Sciences

Nikita Tavengwa

University of Venda School of Mathematical and Natural Sciences

Research Article

Keywords: Response surface methodology, solasodine, optimization, central composite design, multiple reaction monitoring

Posted Date: July 26th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-735334/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Aqueous two phase extraction (ATPE) was applied in the extraction of an allelochemical, solasodine, from an invasive plant, *Solanum mauritianum*. Central composite design was performed which included numerical parameters such as time and mass of plant powder. The categorical factors included the type of salt used in aiding extraction such as the chaotrope (NaCl) and kosmotrope (Na₂CO₃). Fitting the central composite design response surface model to the experimental data generated a quadratic model with a good fit ($R^2 = 0.925$). The linear effect of mass of plant powder was a statistically significant ($p < 0.05$) parameter for solasodine extraction. The optimized conditions for the extraction of solasodine in the presence of NaCl or Na₂CO₃ were time: 10 min and mass of plant powder: 1.2 g. Corresponding to these conditions, the maximal mean extraction based on multiple reaction monitoring (MRM) transition of solasodine (m/z 414 $\rightarrow$ 396) on the UHPLC-qTOF-MS was 233.65 mg kg⁻¹ and 413.50 mg kg⁻¹ for NaCl and Na₂CO₃, respectively. The greater extraction ability of the kosmotrope was due to the higher negative charge density of the carbonate ion during salting-out. Furthermore, the synergistic effect of mass of plant powder and salting-out was shown to enhance extraction of solasodine compared to the chaotrope. The kosmotrope assisted solasodine ATPE extracts from *Solanum mauritianum*, can potentially be applied as antipathogenic agents in medicine while simultaneously limiting the allelopathic impact of *Solanum mauritianum*.

Introduction

Solanum mauritianum Scopoli (Solanaceae), also known as the Bugweed, Tobacco tree or Woolly Nightshade, is an invasive tree of global significance. As an invasive alien plant species in South Africa, for more than a century, it has shown to have harmful impacts on organic matter content and on ecosystem services, thus degrading the lands productive potential (Lottering et al. 2020). The source of the devastating impact of *Solanum mauritianum* to its surroundings is its toxic metabolic composition. *Solanum mauritianum* produces toxic alkaloid allelochemicals that alter biogeochemical cycles, which constrain the growth rates of surrounding forest vegetation (Chornesky et al. 2005; Lottering et al. 2020). Despite the poisonous metabolic composition of *Solanum mauritianum*, tribes however, have used the plant to cure skin borne disorders (Jayakumar et al. 2017). A variety of *Solanum mauritianum* alkaloid secondary metabolites were isolated from herbals and were reported to exhibit antiproliferation and antimetastasis effects on diverse types of cancers both under in vitro and in vivo conditions (Jayakumar et al. 2017).

One class of alkaloid secondary metabolites are steroidal alkaloids (SA), which are nitrogen containing compounds prevalent in potatoes (*Solanum tuberosum*), tomatoes (*Solanum lycopersicum*) and eggplants (*Solanum melongena*). These metabolites include glycoalkaloids (containing mono/polysacharrides) and its hydrolysis products aglycones (without sugar moieties) (Mokgehle et al. 2021). In solanaceous plants, solasonine and solamargine are the most important glycoalkaloids of which the principal aglycone is solasodine (Neves et al. 2012). As a result, solasodine has been employed

as a steroidal precursor within the steroid drug trade for the manufacturing of corticosteroids and antifertility drugs (Kumar et al. 2019). Solasodine which is an aglycone unit of glycoalkaloids such as solasonine and solamargine has been reported to play an important role in the apoptosis of cancer cells (Kumar et al. 2019; Bhattacharya et al. 2013; Jayakumar et al. 2016; Jayakumar et al. 2017). Solasodine has been reported to rupture lysosomes, resulting in lysosomal contents (mainly hydrolytic enzymes) being released into the cytosol causing apoptosis of cancer cells. Solasodine based rhamnosyl glycosides in combination with cisplatin proved more effective against cisplatin-resistant tumor cells, including lung and breast cancer cells (Cham et al. 2008; Jayakumar et al. 2017).

Despite the useful properties of solasodine, it remains a highly toxic compound. For instance, glycoalkaloids containing the solasodine backbone were reported for reducing larval growth of the red flour beetle *Tribolium castaneum* (Weissenberg et al., 1998; Ventrella et al., 2016). Furthermore, according to Smith et al. (2008) a 52-year-old woman who consumed *Solanum torvum* berries experienced vomiting, diarrhea, blurry vision, ataxia, slurred speech, ptosis, muscle fasciculations, diaphoresis, dyspnea and urinary incontinence. Another study by Glover et al. (2016) reported on a case of poisoning of a 54-year-old woman after intake of a *Solanum torvum*. In both cases glycoalkaloids containing solasodine were responsible for poisoning.

Considering the toxic nature of solasodine, this project was directed at optimizing the aqueous two phase extraction (ATPE) of solasodine from the weed, *Solanum mauritianum*. A multivariate optimization approach based on central composite design (CCD) was performed and the responses viewed via response surface methodology (RSM). The CCD and RSM approach is useful as it reduces the number of optimization experiments, making it less laborious and time efficient (Silva et al. 2019). Two variables were investigated which included extraction time and mass of plant powder. Quantification was based on scheduled multiple reaction monitoring (MRM) on the ultra high performance liquid chromatography time of flight mass spectrometer (UHPLC-qTOF-MS), where an m/z transition, from the precursor ion to the product ion, for solasodine (414→396) was explored. Pareto charts were also examined to determine which of the parameters had the greatest influence on extraction of solasodine.

Experimental

Chemicals and reagents

The salts NaCl (anhydrous $\geq 99\%$ purity), Na₂CO₃ (anhydrous $\geq 99\%$ purity) and ethanol (99% CP) were purchased from Associated Chemical Enterprises (Johannesburg, South Africa) and Sigma-Aldrich (Johannesburg, South Africa). Ultra-pure water (0.005 μ S, 18 m Ω) using a Direct-Q 5UV distiller (Massachusetts, United States of America) was applied for the preparation of the salt solutions. The extraction was performed on a DIAB MX-RL-Pro dragon shaker. Chromatographic separation of the metabolites in the extracts was done using a reverse phase Shim-pack Velox C₁₈, 2.1 x 100 mm, 2.7 μ m with a serial number 227-32009-03 (Columbia, USA). The UPLC was connected to a Shimadzu 9030 LC,

qTOF-MS detector (Shimadzu, Kyoto). The solvents used for the chromatographic runs were methanol and formic acid, which were purchased from Romil Pure Chemistry (Cambridge, UK).

Sample collection, preparation and ATPE

The leaves of *S. mauritianum* were obtained from a street vendor within the Thulamela District in Thohoyandou, South Africa. The plants were air dried until a constant weight was obtained, and the leaves were ground into a fine powder with a blender at 2000 rpm and stored in glass containers. The containers were covered in paper bags to prevent light penetration. The powdered leaves were placed in a 250 mL in a centrifuge tube (50 mL). Thereafter, saturated salt concentrations (20 mL) of 30% (w/v) involving Na₂CO₃ (kosmotrope) and NaCl (chaotrope) which were prepared by weighing 15 g of salt in 50 mL of water, were added to the powdered leaves contained in the centrifuge tube (50 mL). The aqueous solution was placed over the dragon shaker for (1 - 10 min) and the range of the mass of plant powder studied included (0.2 - 1.2 g). Thereafter, 99.9% ethanol extraction solvent (20 mL) was added and resulted in an ATPE system. Following this, the extracts obtained were then analysed on a UPLC-QTOF-MS for detection of solasodine extracted.

Chromatographic and mass spectrometry conditions

Solasodine were separated using a Shimpack C₁₈, 2.1 x 100 mm, 2.7 µm column from Shimadzu (Honeydew, South Africa). The column was maintained at 40 °C at a flow rate of 0.4 mL min⁻¹ and the injection volume was 5 µL. Mobile phase A was 0.1% formic acid in ultrahigh purity water (v/v) and mobile phase B was 0.1% (v/v) formic acid in methanol.

An UPLC-QTOF-MS 9030 mass spectrometer (Shimadzu, Japan) was used for all mass spectral measurements. The mass spectrometer was equipped with an electrospray interface (ESI) operating in positive mode. ESI parameters were optimized for solasodine by direct infusion of standard solutions into the mass spectrometer. The mass spectrometer was operated in the multi reaction monitoring (MRM) mode to confirm the identity of solasodine. This was achieved by selecting specific precursor to product ion transitions for each solasodine based on MRM transitions. High-purity nitrogen (N₂) was used as the nebulizing and drying gas. The optimum parameters were as follows: drying gas temperature, 250 °C; drying gas flow, 10 L min⁻¹ and collision energy, 30 - 60V. For the chromatographic separation an Shimadzu 9030 LC instrument (Shimadzu, Japan) was used. The instrument consisted of an autosampler, thermostated column compartment and a binary pump. Lab solutions software was used to control the LC-MS/MS instrument and for data acquisition and the mass range was *m/z* 100-1000.

Preparation of standards

The stock standard solution was prepared in methanol at a concentration of 1000 µg L⁻¹. The stock standard solution was stored at 4°C in amber volumetric flasks. A series of nine working standard solutions at the concentration values of 15 to 1000 µg L⁻¹ were prepared from the stock standard solution by diluting with HPLC grade methanol. The solanine standards were quantified based on scheduled

multiple reaction monitoring (MRM) where one m/z transition, from the precursor ion to the product ion, for solanine (414 $\rightarrow$ 396) was explored. The regression equation was $y = 537.484x + 41.893$, limit of detection (LOD) and limit of quantification (LOQ) were 0.078 and 0.236, respectively. The above mentioned transition was then applied for quantification of solasodine from the ground leaves of *Solanum mauritianum* following ATPE extraction. The parameters evaluated for optimization of solasodine from ATPE were time, mass of plant powder and the salt type (komostrope or chaotrope).

Statistical analysis

The central composite design response surface model (CCD RSM) was fitted to experimental data to obtain the relationship between factors and optimize the response of Z (solasodine yield) in relation to A (time), B (mass of plant powder) using Minitab 17 (UK). A Two-level full factorial CCD was designed, a total of 26 experimental runs (including 2 repetitions) were designed. This included numerical factors such as time (1 min, 5 min 30 sec and 10 min), mass of plant powder (0.2, 0.7, 1.2 g) and 2 categorical factor levels for salts which included the chaotrope (NaCl) and komotrope (Na₂CO₃).

Model parameters and model significance were determined at $p < 0.05$. The fitness of the model was determined by evaluating the coefficient of regression (R^2) obtained from the analysis of variance (ANOVA). The model fit generated the response surface that defined the behaviour of the response variable. By means of these plots, the optimized ranges for each factor that led to the highest response (i.e concentration of solasodine) that can be extracted.

The interaction between the various parameters studied and its resultant effected on the extraction of solanine (mg kg⁻¹) was fitted to experimental data by using a statistical multiple regression approach method of least square (MLS), and resulted in the lowest possible residual (Bas et al. 2007). Model parameters and model significance were determined at $p < 0.05$. The fitness of the model was determined by evaluating the coefficient of regression (R^2) obtained from the analysis of variance (ANOVA). The model fit generated the response surface that defined the behaviour of the response variable. By means of these plots, the optimized ranges for each factor that led to the highest response (i.e concentration of solanine) that can be extracted (Bas et al. 2007; Arteaga-Crespo et al. 2020).

Results And Discussion

MRM quantification of solasodine based on the 414 $\rightarrow$ 396 transition

Solanum mauritianum, an invasive species, is an abundant source of anti-cancer and antifungal metabolites such as solasodine glycosides (Jayakumar et al. 2016; Jayakumar et al. 2017). In this study, we demonstrated the extraction of an aglycone unit of solasodine glycosides, solasodine, from *Solanum mauritianum*, using ATPE. The ATPE technique was performed by evaluating the different factors shown in Table 1 on the recovery of solasodine. The presence of solasodine was reported in *Solanum mauritianum* and other species within the *Solanum* genus (Bhattacharya et al. 2013; Jayakumar et al. 2016; Jayakumar et al. 2017). Using a sensitive and robust tandem MS approach (UHPLC-qTOF-MS) with

settings presented (Mokgehle et al. 2021) it was possible to efficiently fingerprint these solasodine based on m/z 396 product ion (Fig. 1). Thereafter, based on the $414 \rightarrow 396$ transition within the MRM method, solasodine was quantified as shown in Table 1.

Table 1 List of experiments using CCD for ATPE optimization, the response and predicted values.

	Factor 1:	Factor 2:	Factor 3	Solasodine (mg kg ⁻¹)			
Run	Time (min)	mass of plant powder (g)	Salt type	Run 1	Run 2	Mean ± SD	Predicted
1	1	0.2	Na ₂ CO ₃	142.17	109.16	125.66+23	143.14
2	1	0.7	NaCl	287.55	286.09	286.82+1	242.66
3	1	1.2	Na ₂ CO ₃	308.69	309.69	309.19+1	309.60
4	1	0.7	Na ₂ CO ₃	232.21	300.07	266.14+48	248.24
5	1	1.2	NaCl	217.81	198.46	208.14+14	236.85
6	1	0.2	NaCl	137.62	158.38	148.00+15	163.42
7	5.5	0.7	NaCl	226.82	196.81	211.82+18	225.47
8	5.5	0.7	Na ₂ CO ₃	173.56	221.42	197.49+34	215.87
9	5.5	0.7	Na ₂ CO ₃	206.84	172.81	189.83+24	215.87
10	5.5	0.7	NaCl	222.62	208.09	215.35+10	225.47
11	5.5	0.7	Na ₂ CO ₃	196.11	261.55	228.83+46	215.87
12	5.5	1.2	Na ₂ CO ₃	277.18	297.24	287.21+14	308.14
13	5.5	0.7	Na ₂ CO ₃	273.98	251.05	262.52+16	215.87
14	5.5	0.7	NaCl	238.13	223.28	230.71+11	225.47
15	5.5	1.2	NaCl	265.45	288.52	276.99+16	240.13
16	5.5	0.7	Na ₂ CO ₃	188.79	228.15	208.47+28	215.87
17	5.5	0.7	NaCl	209.45	193.59	201.52+11	225.47
18	5.5	0.2	NaCl	137.55	135.20	136.38+2	125.75
19	5.5	0.2	Na ₂ CO ₃	106.62	79.50	93.06+19	79.875
20	5.5	0.7	NaCl	217.94	222.31	220.13+3	225.47
21	10	0.2	Na ₂ CO ₃	104.28	60.35	82.32+31	78.02
22	10	1.2	Na ₂ CO ₃	402.78	424.22	413.50+15	368.10
23	10	0.2	NaCl	88.87	94.34	91.60+4	86.79
24	10	0.7	Na ₂ CO ₃	226.53	212.07	219.30+10	244.93

25	10	0.7	NaCl	221.03	200.30	210.66+15	206.99
26	10	1.2	NaCl	275.69	191.62	233.65+59	242.13

Fit statistics of experimental and predicted data

The model fitted to the data was observed to have a quadratic fit P-values less than 0.001 indicate model terms are significant. In this case mass of plant powder, mass of plant powder² (when NaCl was applied) were significant ($p < 0.05$) model terms and were adequate predictors of the experimental values obtained. The other terms such as time $\times$ mass of plant powder, time² and time were insignificant ($p > 0.05$). The lack of fit of F-value was observed to be 1.70 which indicated that the lack of fit was not significant relative to the pure error. The non-significant lack of fit was desirable. The goodness of fit between the experimental and the predicted values was $R^2 = 0.925$

The Pareto chart of parameter main effects and their interactions produced from ANOVA and resultant box plots

In Fig. 2 (a) and (b) are the pareto charts showing the influences of the parameters time, mass of plant powder, the square of each model and the product of the two parameters. The model term mass of plant powder was shown to be statistically significant ($p < 0.05$) when both NaCl and Na₂CO₃ were applied, showing a linear effect on the yield of solasodine (Fig. 2 (a) and (b)). In Fig. 3 are the box-and-whiskers plots of the effect of time and mass of plant powder on the ATPE extraction of solasodine from leaves of *Solanum mauritianum*. From these plots a proportional increase in solasodine was observed with an increase in the mass of plant powder when NaCl and Na₂CO₃ were applied during extraction (Fig. 3 (a) and (b)). This indicated that the mass of the plant powder played a key role in the recovery solasodine. The increased enrichment of solasodine was more notable when Na₂CO₃ was used compared to NaCl. For instance, the extracted solasodine increased from approximately 85 mg kg⁻¹ (0.2 g) to 383 mg kg⁻¹ (1.2 g) which equated to almost a five fold increase for Na₂CO₃. The observed increment in the yield of solasodine with increase in mass can be attributed to the increased mass transfer of metabolites from the plant matrix to the solvent when larger weights of the plant material were used. This observation is in agreement with what was reported by Doulabi et al (2020).

It was also observed from Fig. 3 (c) and (d) that an increase time generally had no impact on the extraction of solasodine. For instance, the median for extraction of solasodine settled at approximately 223 mg kg⁻¹ as time was increased from 1 to 10 min, when NaCl was applied as to aid extraction, Fig. 3 (c). This correlates with the level of significance values for the linear effect of time when both NaCl and Na₂CO₃ were applied which indicated that time was statistically insignificant ($p > 0.05$) on the extraction of solasodine, as shown in Fig. 2 (a) and (b). Factors involving the quadratic effect of time as well as the product of time and mass of plant powder were also insignificant on the extraction of solasodine from

Solanum mauritianum. Similar observations of the insignificance of time for extraction of metabolites based on a response surface methodology were reported by Pandey et al. (2018).

Chromatographic profile of MRM based quantification of solasodine

Chromatograms depicting the highest and lowest concentrations of solasodine (mg kg⁻¹) obtained when NaCl and Na₂CO₃ was applied are shown in Fig. 4 (a), (b), (c) and (d). The chromatogram indicates an MRM transition of solasodine *m/z* 414 → 396 and is observed at a retention time of 3.825 min. The fragmentation profile showing the product ions of solasodine are also included in Fig. 4 (f). As seen in Fig. 1, solasodine underwent a dehydration reaction from the precursor ion *m/z* 414 to the product ion *m/z* 396. From the experimental results dehydration of solasodine seems to be more prominent at extraction parameters involving longer times in combination with larger masses of plant powder. Under such conditions, solasodine concentrations of 402.78 and 275.69 mg kg⁻¹ for Na₂CO₃ and NaCl were obtained, respectively, as shown in Fig. 4 (c) and (d). This is also in agreement with the significant effect of mass of plant powder, as seen in the pareto charts in Fig. 2 (a) and 2(b), and the box plots in Fig. 3.

Response surface equations and corresponding for NaCl and Na₂CO₃ and the resultant optima

Response equations, Eqs 1 and 2, corresponding to NaCl and Na₂CO₃, respectively, and the resultant response surfaces evaluating the bivariate interaction between the time and mass of plant are shown in Fig. 5. In this case, Z was the dependent variable (solasodine concentration) and x (time) and y (mass of plant powder) the independent variables. From the quadratic fit of *r*² = 0.925, as reported earlier, Eqs 1 and 2 were obtained

$$z(x,y) = 132.09 - 11.18 x + 338.74 y - 0.035 x^2 - 190.50 y^2 + 10.19 xy \dots\dots\dots(1)$$

$$z(x,y) = 127.68 - 29.86 x + 308.22 y + 1.69 x^2 - 97.97 y^2 + 15.38 xy\dots\dots\dots(2)$$

As the mass of plant powder was increased, in the presence of NaCl and Na₂CO₃ a proportional increase in the yield of solasodine was obtained (Fig. 5 (a) and (b)). This concurs with observations from pareto chart, Fig. 3 (a) and (b), which indicates the significant linear effect (*P* < 0.05) of mass of plant powder. In Fig. 6 (a) and (b) the predicted optimal extraction of solasodine in the presence of NaCl and Na₂CO₃ was 261.75 mg kg⁻¹ and 347.32 mg kg⁻¹, with a desirability score of 0.58 and 0.86 respectively. The higher desirability score of Na₂CO₃ compared to NaCl indicated its closeness to the target requirement of 1, and hence the greater reliability of this optimum for maximal enrichment of solasodine. Additionally, comparisons of the concentrations of solasodine obtained in Table 1 and Fig. 6, indicated that more of solasodine was extracted for Na₂CO₃ compared to NaCl. This suggested that solasodine extraction during ATPE was probably favored by the presence of the multiply charged carbonate ion from Na₂CO₃ compared to the singly charged chloride ion in NaCl. The doubly charged carbonate ions from Na₂CO₃, probably formed strong hydrogen bonds with the water molecules surrounding the solasodine, weakening the solasodine-water interaction, and enhancing the extent of solasodine precipitation (salting-out) from

the hydration sphere. The salted-out solasodine was subsequently extracted by ethanol into the organic phase. Hence, extraction of solasodine during ATPE occurred through the process of salting out. Furthermore, the extent of salting out was further driven by the increase in mass of plant powder due to mass transfer under the conditions studied. The salting-out effect in ATPE was also reported by Sazali et al. (2019) and Mokgehele et al. (2021). Additionally, the higher extraction of solasodine via Na_2CO_3 compared to NaCl can correlate with the Hoffmeister series. Larger multivalent anions have a propensity for salting out proteins than smaller monovalent anions (Kang et al., 2020; Dogra et al., 2020; Wang et al., 2021). Similarly, the Hoffmeister principle was evident in this study as the multivalent carbonate ion precipitated greater concentrations of solasodine under the optimized ATPE conditions of time: 10 min and mass of plant powder: 1.2 g.

Conclusions

The application of ATPE in the presence of a chaotrope and kosmotrope was shown to be a viable technique for extraction of an allelochemical, solasodine, from the weed, *Solanum mauritianum*. On average the maximal MRM transition based extraction of solasodine was $233.65 \text{ mg kg}^{-1}$ and $413.50 \text{ mg kg}^{-1}$ for NaCl and Na_2CO_3 , respectively. This indicated that the higher charge density of the carbonate ion relative to the chloride ion was responsible for the greater extent of salting-out of solasodine. Furthermore, the application of ATPE using the kosmotrope was shown to enhance the extraction of solasodine compared to the chaotrope due to the synergistic effect of mass of plant powder, which was shown to be significant effect ($p < 0.05$), and salting-out. The effect of time and the paired factors were shown to have an insignificant effect ($p > 0.05$) on the enrichment of solasodine. In view of the better extraction performance of the kosmotrope in ATPE, this could possibly be extended to extraction of other toxic allelochemicals from other invasive plants which could better preserve the natural environment. Furthermore, the enriched solasodine ATPE extracts could potentially be a reliable source of antipathogenic agents in medicine.

Declarations

Acknowledgements

The authors would also like to express their gratitude to the Council for Scientific and Industrial Research studies for their technical support. The University of Venda is also thanked for financial support.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and material (data transparency)

Not applicable

Code availability (software application or custom code)

Not Applicable

Contributions

TM, NT and NEM conceived the study, TM and NEM conducted the experiments and data analyses. NT, NM and WG supervised the project. WG helped to draft the manuscript. All authors read and approved the final manuscript.

References

- Bhattacharya S, Kohli S, Chaudhary AS (2013). Isolation of solasodine from the unripe fruits of *Solanum xanthocarpum* Schrad and Wendl. (*Solanaceae*) and its anti cancer activity against HeLa and U937 cell lines. *Austral-Asian J. Cancer* 12:199-213.
- Cham BE. (2008) Cancer intralesion chemotherapy with solasodine rhamnosyl glycosides. *Res. J. Biol. Sci.* 3:1008-1017. <https://medwelljournals.com/abstract/?doi=rjbsci.2008.1008.1017>
- Chornesky EA, Bartuska AM, Aplet GH., Britton KO, Cummings-Carlson J, Davis FW, Eskow J, Gordon DR, Gottschalk KW, Haack RA, Hansen AJ, Mack AN, Rahel FJ, Shannon, MA, Wainger, LA, Wigley TB (2005) Science priorities for reducing the threat of invasive species to sustainable forestry. *Bioscience* 55:335-348 <https://doi.org/10.1016/j.jep.2009.03.018>
- de Mesquita ML, de Paula, JE, Pessoa C, de Moraes, MO, Costa-Lotufo, LV, Grougnet R., Michel S, Tellequin F, Espindola LS (2009) Cytotoxic activity of Brazilian Cerrado plants used in traditional medicine against cancer cell lines. *J Ethnopharmacol*, 123, 439-445. <https://doi.org/10.1016/j.jep.2009.03.018>
- Dogra P, Roy SS, Joshi A, Mukhopadhyay S (2020) Hofmeister ions modulate the autocatalytic amyloidogenesis of an intrinsically disordered functional amyloid domain via unusual biphasic kinetics. *J Mol Biol* 432:6173-86. <https://doi.org/10.1016/j.jmb.2020.10.015>
- Doulabi M, Golmakani MT, Ansari S (2020). Evaluation and optimization of microwave-assisted extraction of bioactive compounds from eggplant peel by-product. *J Food Process Pres* 44:1-13. <https://doi.org/10.1111/jfpp.14853>
- Glover RL, Connors NJ, Stefan C, Wong E, Hoffman RS, Nelson LS, Milstein M, Smith SW, Swerdlow M. (2016) Electromyographic and laboratory findings in acute *Solanum torvum* poisoning. *Clin. Toxicol.* 54:61-65 <https://doi.org/10.1016/j.toxicol.2008.07.016>
- Jayakumar K, Murugan K (2017) Purified solasodine and caulophyllumine: a from *Solanum mauritianum* Scop. against MCF-7 breast cancer cell lines in terms of cell growth, Cell Cycle and Apoptosis. *J. Pharmacogn. Phytochem.* 6:472-478.

- Kang B, Tang H, Zhao Z, Song S (2020) Hofmeister series: Insights of ion specificity from amphiphilic assembly and interface property. *ACS omega*, 5:6229-6239. <https://doi.org/10.1021/acsomega.0c00237>
- Kumar R, Khan MI, Prasad M (2019) Solasodine: A Perspective on their roles in health and disease. *Res J Pharm Technol* 12:2571-2576. <https://doi.org/10.5958/0974-360X.2019.00432.3>
- Lottering RT, Govender M, Peerbhay K, Lottering S (2020) Comparing partial least squares (PLS) discriminant analysis and sparse PLS discriminant analysis in detecting and mapping *Solanum mauritianum* in commercial forest plantations using image texture. *ISPRS J. Photogramm. Remote Sens.* 159:271-280. <https://doi.org/10.1016/j.isprsjprs.2019.11.019>
- Mokgehle T, Madala N, Gitari W, Tavengwa N (2021) Deciphering the effects of kosmotrope and chaotrope salts during aqueous two phase extraction (ATPE) of polyphenolic compounds and glycoalkaloids from the leaves of a nutraceutical plant, *Solanum retroflexum*, with the aid of UPLC-QTOF-MS. *Appl. Biol. Chem.* 64:1-15. <https://doi.org/10.1186/s13765-021-00603-8>
- Munari CC, de Oliveira PF, Campos JCL, Martins SDPL, Da Costa JC, Bastos JK, Tavares DC (2014) Antiproliferative activity of *Solanum lycocarpum* alkaloidic extract and their constituents, solamargine and solasonine, in tumor cell lines. *J Nat Med* 68:236-241. <https://doi.org/10.1007/s11418-013-0757-0>
- Neves, MP, Cravo S, Lima R T, Vasconcelos MH, Nascimento MSJ, Silva AM, Corrêa AG (2012) Solid-phase synthesis of 2'-hydroxychalcones. Effects on cell growth inhibition, cell cycle and apoptosis of human tumor cell lines. *Bioorg Med Chem* 20:25-33. <https://doi.org/10.1016/j.bmc.2011.11.042>
- Nussbaumer S, Bonnabry P, Veuthey JL, Fleury-Souverain S (2011) Analysis of anticancer drugs: a review. *Talanta* 85:2265-2289. <https://doi.org/10.1016/j.talanta.2011.08.034>
- Pandey A, Belwal T, Sekar KC, Bhatt ID, Rawal RS (2018) Optimization of ultrasonic-assisted extraction (UAE) of phenolics and antioxidant compounds from rhizomes of *Rheum moorcroftianum* using response surface methodology (RSM). *Ind Crops Prod.* 119:218-225. <https://doi.org/10.1016/j.indcrop.2018.04.019>
- Sazali NH, Alshishani A, Saad B, Chew KY, Chong MM, Miskam M (2019) Salting-out assisted liquid-liquid extraction coupled with high-performance liquid chromatography for the determination of vitamin D3 in milk samples. *R. Soc. Open Sci.* 6:1-8. <https://doi.org/10.1098/rsos.190952>
- Silva CL, Perestrelo R, Silva P, Tomás H., Câmara JS (2019) Implementing a central composite design for the optimization of solid phase microextraction to establish the urinary volatome expression: a first approach for breast cancer. *Metabolomics* 15:1-13. <https://doi.org/10.1007/s11306-019-1525-2>
- Smith SW, Giesbrecht E, Thompson M, Nelson LS, Hoffman RS. (2008) Solanaceous steroidal glycoalkaloids and poisoning by *Solanum torvum*, the normally edible susumber berry. *Toxicon.* 52:667-676. <https://doi.org/10.1016/j.toxicon.2008.07.016>

Ventrella E, Adamski Z, Chudzińska E, Miądowicz-Kobielska M, Marciniak P, Büyükgüzel E, Büyükgüzel K, Erdem M, Falabella P, Scrano L, Bufo SA (2016) *Solanum tuberosum* and *Lycopersicon esculentum* leaf extracts and single metabolites affect development and reproduction of *Drosophila melanogaster*. PloS one 11:1-16 <https://doi.org/10.1371/journal.pone.0155958>

Wang X, Qiao C, Song K, Jiang S, Yao J (2021). Hofmeister effect on the viscosity properties of gelatin in dilute solutions. Colloids and Surfaces B: Biointerfaces, 111944. <https://doi.org/10.1016/j.colsurfb.2021.111944>

Weissenberg M, Levy A, Svoboda JA, Ishaaya I (1998) The effect of some *Solanum* steroidal alkaloids and glycoalkaloids on larvae of the red flour beetle, *Tribolium castaneum*, and the tobacco hornworm, *Manduca sexta*. Phytochemistry 47:203-209 [https://doi.org/10.1016/S0031-9422\(97\)00565-7](https://doi.org/10.1016/S0031-9422(97)00565-7)

Figures

Figure 1

Molecular transition of solasodine (m/z = 414) to [solasodine – H_2O] (m/z = 396) after the loss of water

Figure 2

Pareto chart of standardized effects of time, mass of plant powder and power on the extraction solasodine at 414 → 396 (a) NaCl and (b) Na₂CO₃

Figure 3

Box-and-whiskers plots evaluating the effect of mass of plant powder on (a) NaCl (b) Na_2CO_3 and the effect of time on (c) NaCl and (d) Na_2CO_3 on the extraction of solasodine from leaves of *Solanum mauritianum*

Figure 4

Chromatogram of the lowest (a) Na_2CO_3 , (b) NaCl and highest (c) Na_2CO_3 , (d) extraction yields of solasodine for run 1. (f) mass spec of solasodine

Figure 5

Response surfaces plots evaluating the effect of time and mass of plant powder in the presence of (a) Na_2CO_3 and (b) NaCl on the extraction of solasodine

Solasodine maximum
 $Y = 347.32$
 $d = 0.864$

Solasodine maximum
 $Y = 261.75$
 $d = 0.5842$

Figure 6

Optimal conditions for extraction of solasodine in the presence of (a) NaCl and (b) Na₂CO₃