

UHPLC-DAD-ESI-MS/MS of Ethanolic Extract of the Flowers from *Spathodea Campanulata* Beauv. in Cuba

José González^{1*}, Pilar A. Soledispa², Enrique Gómez³, Max Monan⁴

¹Facultad de Educación en Ciencias Técnicas, UCP "Enrique José Varona", La Habana, Cuba

²Facultad de Ciencias Químicas, Universidad de Guayaquil, Ecuador

³Instituto de Farmacia y Alimentos, Universidad de La Habana, Cuba

⁴ARVARNAM, 16 lot. les Rosiers, Quartier Thoraille, 97215, Rivière-Salée, Martinica

*Corresponding Author

Received: 23 July 2021/ Revised: 15 August 2021/ Accepted: 31 August 2021/ Published: 30-09-2021

Copyright © 2021 International Journal of Engineering Research and Science

This is an Open-Access article distributed under the terms of the Creative Commons Attribution

Non-Commercial License (<https://creativecommons.org/licenses/by-nc/4.0>) which permits unrestricted Non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

Abstract— A sample of ethanolic extract of the flowers from *Spathodea campanulata* Beauv. was scrutinized using a UHPLC-DAD-MS/MS Thermo scientific Dionex Ultimate 3000 RS coupled to a Thermo scientific LTQ XL mass spectrometer. In addition, about nine different chemical compounds were tentatively identified in this species based on chromatography retention time (Rt), UV and MS/MS spectra and compared with those of isolated authentic compound and literature data in our country for the first time. Information obtained from these studies can be used as biomarkers in the identification and standardization of this flower as an herbal remedy and also towards monograph development on the plant.

Keywords— UHPLC, Flowers, Chemical compounds, Ethanolic extract, *Spathodea campanulata*.

I. INTRODUCTION

Spathodea campanulata P. Beauvais tree is native to Africa. In tropical Africa it is planted as an ornamental plant, e. g. in Cape Verde, Zimbabwe and Madagascar. It is widely grown in tropical and subtropical regions outside Africa [1]. This plant is also commonly found in India as an ornamental plant. This plant is also called as African tulip tree or Flame tree, *Spathodea campanulata* P. Beauv is the botanical name. It is a single species of the monotypic genus *Spathodea* in the flowering plant family Bignoniaceae which is composed of around 800 species distributed in 112 genera [2].

The flowers are used as diuretic and anti-inflammatory, while the leaves are against kidney diseases, urethra inflammations and as an antidote against animal poisons [3]. Several phytochemical studies were performed with different parts of *Spathodea campanulata* including stem barks, leaves, flowers and fruits. The leaves contain spathodol, caffeic acid, other phenolic acids and flavonoids, while fruits contain polyphenols, tannins, saponins and glycosides [4]. The plant leaves reported to have antiplasmodial, analgesic, anti-inflammatory and anti-larvicidal activity. The stem bark decoction of *Spathodea campanulata* have been displayed hypoglycemic, anti-complementary, antimalarial and anti-HIV activity [5].

The large, orange-red flowers are arranged in dense clusters (8-10 cm long) at the tips of the branches. The reddish-orange colored petals are also fused together and are shaped somewhat like a tulip flower i.e. tubular (Figure 1). Flowering occurs throughout the year, but usually peaks during spring [6].



FIGURE 1: Flowers of *Spathodea campanulata* Beauv

Preliminary phytochemical screening of *S. campanulata* revealed the presence of alkaloids, reducing sugars, carbohydrates, flavones, glycosides, and phenolic compounds [7]. Several compounds have been isolated from different parts of the plant. From the flowers has been isolated 1,1-diethoxy-3-methyl-butane, N-hexadecanoic acid, 1,2-benzenedicarboxylic acid diisooctyl ester, and oleic acid [8]. Phytol, α -methyl cinnamaldehyde, β -sitosterol-3-acetate, naringenin, catechin-3- O - α -rhamnopyranoside and 5, 6, 4'-trihydroxy flavonol-7- O - α -rhamnopyranoside, and anthocyanins [9].

The floral nectar contains a complex mixture of triterpenoids and steroids [Rev. Bras. Pl. Med., 2012. (10)]. The aim of this study therefore, was to isolate and characterize the active phytochemical(s) of *S. campanulata* flowers ethanolic extract.

II. MATERIAL AND METHODS

2.1 Sample Collection and Processing

The flowers were freshly collected in the morning between 9 and 10 AM during five days in April of 2018 in the gardens of the Faculty of Pharmacy and Foods at Havana University. Authors are waiting for identification at the herbarium of National Botany Garden of Havana, where the voucher specimen number will be deposited.

2.2 Extract and Samples Preparation

The flowers used were dried in an oven with controlled temperature at 40°C, during 5 days. The extracts were prepared with the ground material (100 g), using a Soxhlet apparatus and 95% ethanol (675 mL) for 20 hours, by triplicated. The ethanolic extracts were concentrated and rotoevaporated under vacuum to 200 mL at 120 rpm, 70°C, and 500 mbar. The concentrated extract was put on to the Lab table at room temperature until an abundant solid appear and it was recuperated by filtration.

2.3 UHPLC-DAD-ESI-MS/MS Procedures, Instrumentation, and Parameters

The LC system consisted of an UPLC/DAD/MS Thermo scientific Dionex Ultimate 3000 RS with quaternary pump, autosampler, DAD (diode array detector) Dionex with a UV-VIS at 200 nm (UV1), 250 nm (UV2), 280 nm (UV3), and 330 nm (UV4) coupled to a mass spectrometer Thermo scientific LTQ XL with ESI (ion trap analyzer) in negative ionization mode. Conditions of detection were optimizing with a Tune archive based on the behavior of quercetin. Temperature: 225°C, Voltage 5 KV, Capillary voltage 50 V. Column: Accucore RP-MS (100 × 2.1 mm × 2.6 μ m). Temperature: 35°C. Chromatographic system: eluent CAN (5%); HCOOH (0.1%). Isocratic 20 min. Flow: 0.4 mL/min (Table 1). Nitrogen gas flow: 34, auxiliary gas: 16, barrier gas: 3. Induced fragmentation gas: Helium. Was realized an experiment Full Scan in independent mode to identify the principal ions (TIC) and get the MS and MS². The sample was dissolved in methanol HPLC grade at 1 mg/mL filtered by a nylon filter of 0.20 μ m. Injection volume: 2 μ L. Mass scan between 200 - 700 u.m.a.

TABLE 1
CHROMATOGRAPHIC CONDITIONS USED IN THE STUDY

Time (min)	ACN	Formic acid 0,1 %
0	12	88
2	27	73
3	27	73
5	30	70
6	30	70
9	35	65
10	95	5
11	12	88
17	12	88

III. RESULTS AND DISCUSSION

3.1 MS-ESI Identification of phytochemicals in *S. campanulata* flowers from Cuba

Several phytochemical components in *S. campanulata* flowers were detected and tentatively identified using UHPLC with UV-visible data and ion trap electrospray mass spectrometry (IT-ESI-MS). Figure 3 shows the TIC chromatogram fingerprint at 250 nm. All of them were analyzed in negative ion mode between 0.17 and 11.38 min of retention time.

D:\E SH\ASAS\RESULTADOS\Pilar\P4pilar6

19/6/2018 11:20:04

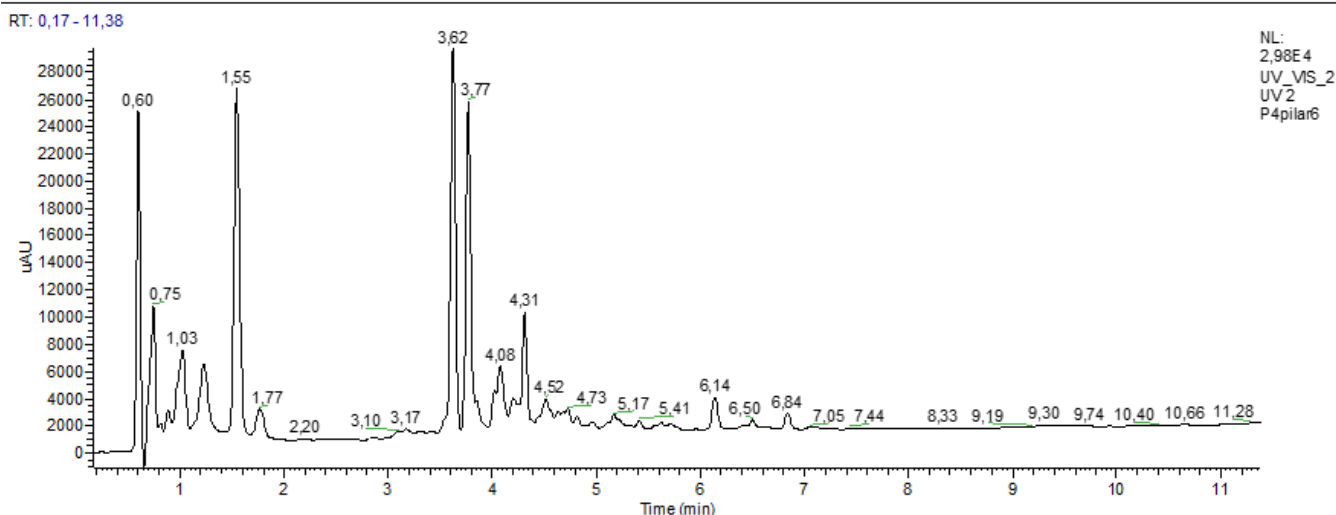


FIGURE 3: Current chromatogram at 250 nm of ethanolic extract from *S. campanulata*.

Fifteen chemical components were detected after sample running, but among them, only 9 compounds were tentatively identified, mainly glycosylated flavones, glycosylated flavonols and alkaloids (Table 2). Below is the detailed explanation and identification of the compounds using UHPLC and DAD-ESI and MSⁿ analysis.

TABLE 2
HPLC-DAD-ESI-MSⁿ DATA OF *S. CAMPANULATA* ETHANOLIC EXTRACTS

Peak No.	Retention time	UV Max	[M-H]	Other ions	Identification
1	0.61	322, 208	—	—	Unknown
2	0.74	296, 206	—	—	Unknown
3	1.02	218, 258	—	—	Unknown
4	1.22	326, 301, 247	—	—	Unknown
5	1.54	256	—	—	Unknown
6	1.77	318, 246, 222	—	—	Unknown
7	3.62	260	481	319, 205	Flavonoid glucoside
8	3.78	327, 245	523	361, 179, 163	Flavonoid glucoside
9	4.31	315, 247	523	361, 179, 163	Flavonoid glucoside
10	4.14	—	593	327, 285, 257	Flavonoid glucoside
11	4.54	—	507	345, 231, 163	Flavonoid glucoside
12	5.02	—	507	345, 231, 163	Flavonoid glucoside
13	6.65	—	582	462, 342	Alkaloid
14	6.88	—	582	462, 342	Alkaloid
15	7.09	—	612	492, 466 (462), 342	Alkaloid

**According to the data reported by the technician.*

3.2 Flavonoids and Derivatives

Explanation was done taking into account the retention times, spectral data and the grouping order in which the phytochemicals were reported by the technician. The three first chemical components were registered at 3.66; 3.81 and 4.35 minutes of retention time, respectively, as is showed in Figure 4, two of them with a molecular mass of 523 Da, but with different retention times, at 3.81 and 4.35 min, respectively, indicating that both chemical components are closely related or perhaps they are isomers.

The first compound detected at 3.66 min have a molecular mass of 481 Da, having a similar molecular mass of flavonoids related with some of the four gossypetin glycosylated derivatives isolated and characterized from spices that belong to *Hibiscus* and *Talipariti* genders, but the confirmation is still pendant by NMR spectroscopy (data not shown here). We are assuming that only two aglycon moieties in nature have a MM of 318, there are gossypetin and hibiscetin. The first one is a flavonol and the second one a flavone.

As is known, four gossypetin glucosides have been isolated and characterized from different plants like *Hibiscus vitifolius* and *Hibiscus sabdariffa* L. (Gossypetin-8-O-glucoside or gossypin); from *Hibiscus sabdariffa* L., *Talipariti elatum* (Sw.) and *Talipariti tiliaceum* (Sw.) (Gossypetin-7-O-glucoside or gossypitrin or gossypetrin); from *Hibiscus sabdariffa* L. and also from *Talipariti tiliaceum* (Sw.) (Gossypetin-3-O-glucoside or gossytrin) and finally from *Abelmoschus manihot* (initially *Hibiscus manihot*) and *T. elatum* (Sw.) in Martinica and Cuba (Gossypetin-3'-O-glucoside) (11, 12, 13).

D:\ESI-MASAS\RESULTADOS\Pilar\P4pilar6

19/6/2018 11:20:04

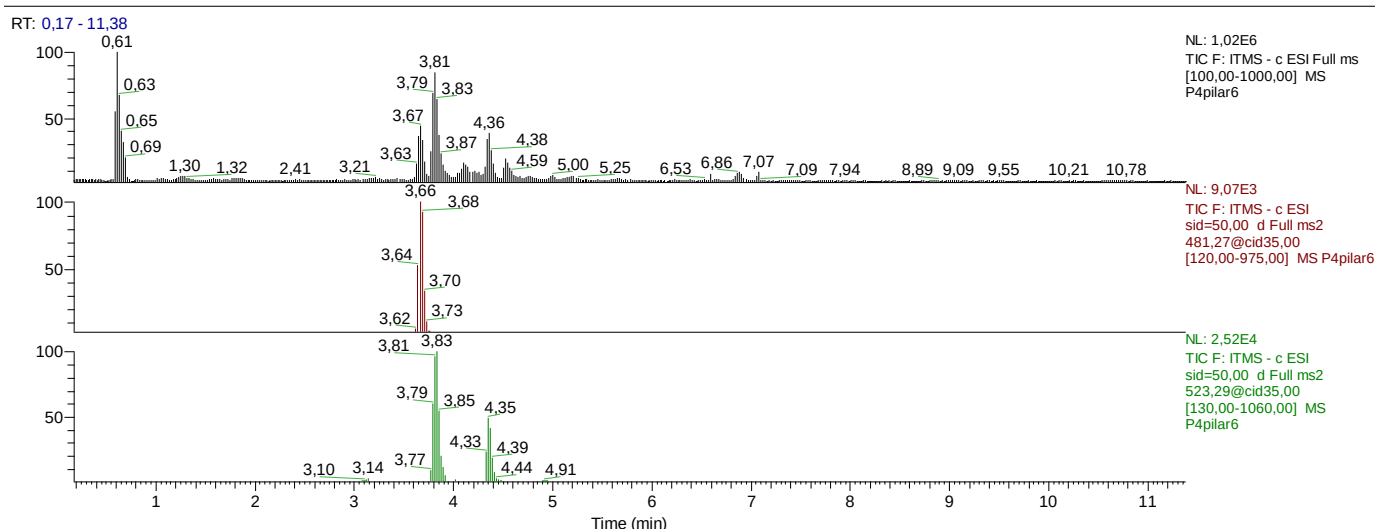


FIGURE 4: Full mass chromatogram of the three first chemical components registered.

Beside the retention time, both chemical compounds with the same molecular masses of 523 Da, showed different fragmentation pathway suggesting the presence of two isomeric forms from the same chemical component as is represented in Figure 5.

D:\ESI-MASAS\RESULTADOS\Pilar\P4pilar6

19/6/2018 11:20:04

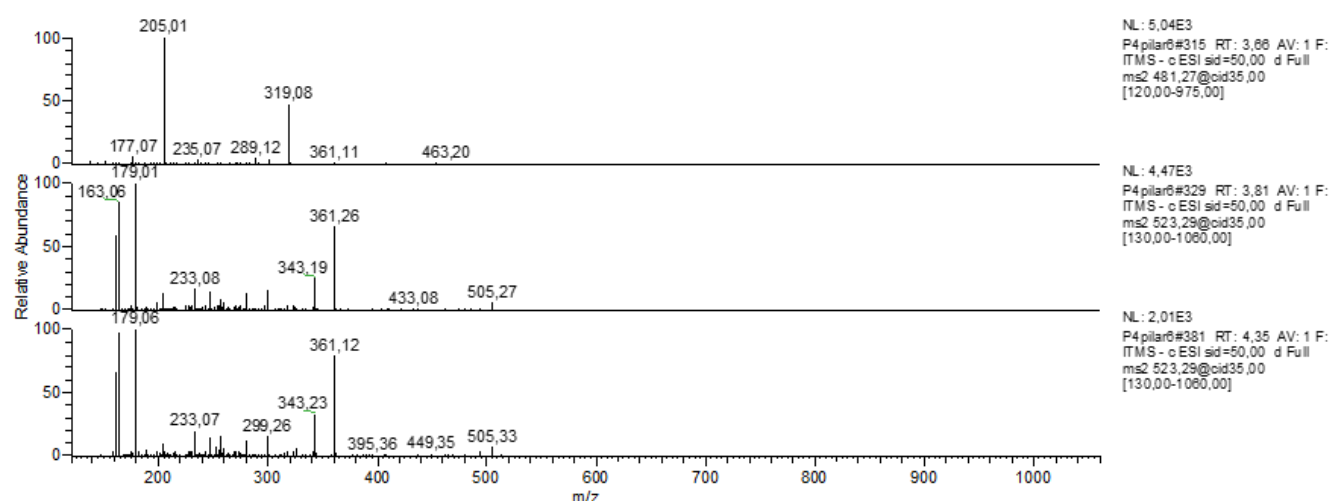


FIGURE 5: MS/MS spectrums of the three first chemical components registered.

Both compounds with MM of 507 amu have a little different behavior in their fragmentation pathway, but with the same daughter ions at m/z 345, 231 and 163. Seems to indicate that they are glucoside derivatives from 5,7-dihydroxy-3',4',5'-trimethoxyflavone with m/z 344 and [M-H]⁻ 343, with the glucose moiety in C8 in both isomeric forms: α and β . So, we are

proposing the structure of 8- α -D-glucopiranosyl-5,7-dihydroxy-3',4',5'-trimethoxyflavone ($[M]^+$ 524; $[M-H]^-$ 523) or instead, the structure of 8- β -D-glucopiranosyl-5,7-dihydroxy-3',4',5'-trimethoxyflavone ($[M]^+$ 524; $[M-H]^-$ 523).

Three second chemical components were then registered between 4.14 and 5.02 min, keeping the same behavior like previous case, the first one of them at 4.14 min with a molecular mass of 593 Da, following by another two compounds at 4.52 and 5.02 min, respectively, but with the same molecular masses of 507 Da and different fragmentation pathway, so we can consider that both phytochemicals are isomers too (Figures 6 and 7).

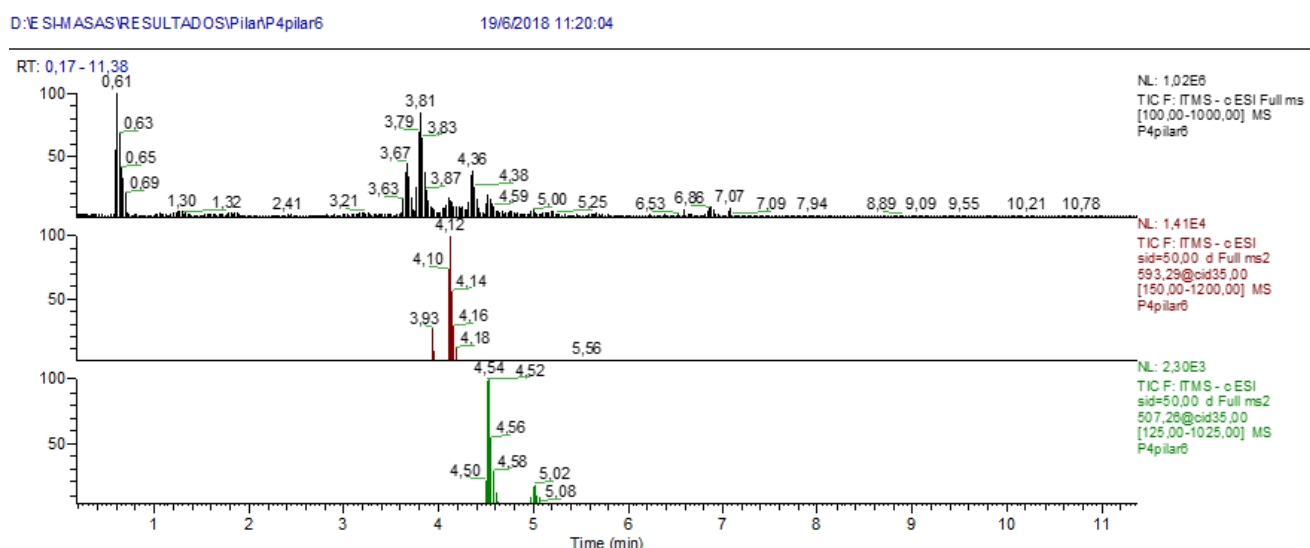


FIGURE 6: Full mass chromatogram of the three second chemical components registered.

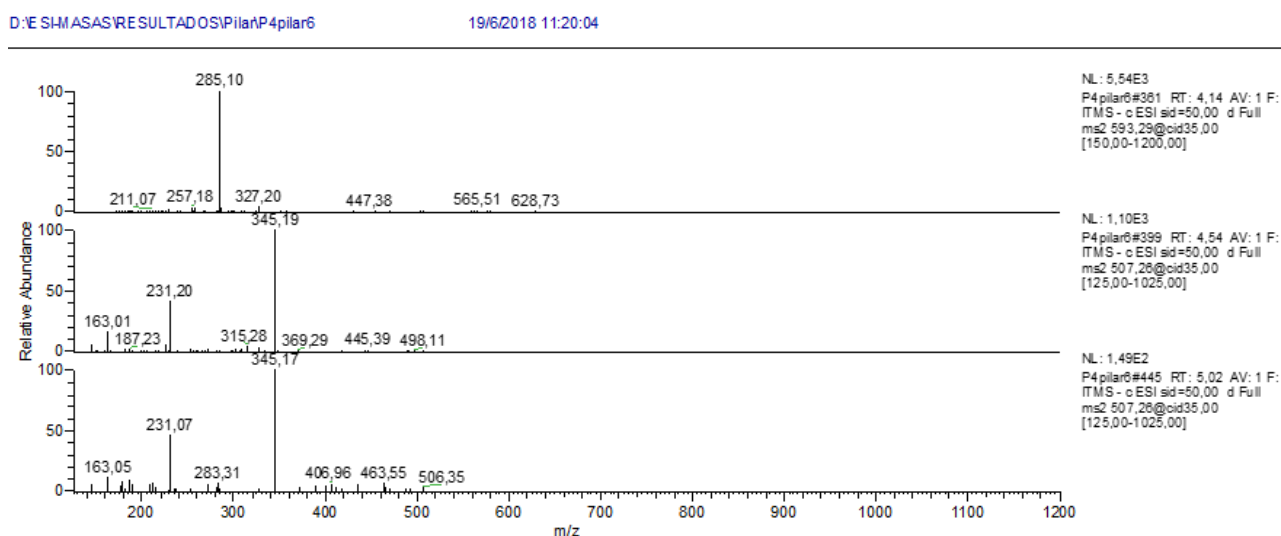


FIGURE 7: MS/MS spectra of the three second chemical components registered.

Four different secondary metabolites in nature produce a parent ion at m/z 593: luteolin-7-*O*-rutinose, kaempferol-3-*O*-rutoside, kaempferol-3(*p*-coumarylglucoside) and glycosylated sitosterol, this one, identified in the bark and flowers extracts of *S. campanulata* by Heim et al., 2012. According to Rodriguez et al., 2009 [14], we are tentatively proposing the structure of kaempferol-3(*p*-coumarylglucoside) for the component at 4.14 min of retention time, because of its present the same fragmentation pathway of that phytocompound analyzed and daughter ions at m/z 447 and m/z 285 $[M-H-coumarylglucoside]^-$.

Both chemical components with parent ion at m/z 507 are closely related with 3-hydroxy-4',5,7- trimethoxyflavone that have a parent ion at m/z 328 to be the precursor moiety to those compounds. In such case, both structures keeping the stereochemical configuration α or β , but in this case, having one OH group attached at the flavone moiety. They presented a pseudomolecular ion at m/z 507, which experienced a hexoside loss (162 u) to produce a flavone ion at m/z 327 and, thus, was identified as 8- α -D-*O*-glucosyl-3-hydroxy-4',5,7-trimethoxyflavone (or its isomeric form β).

3.3 Alkaloids

Finally, the three third chemical components were detected at 6.65; 6.88 and 7.09 minutes, respectively. The first and second one with a molecular mass of 582 Da, probably two isomeric forms of the same phytochemical compound, and the third one with a molecular mass of 612 Da (Figures 8 and 9).

Obviously, those three phytocompounds perhaps belong to alkaloids group due to their molecular masses, according to Brindha et al., 2012, who previously reported that preliminary phytochemical screening of *Spathodea campanulata* P. Beauvais revealed the presence of alkaloids.

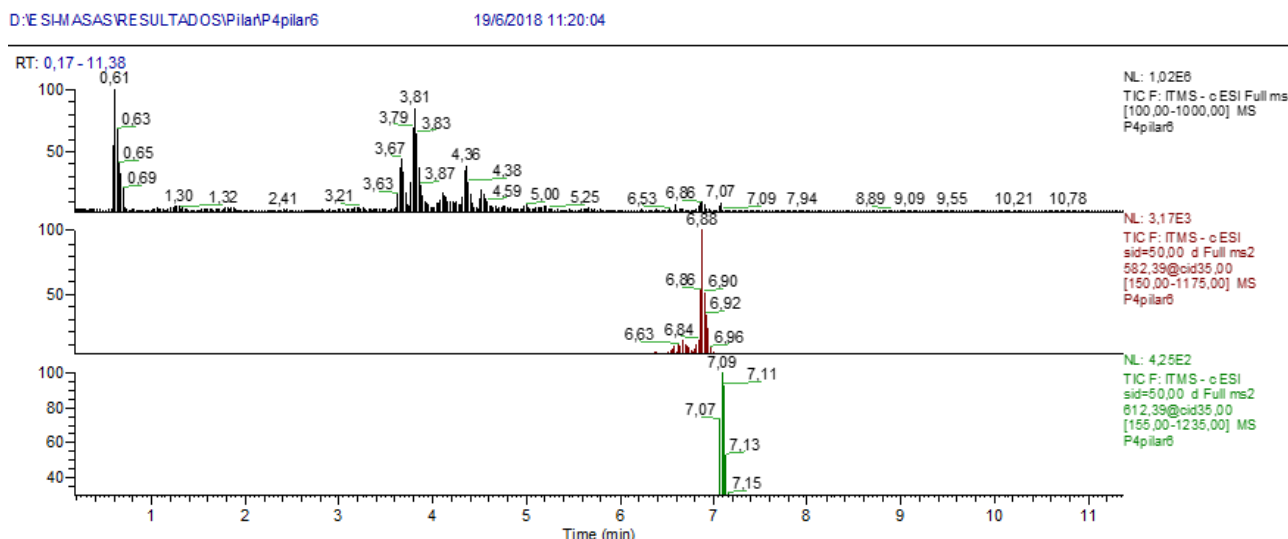


FIGURE 8: Full mass chromatogram of the three third chemical components registered.

Compounds with pseudomolecular mass at m/z 582, producing daughter ions at m/z 462 and 342, exhibit the same molecular formula $C_{35}H_{38}N_2O_6$, $[M]^+$ m/z 582. They both carry a secondary amino function. According to Fournet et al., 1987 [15], two *bis*-benzylisoquinolines were isolated and characterized from *Abuta pahni*, belonging to the genus *Abuta* (Menispermaceae, Anomospermae) spreads widely throughout tropical America. *A. pahni* is part of Amazonian curare mixtures.

The parent ion at m/z 582 are in correspondence with those both chemical components found out in this research but more experiments will be needed to confirm their presence in ethanolic extracts from the flowers of *S. campanulata* in our country.

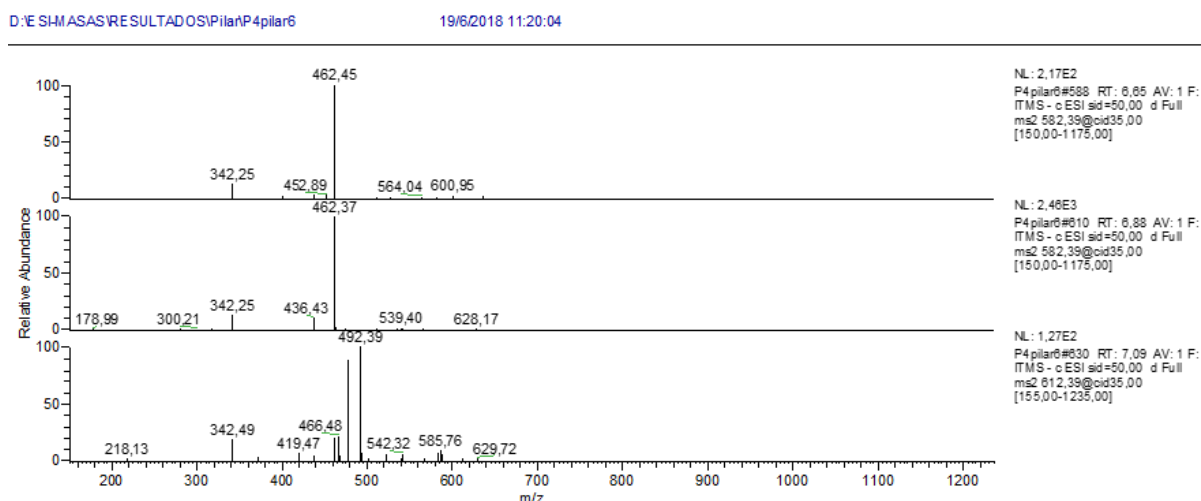
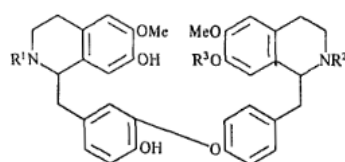


FIGURE 9: MS/MS spectrums of the three third chemical components registered.

Figure 10 shows the structures of both *bis*-benzylisoquinolines. They are in correspondence with structures 4 and 5, identified by comparison with authentic samples and the respective positions of the secondary amino and tertiary amino groups in alkaloid 4 are determined through NOE measurements (cf. values in the Experimental). It can then be given the structure 2-*N*-methyllindoldhamine. Consequently, alkaloid 5 corresponds to 2'-*N*-methyllindoldhamine.



- 1 $R^1 = \text{Me}, R^2 = \text{Me}, R^3 = \text{Me}$
 2 $R^1 = \text{Me}, R^2 = \text{H}, R^3 = \text{Me}$
 3 $R^1 = \text{H}, R^2 = \text{H}, R^3 = \text{H}$
 4 $R^1 = \text{Me}, R^2 = \text{H}, R^3 = \text{H}$
 5 $R^1 = \text{H}, R^2 = \text{Me}, R^3 = \text{H}$
 6 $R^1 = \text{Me}, R^2 = \text{Me}, R^3 = \text{H}$

FIGURE 10: Structural representation of bis-benzylisoquinolines from *Abuta pahni*.

Surprisingly, another two chemical components belonging to alkaloid group are in correspondence of those molecular masses: ergotamine (m/z 582) and ergocryptine (m/z 610). They were found in Ergot (*Claviceps purpurea*). Ergot alkaloids are mycotoxins generated by grass and grain pathogens such as *Claviceps*, for example. Ergot alkaloid-poisoning syndromes, such as tall fescue toxicosis from endophyte-infected tall fescue grass, are important veterinary problems for cattle, horses, sheep, pigs and chickens, with consequent impact on food, meat and dairy industries. Damage to livestock is of the order of a billion dollars a year in the United States alone (Lehner et al., 2005 [16]).

HPLC with UV and fluorescence detection are the predominant means of ergot alkaloid. Lehner et al., 2005, demonstrated the facility of using electrospray(+) mass spectrometry with multiple reaction monitoring (MRM) detection during chromatographic examination of ergot alkaloid standards of lysergic acid, lysergol, ergonovine, ergovaline, ergotamine, ergocornine, ergocryptine and ergocryptine by HPLC.

Figure 11 shows the general structure of Ergot alkaloids. The ergocryptine dehydrate was studied with settings similar to those of parental ergocryptine.

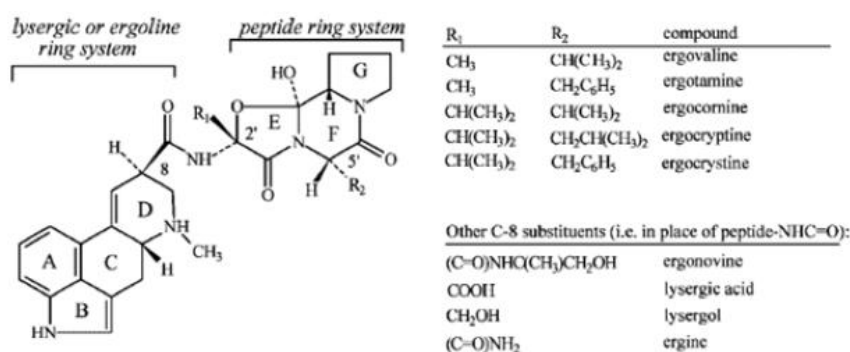
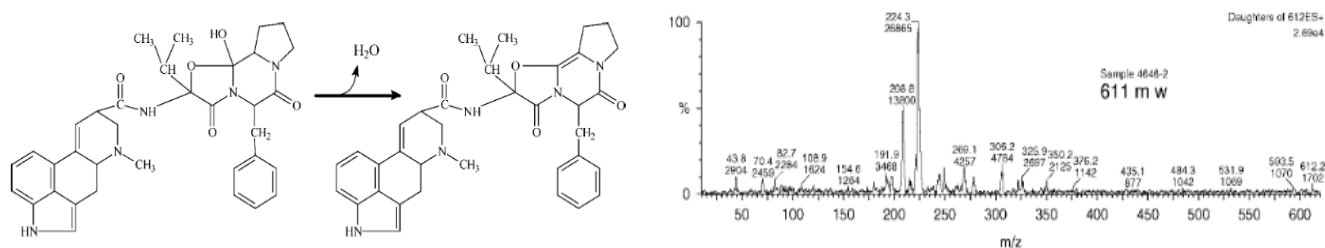


FIGURE 11: Ergot alkaloid general structure, including stereochemistry and crucial position numbering and ring labeling (rings A–D constitute lysergic or ergoline; E–G, peptide ring systems). R_1 and R_2 alkyl and aralkyl substituents are listed in tabular form with corresponding compound names (taken from Lehner et al., 2005).

According with that, our results are in concordance with those authors when explained the presence of ergocryptine (m/z 612) and its dehydrated form (dyhydroergocryptine, m/z 592) due to the loss of 18 u.m.a. [M-18] (Scheme 1).



SCHEME 1: Dehydration of the ergocryptine 12'-hydroxyl group in the peptide ring system (from Lehner et al., 2005)

Mass spectrum of chemical compound detected at 7.07 min shows that the phytochemical identified in *S. campanulata* in Cuba have the same fragmentation pathway of that one found in perennial ryegrass (*Lolium perenne*) and tall fescue grass (*Festuca arundinacea* Schreb) (Figure 12). For that reason, we are proposing the structure of ergocryptine that showed a $[M - H]^-$ ion at m/z 612 and MS^1 ion at m/z 592 ($[M - H - H_2O]^+$) that belongs to the dehydrated product dyhydroergocryptine.

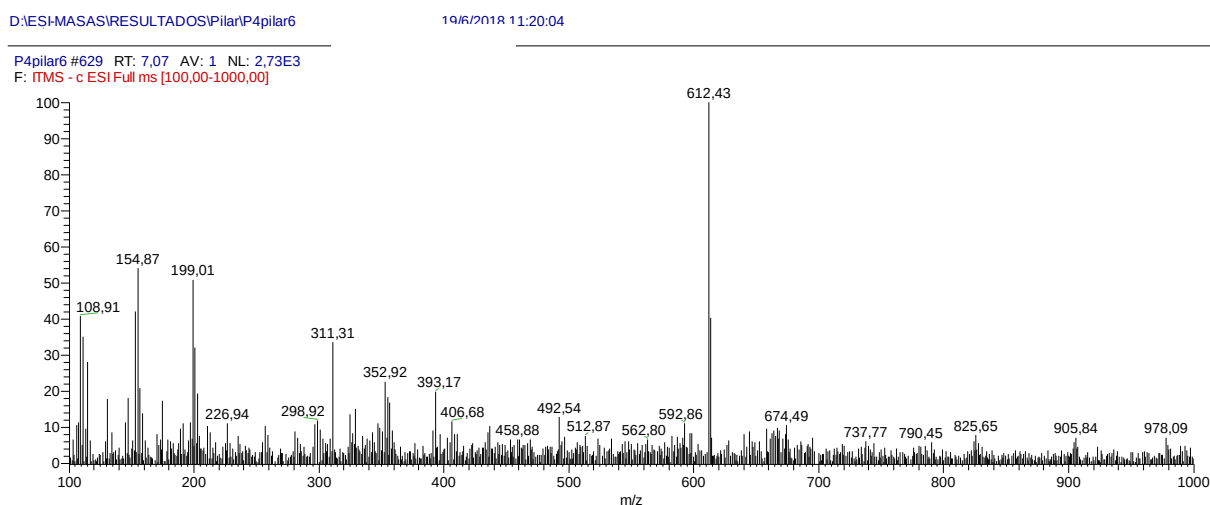


FIGURE 12: Full mass spectrum of compound with m/z 612 at 7.07 min.

Probably, degradation products in the spectrometric measurement carry out to obtain another ergot alkaloid component: ergocornine, after the loss of 30 u.m.a ($[M-30]^+$, m/z 562). Those losses are typically of alkaloids (NH_2CH , CH_2O , NO). Obviously, parent ion at m/z 582 $[M-H]^-$ could be assigned to 9,10-dyhydroergotamine or ergotamine (m/z 581), and consequently, the peak at m/z 462 correspond to ergocornine after the same loss of 30 u.m.a.

Summarizing, Table 3 relate the chemical compounds proposed to be present into ethanolic extracts of flowers from *S. campanulata* in our country. Further experiments will be done to increase the information about the existence of such phytochemicals in ethanolic extracts of the flowers of this plant, including the use of NMR spectroscopy.

TABLE 3
PROPOSAL COMPONENTS IN ETHANOLIC EXTRACTS OF THE FLOWER FROM *S. CAMPANULATA*

Compound	Ret. time	Parent ion	Daughter ions	Identification
1	3.62	481	319, 205	Gossypetin glucosides derivatives or Hibiscetin-3-O-rhamnopyranoside*
2	3.78	523	361, 179, 163	8- α -D-glucopyranosyl-5,7-dihydroxy-3',4',5'-trimethoxyflavone
3	4.31	523	361, 179, 163	8- β -D-glucopyranosyl-5,7-dihydroxy-3',4',5'-trimethoxyflavone
4	4.14	593	327, 285, 257	Kaempferol-3-(<i>p</i> -coumaroylglucoside)
5	4.54	507	345, 231, 163	8- α -D-glucopyranosyl-3-hydroxy-4',5,7-trimethoxyflavone
6	5.02	507	345, 231, 163	8- β -D-glucopyranosyl-3-hydroxy-4',5,7-trimethoxyflavone
7	6.65	582	462, 342	2-N-methylindolamine *9,10-dyhydroergotamine
8	6.88	582	462,342	2'-N-methylindolamine *9,10-dyhydroergotamine
9	7.09	612	592, 562, 466, 342	Ergocryptine

*more probably expected.

IV. CONCLUSIONS

The present study point outs the essentiality of collecting similar data for different plants and their flowers, as well as other parts. For the first time in our country nine different chemical compounds were tentatively identified using UHPLC-DAD-ESI-MS/MS experiments. Six of those components belong to flavonoid group (flavones and flavonol subgroup) and 3 of them to alkaloid group. All stretching related with biological activities associated to this part of the plant (antioxidant, anti-

solar, diuretic and anti-inflammatory activity). Further investigation to explore the therapeutic action of the individual phytochemicals and their mechanism of action can be encouraged.

CONFLICT OF INTEREST

Authors declare that we have not conflict of interest.

REFERENCES

- [1] Bosch CH. *Spathodea campanulata* P. Beauv. [Internet] Record from PROTA4U. Oyen, L. P. A. and Lemmens, R. H. M. J. (Editors); 2002.
- [2] Spangler RE, Olmstead RG. Phylogenetic analysis of Bignoniaceae based on the cp DNA gene sequences *rbcl* and *ndhF*. *Ann Mo Bot Gard* 1999; 86:33-46.
- [3] Mensah AY, Houghton PJ, Dickson RA, Fleischer TC, Heinrich M, Bremner P. In vitro evaluation of effects of two Ghanaian plants relevant to wound healing. *Phytother Res* 2006; 20:941-4.
- [4] Ngouela S, Tsamo E, Sondengam BL. Extractives from Bignoniaceae: constituents of the stem bark of *Spathodea campanulata*. *Planta Med* 1988; 54:476.
- [5] Niyonzima G, Laekernan G, Witvrouw M, Van Poel B, Pieters L. Hypoglycemic, anti-complement and anti-HIV activities of *Spathodea campanulata* stem bark. *Phytomedicine* 1999; 6:45-9.
- [6] Parul T, Aditi S. Natural resources from plants in the treatment of cancer: an update. *Asian J Pharm Clin Res* 2017; 10:13-22.
- [7] Brindha P, Nagarajan A, Saralla RP, R Narendran R, Sridharan K. A study on chemical and botanical standards of a traditional drug source *Spathodea campanulata* Beauv. *Int J Pharm Sci* 2012; 4:157-60.
- [8] Kumaresan M, Palanisamy PN, Kumar PE. Chemical investigation of the flower of *Spathodea campanulata* by GCMS. *J Nat Prod Plant Resour* 2011; 1:14-7.
- [9] Naglaa GS, Hanaa HE, Soheir M. Bioactivity and composition of the flowers of *Spathodea campanulata* p. Beauv. *World J Pharm Res*, 2014;3:213-30.
- [10] Heim, S.C., Guarnier, F.A., Ferreira, D.T., Braz-Filho, R., Cecchini, R., Cecchini, A.L. Antioxidant activity of *Spathodea campanulata* (Bignoniaceae) extracts. *Rev. Bras. Pl. Med.*, v.14, n.2, 2012;14:287-92.
- [11] Yaque, J.G., Cuéllar, A., Massi, L., Monan, M., Nossin, E. and François-Haugrin, F. (2016) Isolation and Characterization of Flavonols by HPLC-UV-ESI-MS/MS from *Talipariti elatum* S.w. *American Journal of Plant Sciences*, 7, 1198-1204. <http://dx.doi.org/10.4236/ajps.2016.78115>.
- [12] François-Haugrin F., Monan M., Nossin E., Smith-Ravin J. and Odile Marcelin. Antioxidant activity of an isomer of gossypitrin (gossypetin-3'-O-glucoside) isolated in the petals of *Talipariti elatum* Sw., and determination of total phenolic content of the total flower. *Journal of Pharmacognosy and Phytochemistry* 2016; 5(5): 200-208.
- [13] Yaque, J.G., Cuéllar, A., Gaysinski, M., Monan, M., Nossin, E. and François-Haugrin, F. (2016) New Reported Flavonol Characterized by NMR from the Petals of *Talipariti elatum* S. w. in Cuba. *American Journal of Plant Sciences*, 7, 1564-1569. <http://dx.doi.org/10.4236/ajps.2016.711148>.
- [14] Rodriguez I. C. et al. Medina Direct characterization of aqueous extract of *Hibiscus sabdariffa* using HPLC with diode array detection coupled to ESI and ion trap. *MS J. Sep. Sci.* 2009, 32, 3441-3448.
- [15] Pascale D., Weber J-F., Fournet A., Cavé A., and Brutenon J. Bis-benzylisoquinolines alkaloids from *Abuta pahnii*. *Phytochemistry*, Vol. 26, No. 7, pp. 2136-2137, 1987.
- [16] Lehner F. A., Craig M., Fannin N., Bush L., and Tobin T. Electrospray [+] tandem quadrupole mass spectrometry in the elucidation of ergot alkaloids chromatographed by HPLC: screening of grass or forage samples for novel toxic compounds. *J. Mass Spectrom.* 2005; 40: 1484-1502.