



Revista Colombiana de Química
ISSN: 0120-2804
ISSN: 2357-3791
rcolquim_fcbog@unal.edu.co
Universidad Nacional de Colombia
Colombia

Emetine and cephaeline content in plants of *Psychotria ipecacuanha* in Costa Rica

Rosales- López, Catalina; Muñoz-Arrieta, Rodrigo; Abdelnour-Esquivel, Ana
Emetine and cephaeline content in plants of *Psychotria ipecacuanha* in Costa Rica
Revista Colombiana de Química, vol. 49, no. 2, 2020
Universidad Nacional de Colombia, Colombia
Available in: <https://www.redalyc.org/articulo.oa?id=309063462003>



This work is licensed under Creative Commons Attribution 4.0 International.

Emetine and cephaeline content in plants of *Psychotria ipecacuanha* in Costa Rica

Contenido de emetina y cefaelina en plantas de *Psychotria ipecacuanha* en Costa Rica

Emetina e conteúdo de cephaelina em plantas de *Psychotria ipecacuanha* na Costa Rica

Catalina Rosales- López

Instituto Tecnológico de Costa Rica, Costa Rica

crosales@itcr.ac.cr

Redalyc: [https://www.redalyc.org/articulo.oa?](https://www.redalyc.org/articulo.oa?id=309063462003)

id=309063462003

Rodrigo Muñoz-Arrieta

Centro Nacional de Alta Tecnología, Costa Rica

Ana Abdelnour-Esquivel

Instituto Tecnológico de Costa Rica, Costa Rica

Received: 08 March 2019

Accepted: 13 March 2020

ABSTRACT:

The objective of this study was to identify the emetic metabolites in different parts of the *P. ipecacuanha*, a plant with emetic properties. Partial phytochemical analysis was performed to determine the presence of emetine and cephaeline in leaves, stems and roots. Both alkaloids were detected in the three plant parts analyzed. Highest alkaloid content was found in roots (8.55 mg/g), followed by stems (4.05 mg/g), and the lowest was found in leaves (2.4 mg/g). The cephaeline content (8.35 mg/g) was higher than that of emetine (6.65 mg/g) in all the three organs analyzed. Toxicity analysis of the crude extract showed a LD₅₀ of 500 mg/kg.

KEYWORDS: Second metabolites, ipecac, emetic, chemical analysis, HPLC, TLC.

RESUMEN:

El objetivo de este estudio fue identificar los metabolitos eméticos en diferentes partes de *P. ipecacuanha*, una planta con propiedades eméticas. Se realizó un análisis fitoquímico parcial, donde se determinó la presencia de emetina y cefalina en hojas, tallos y raíces. Ambos alcaloides se detectaron en las tres partes de la planta analizadas. El mayor contenido de alcaloides se encontró en las raíces (8.55 mg/g), seguido de los tallos (4.05 mg/g), y el más bajo se encontró en las hojas (2.4 mg/g). El contenido de cefalina (8.35 mg/g) fue mayor que el de emetina (6.65 mg/g) en los tres órganos analizados. El análisis de toxicidad del extracto crudo mostró una DL₅₀ de 500 mg/kg.

PALABRAS CLAVE: metabolitos secundarios, ipecacuana, emético, análisis químico, HPLC, TLC.

RESUMO:

O objetivo deste estudo foi identificar os metabólitos eméticos em diferentes partes do *P. ipecacuanha*, uma planta com propriedades eméticas. Foi realizada análise fitoquímica parcial para determinar a presença de emetina e cefalina nas folhas, caules e raízes. Ambos os alcalóides foram detectados nas três partes da planta analisadas. O maior teor de alcalóides foi encontrado nas raízes (8,55 mg/g), seguido dos caules (4,05 mg/g), e o menor foi encontrado nas folhas (2,4 mg/g). O conteúdo de cephaelina (8,35 mg/g) foi superior ao de emetina (6,65 mg/g) nos três órgãos analisados. A análise de toxicidade do extrato bruto mostrou um LD₅₀ de 500 mg/kg.

PALAVRAS-CHAVE: metabolitos secundários, ipecacuana, emética, análise química, HPLC, TLC.

AUTHOR NOTES

crosales@itcr.ac.cr

INTRODUCTION

Plants synthesize secondary metabolites that can be isolated and used for medicinal, industrial, cosmetic, nutritional, and other purposes [1]. *Psychotria ipecacuanha* (or *Carapichea ipecacuanha*) is native to Central and South America. It is known as “raicilla” in Costa Rica and Nicaragua, and as “ipecacuana” in the international market. It is an herbaceous plant with a thin, twisted semi-woody stem that is 20 to 30 cm in length [2] (Figure 1). In Costa Rica, *Psychotria ipecacuanha* is of great economic importance. It was the first medicinal plant from the tropical forest to be exploited rationally in Costa Rica [2]. It is grown in the Huetar Norte region, which borders Nicaragua. This species is critically endangered due to both over-exploitation since it is used in traditional medicine, and the negative effect caused by climate change on its survival [3]. It is produced in commercial plantations in the Huetar Norte region of Costa Rica for export to countries in Asia [2]. Roots are known to have various medicinal properties (emetic, expectorant, and amoebicidal) [5] due to the presence of bioactive compounds, particularly isoquinolic alkaloids (bis-isoquinolines). These molecules have a monoterpenoid-tetrahydroisoquinoline skeleton and are formed by the condensation of dopamine and secologanin [6] with conversion to cephaeline and emetine [5].



FIGURE 1.

Ipecacuana plants collected in northern Costa Rica. Roots are exported to China.

Cephaeline is twice as potent as emetine. Structural differences between the two compounds are due to the additional methoxyl group in emetine (emetine has two isoquinoline nuclei and four methoxyl groups, while cephaeline has three methoxyl groups and one free OH). Both alkaloids exert a direct irritant action on the gastric mucosa and generally cause vomiting within 30 min of administration of ipecacuana to the patient [2]. They also inhibit the synthesis and activity of proteins, ribosomal, and mitochondrial DNA and RNA, as shown in mammalian, yeast, and plant cells [4]. Recently, emetine was reported to have significant antiviral activity against the dengue virus [3]. Typically, only the roots are used as raw material for the international pharmaceutical industry and the entire plant is destroyed at harvest [2, 4]. Based on this commercial practice, the objective of this study was to evaluate the phytochemical profiles of emetic alkaloids in whole plants of *Psychotria ipecacuanha* grown in Costa Rica in order to prevent the destruction of plantations.

MATERIALS AND METHODS

Plant material

Plants were collected in a commercial plantation in Moravia de Cutris in San Carlos in northern Costa Rica (10° 25' 00" N 84° 34' 00" W). Entire plants approximately one year old were collected randomly. Plants were washed, and leaves, stems and roots were separated. Plant material was dried in a convection oven (Digisystem, model DSI-300D, Taiwan) at 50 °C for 48 h, then ground in a laboratory mortar and sifted with #18 mesh.

Extraction and preparation

Different extraction procedures were evaluated using leaves, stems, and roots. Dried ground plant material (1.0 g) of each plant organ was used. The following solvents were evaluated for extraction: Ethanol 70% (*v/v*) (J. T. Baker, Spain), methanol (J. T. Baker, Spain), acetone (Merck, United States), ethyl ether (Sigma Aldrich, United States) and hexane (Merck, United States). A volume of 15 mL of each solvent was used. Extraction techniques included vortex agitation, maceration without agitation, maceration with agitation, and ultrasonic bath. Extracts were filtered and concentrated by rota-evaporation (Buchi, Switzerland) to eliminate solvents prior to the evaluation of metabolites in samples of each organ type.

Identification of emetic metabolites

Dragendorff test

For the qualitative identification of alkaloids, extracts were subjected to a rapid chemical test using the Dragendorff reagent as described by Dominguez (1973) [7]. Samples of dried plant material (0.1 g) were placed in test tubes prepared in triplicate. Five drops of 5% (*v/v*) HCl were added to each sample so that the entire sample was in contact with the acid solution. Samples were maintained at 50 °C in a water bath for 5 min, and then filtered. The filtrate (acid) was recovered in another test tube and one drop of Dragendorff reagent was added [$\text{Bi}(\text{NO}_3)_3$ in HNO_3 -KI in distilled water]. The formation of a precipitate or turbidity indicated the presence of alkaloids.

Thin layer chromatography (TLC)

Extracts were separated on silica gel plates (60 F254, Merck). The mobile phase consisted of chloroform : methanol : 10% ammonium hydroxide 100:10:1 [8]. Chromatographs were observed under ultraviolet light (254 nm and 366 nm), and then stained with Dragendorff reagent.

High-Performance Liquid Chromatography (HPLC)

Alkaloids were quantified by HPLC using the method described by Han et al. (2013) [9]. 3.0 mL of aqueous 70% (*v/v*) methanol and 0.5 mL of 0.1 M NaOH were added to 100 mg of powdered plant material. Samples were incubated in an ultrasonic bath at 25 °C for 10 min, then centrifuged at 1840 rpm for 10 min, and the supernatant was collected. The process was repeated two more times. The three extracts obtained were combined in a volumetric flask and the volume was brought to 10 mL with the extraction solution. Two emetic standards were used for identification and quantification: Emetine hydrochloride (purity > 99.5%, lot No.061M1826V) and cephaeline hydrochloride (purity > 97.8%) from Sigma Aldrich. The reference substances were dissolved in methanol, and then diluted quantitatively with the mobile phase to obtain 10 µg/mL emetine hydrochloride and 10 µg/mL cephaeline hydrochloride. A high-performance liquid chromatograph (Shimadzu, Japan, USA) equipped with a diode array detector (DAD) and a 150 m, 4.6 mm, and 5 mm particle size Gemini-NX C18 column was used (Phenomenex, USA). Mobile phase consisted of methanol:acetonitrile:0.1% phosphoric acid 9:3:88 for an isocratic elution at a flow rate of 1.0 mL/min. During all the runs both the oven temperature (40 ± 1 °C) and the wavelength detection (245 nm) were kept constant. The injection volume was 10 µL. Emetine and cephaeline present in the samples were identified by

comparing retention times with known standards. The concentration of each alkaloid was estimated using calibration curves from 0.005 to 0.03 mg/mL.

Toxicity Analysis

The median lethal dose (LD_{50}) was determined for each test substance in Hsd: Sprague-Dawley® SD® rats, in compliance with OECD Test Guideline 423: Acute Oral Toxicity–Acute Toxic Class Method. There were no deviations from the protocol that would affect test quality or results. All procedures used in the study were in compliance with Costa Rican Animal Welfare Law N° 7451 (<https://www.mep.go.cr/sites/default/files/page/adjuntos/ley-no-7451-bienestar-animal.pdf>).

Statistical Analysis

The results obtained were analyzed using the Kruskal-Wallis test, where the medians were compared, with a level of significance of $\alpha \leq 0.05$ in the Minitab 17 software.

RESULTS AND DISCUSSION

The extraction method with the highest yield was the ultrasonic bath using 70% ethanol as solvent. This method is commonly used and is inexpensive. The vibrations penetrate the plant cells and achieve efficient cell disruption. Moreover, all the methods using agitation had higher yields compared with the extraction by maceration without agitation. Agitation shifts the equilibrium towards solvent saturation and increases the surface contact between solute and solvent, thereby increasing extraction efficiency [10].

The formation of an orange precipitate upon addition of the Dragendorff reagent revealed the presence of alkaloids in root, stem and leaf samples. The presence of emetine and cephaeline in all parts of the *P. ipecacuanha* plant was confirmed by TLC. The compounds were identified making a comparison with the standards. This technique also allowed the separation of compounds of interest from other compounds present in the samples. The concentration of each compound was estimated based on the intensity of the spots (Figure 2).

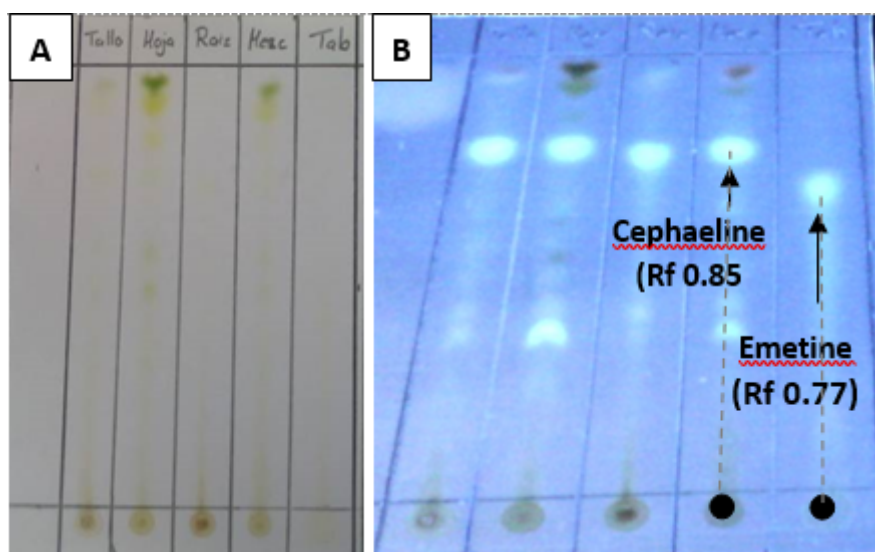


FIGURE 2.

Figure 2. Detection of emetine and cephaeline in the extract of *P. ipecacuanha* plant by Thin Layer Chromatography.

A. Visible light B. UV light. From left to right: stems, leaves, roots, cephaelin standard, and emetine standard.

Visualization of TLC plates under visible and ultraviolet light revealed the presence of emetic alkaloids in roots, stems and leaves (Figure 2, A and B). The concentration of cephaline was higher than that of emetine in all of the sampled plant parts, as evidenced by the more intense staining of the cephaline spots. TLC allowed the qualitative characterization and establishment of a chromatographic profile of the extracts. This technique is used in numerous studies because of its low cost and simplicity [11]. HPLC chromatogram of the standards (Figure 3) was used to identify the cephaeline and emetine retention peaks: 9.0 and 12.0 min respectively. The compounds quantification present in the extracts was more precise (the standard deviations ± 0.09) with HPLC than with TLC (± 0.25). These results coincide with those reported by other researchers, using these same techniques for identification, separation and quantification of relevant compounds [12, 13].

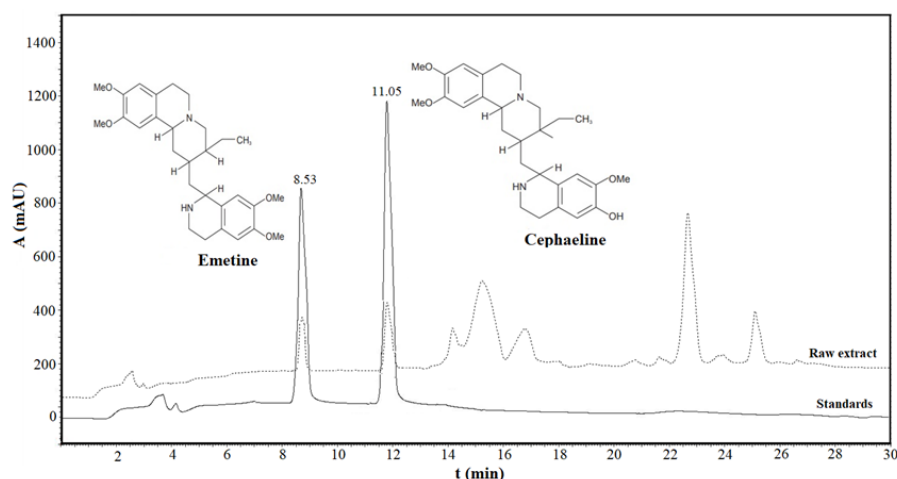


FIGURE 3.

HPLC chromatogram of emetine and cephaeline standards (black uniform line) and chromatogram of the extract (pointed line).

Psychotria spp are known to have high concentrations of indolic alkaloids. This project focused on emetine and cephaeline, the two main alkaloids present in this genus [14]. Regarding the alkaloid concentration in different plant organs, the concentration of both alkaloids, emetine and cephaeline, was higher in roots and

stems and lower in leaves (Tables 1 and 2). Although secondary metabolites accumulate in different organs, accumulation is generally higher in roots and is influenced by climatic conditions at harvest [15, 16].

TABLE 1.

Sample	Concentration (mg/g)	Standard Deviation
Leaves	0.95 b	0.02
Stems	1.80 ab	0.07
Roots	3.9 a	0.85
Total	6.65	0.94

Emetine concentration in different organs of *Psychotria ipecacuanha* (ipecacuana).

*Values are means of 3 replicates.

**Numbers with the same letter are not statistically different.

TABLE 2.

Sample	Concentration (mg/g)	Standard Deviation
Leaves	1.45 b	0.09
Stems	2.25 ab	0.08
Roots	4.65 a	0.1
Total	8.35	0.27

Cephaeline concentration in different organs of *Psychotria ipecacuanha* (ipecacuana).

*Values are means of 3 replicates.

**Numbers with the same letter are not statistically different.

Ocampo reported a 1.7 to 2.3% total alkaloid content in two to three year old ipecacuana plants grown in northern Costa Rica [2]. In this study, emetine represented 60 to 70% of the total alkaloid content and it was the only alkaloid analyzed. In contrast, both emetine and cephaeline were identified in the material collected in the same region in our study; the cephaeline concentration was higher than that of emetine (Figure 4). Other studies carried out in Costa Rica [17] confirm the high genetic variability related to alkaloid content. Differences in alkaloid content related to morphological variations and plant age have also been reported. Genetic variability within the region of origin of Ipecacuana represents an important element for domestication of the species within agroecological systems [17]. The need for high quality raw materials to supply the pharmaceutical industry has created incentives for Ipecacuana production in the region [2].

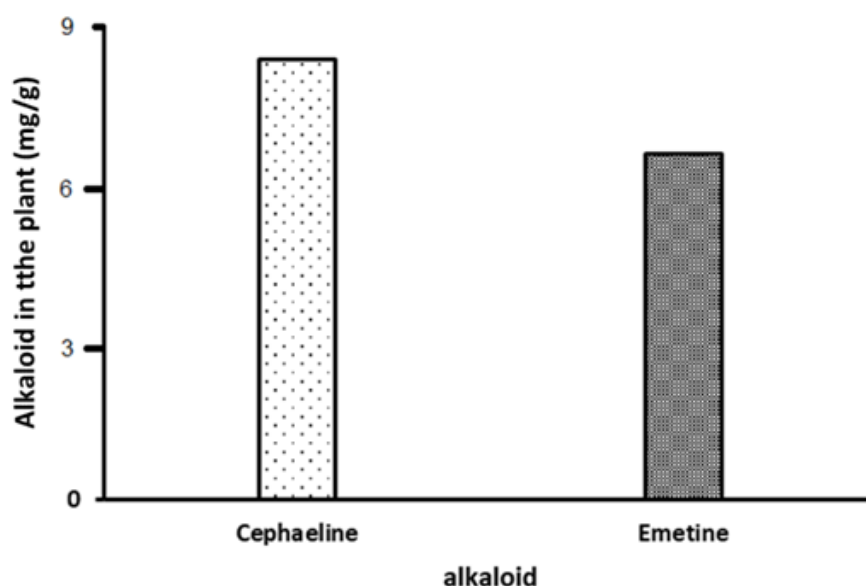


FIGURE 4.

Emetic alkaloid content in plant of *Psychotria ipecacuanha* (ipecacuana).

Although the roots are used for medicinal and commercial purposes, emetic alkaloids are also present in leaves and stems of the Ipecacuana plant. In leaves, the content is higher in younger tissues [18]. These isoquinoline alkaloids are believed to be synthesized in Ipecacuana leaves and accumulate in stems and roots as plant age increases. The content also varies throughout the year [18, 19]. The presence of these emetic alkaloids in leaves, although in a lower concentration than in roots, could be advantageous for growers because the Ipecacuana plant produces abundant foliage and small, thin roots (Figure 1). Emetic alkaloids could be extracted from large quantities of leaves without the need to eliminate entire plants in order to harvest the roots.

Given that alkaloids are secondary metabolites whose toxicity depends on the type and concentration, plant extracts were tested for acute toxicity. In this study, analysis of the crude ipecacuana extract showed that, due to its toxicity (LD_{50} : 500 mg/kg), the daily dose of emetine should not exceed 1.0 g/kg of patient body weight. Reported symptoms in animals exposed to this metabolite include heart, liver, kidney, intestinal tract and skeletal muscle damage, profound keratitis with lymphocyte infiltration in the cornea of dogs and rabbits, and cutaneous irritation in rabbits [20].

CONCLUSIONS

Partial Phytochemical analysis of *Psychotria ipecacuanha* plants grown in northern Costa Rica showed the presence of the alkaloids emetine and cephaeline in leaves, stems, and roots. Although the concentration was greater in roots (4.65 mg/g of cephaeline and 3.9 of emetine), the lush foliage is also an important source of emetic alkaloids (3.7 mg/g of cephaeline and 2.75 of emetine). Therefore, extraction from leaves and stems would be advantageous to avoid the waste of the entire plants in the process.

ACKNOWLEDGMENT

The authors gratefully acknowledge financial support for this project provided by the Office of the Vice Rector for Research of the Instituto Tecnológico de Costa Rica through Grant No.1510092.

REFERENCES

- [1] E. Reyes y J.M. Moreira Castro, "Metabolitos secundarios en plantas medicinales usadas para problemas gastrointestinales. Una revisión sobre medicina ancestral ecuatoriana". Rev. Bases de la Ciencia, vol 2, no. 3, pp.1-16, 2017. DOI: https://doi.org/10.33936/rev_bas_de_la_ciencia.v2i3.1036
- [2] R. Ocampo, "Ipecacuana: Un Producto no maderable cultivado bajo el bosque en Costa Rica en 1980-2000". Agron. Costarricense, vol. 31, no. 1, pp. 113-119, 2007.
- [3] C. Botero, A. Urrea, E. Naranjo, "Potencial de regeneración de *Psychotria ipecacuanha* (Rubiaceae) a partir de capas delgadas de células". Acta Biológica Colombiana, vol. 20, no. 3, pp. 181, 2015. DOI: <https://doi.org/10.15446/abc.v20n3.47354>
- [4] R. M. A. Garcia, L. O. de Oliveira, M. A. Moreira, W. S. Barros, "Variation in emetine and cephaeline contents in roots of wild Ipecac (*Psychotria ipecacuanha*)". Biochemical systematics and ecology, vol. 33, no. 3, pp. 233-243, 2005. DOI: <https://doi.org/10.1016/j.bse.2004.08.005>
- [5] T. Kutchana, and T. Nomuraa, "Three New O-Methyltransferases Are Sufficient for All O-Methylation Reactions of Ipecac Alkaloid Biosynthesis in Root Culture of *Psychotria ipecacuanha*" Journal of Biological Chemistry, vol. 285, no. 10, pp. 7722-7738, 2010. DOI: <https://doi.org/10.1074/jbc.m109.086157>
- [6] T. Nomura, A. L. Quesada, and T. M. Kutchan, "The New β -D-Glucosidase in Terpenoid-Isoquinoline Alkaloid Biosynthesis in *Psychotria ipecacuanha*". Journal of Biological Chemistry, vol. 283, no. 50, pp. 34650–34659, 2008. DOI: <https://doi.org/10.1074/jbc.m806953200>
- [7] X. A. Domínguez, "Métodos de investigación fitoquímica". Editorial Limusa. México, D.F. pp. 81-226, 1973.
- [8] K. Yoshimatsu and K. Shimomura, *Cephaelis ipecacuanha* A. Richard (Brazilian Ipecac): Micropropagation and the Production of Emetine and Cephaeline. In: Bajaj Y.P.S. (eds) Medicinal and Aromatic Plants IV. Biotechnology in Agriculture and Forestry. 1993, vol 21. Springer, Berlin, Heidelberg. DOI: https://doi.org/10.1007/978-3-642-77004-3_6
- [9] G.R. Han, Y.F. Wang, S.H. Feng and Y.X. JIA, "Simultaneous Determination of Cephaeline and Emetine in Ipecac and Its Preparations Using RP-HPLC". Chinese Herbal Medicine, vol. 5, no. 4, pp. 286-291, 2013. DOI: [https://doi.org/10.1016/S1674-6384\(13\)60042-8](https://doi.org/10.1016/S1674-6384(13)60042-8)
- [10] C. L. Rivero, J. Quiñones-Gálvez, A. Martínez, C. Ortiz, M. R. Paneca, G. Valdéz, L. Pérez Gómez, S. La Rosa González, and Y. Ruiz, "Obtención de extractos fenólicos foliares de *Moringa oleifera* Lam mediante el uso de diferentes métodos de extracción". Biotecnología Vegetal, vol. 18, no. 1, pp.47-56, 2018.
- [11] A. D. Talukdar, M. D. Choudhury, M. Chakraborty and B. K. Dutta, "Phytochemical screening and TLC profiling of plant extracts of *Cyathea gigantea* and *Cyathea brunoniana*". Journal of Science and Technology, vol. 5, no. 1, pp. 70-74, 2010.
- [12] V. Devi and K. Mallikarjuna, "Screening and quantification of phytochemicals in leaf extracts of *Schrebera swietenoides* and *Homalium zeylanicum*", Int J Pharm Bio Sci, vol. 6, no. 4, pp. 521 – 529, 2015.
- [13] A. Arulkumar, G. Karthik, S. Paramasivam and M. A. Rabie, "Histamine levels in Indian fish via enzymatic, TLC and HPLC methods during storage". Journal of Food Measurement and Characterization, vol. 11, no. 1, pp. 281-289, 2017. DOI: <https://doi.org/10.1007/s11694-016-9395-z>
- [14] C. Carvalho, and L. de Oliveira, "Ipecac - *Carapichea ipecacuanha*, Genetic Resources, Chromosome Engineering, and Crop Improvement". Medicinal Plants. Boca Raton, USA: CRC Press. pp. 911-922, 2011.
- [15] J. L. Hou, W. D. Li, Q. Y. Zheng, W. Q. Wang, B. Xiao, and D. Xing, "Effect of low light intensity on growth and accumulation of secondary metabolites in roots of *Glycyrrhiza uralensis*". Biochemical Systematics and Ecology, vol. 38, no. 2, pp. 160-168, 2010.
- [16] L. Botha, G. Prinsloo and M. Deutschländer, "Variations in the accumulation of three secondary metabolites in *Euclea undulata* Thunb. var. *myrtina* as a function of seasonal changes". South African Journal of Botany, vol. 117, pp. 34-40, 2018. DOI: <https://doi.org/10.1016/j.sajb.2018.04.016>
- [17] T. Palma, y N. Hidalgo, "Biotecnología: Elemento importante en la domesticación de plantas Medicinales". CATIE, Turrialba, Costa Rica: Informe Técnico, no.245, pp. 17, 1994.

- [18] M. Bernhard, H. Fasshuber, W. Robien, L. Brecker and H. Greger, "Dopamine-iridoid alkaloids in *Carapichea affinis* (*Psychotria borucana*) confirm close relationship to the vomiting root *Ipecac*". *Biochemical systematics and ecology*, vol. 39, no. 3, pp. 232-235, 2011.
- [19] N. Guaycha, C. Jaramillo, S. Cuenca, J. Tocto, y I. Márquez-Hernández, "Estudios farmacognósticos y toxicológicos preliminares de hojas, tallo y raíz de moringa (*Moringa oleifera* Lam.)" *Ciencia Unemi*, vol. 10, no. 22, pp. 60-68, 2017.
- [20] N. Bharti, P. Mohapatra and S. Singh, "Plant-Based Antiamoebic Natural Products: Literature Review and Recent Developments". *Frontiers in Natural Product Chemistry*, vol. 4, no. 4, pp. 1-40, 2018. DOI: <https://doi.org/10.2174/9781681087252118040003>