Article

In Silico Molecular Studies of Antiophidic Properties of the Amazonian Tree *Cordia nodosa* Lam.

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Abstract: We carried out surveys on the use of *Cordia nodosa* Lam. in the jungles of Bobonaza (Ecuador). We documented this knowledge to prevent its loss under the Framework of the Convention on Biological Diversity and the Nagoya Protocol. We conducted bibliographic research and identified quercetin as a significant bioactive molecule. We studied its in silico biological activity. The selected methodology was virtual docking experiments with the proteins responsible for the venomous action of snakes. The molecular structures of quercetin and 21 selected toxins underwent corresponding tests with SwissDock and Chimera software. The results point to support its antiophidic use. They show reasonable geometries and a binding free energy of $-7$ to $-10.03$ kcal/mol. The most favorable values were obtained for the venom of the Asian snake *Naja atra* (5Z2G, $-10.03$ kcal/mol). Good results were also obtained from the venom of the Latin American *Bothrops pirajai* (3CYL, $-9.71$ kcal/mol) and that of Ecuadorian *Bothrops asper* snakes (5TFV, $-9.47$ kcal/mol) and *Bothrops atrox* (5TS5, $-9.49$ kcal/mol). In the 5Z2G and 5TS5 L-amino acid oxidases, quercetin binds in a pocket adjacent to the FAD cofactor, while in the myotoxic homologues of PLA2, 3CYL and 5TFV, it joins in the hydrophobic channel formed when oligomerizing, in the first one similar to $\alpha$-tocopherol. This study presents a case demonstration of the potential of bioinformatic tools in the validation process of ethnobotanical phytopharmaceuticals and how in silico methods are becoming increasingly useful for sustainable drug discovery.

Keywords: *Cordia*; in silico; antiophidic; quercetin; docking; validation

1. Introduction

*Cordia* is a tropical genus of arbustive *Boraginaceae* and is quite interesting from a pharmacological point of view [1]. More than thirty species are referenced as medicinal [2], having bioactive compounds such as rosmarinic acid, cordiaquinones and cordiachromes [3,4]. *Cordia alliodora* Cham., one of the most important timber trees in the Amazon, has an interesting chemical profile [5–11] with antimicrobial, antifungal, larvicidal [12] and cytotoxic activities [13] which have been experimentally tested. *Cordia verbenacea* DC has been studied as anti-inflammatory [14], analgesic [15], antibacterial [16], antiallergic [17] and antitumoral [18]; the latter activity is attributed to the rosmarinic acid (Figure 1) [19].
Another Cordia with promising properties is Cordia nodosa Lam, (= Cordia collococa Aubl [20]) a Pan-Amazonian species that contains [13] quercetrin (Figure 2), a strong antiproliferative in vitro.

In Amazonian Ecuador, many ethnic groups (cofan, redwood, siona, wao, shuar, achuar and kichwa) have reported references to the ancestral use [21]. The fruit is edible, the wood is employed for the construction of their houses in the jungle, and cultural ceremonies and rites are prepared with the leaves [21]. Cordia nodosa Lam contains phenols that justify its anti-inflammatory and analgesic applications and its moderate bactericidal action [22]. However, the most interesting ancestral knowledge of this plant is its ability to act as an antidote to snake bites [23]. This problem, seldom considered in Europe, affects millions of inhabitants of tropical areas of the planet and has not developed a pharmaceutical research according to its dimension [24]. Every year, about 5.4 million snake bites produce 1.8–2.7 million cases of poisoning, 81,410–137,880 deaths and about three times as many amputations and other permanent disabilities [24]. The World Health Organization has included snake bites in the category of “Neglected and Forgotten Diseases” [24].

In the context of ethnobotanical research conducted by our team in the Ecuadorian Amazon [21], we carried out surveys on the use of C. nodosa under the Framework of the Convention on Biological Diversity and the Nagoya Protocol [25]. We had two specific aims: (a) to describe the current use as an antivenom of C. nodosa in the Bobonaza Basin (Pastaza, Ecuador) and (b) to offer an in silico validation by searching the scientific literature and by overall performing docking tests.

2. Results

2.1. Ethnobotanical Survey

The medicinal uses given to the species retrieved from our fieldwork prospectons and literature review are summarized in Table 1, which shows that the use of C. nodosa as an antiophidic is currently

![Figure 1. Rosmarinic acid.](image1)

![Figure 2. Quercetrin.](image2)
in force in indigenous Amazonian ethnic groups. Names like kuchamanku, awas, putumuyu, machakuymisunsal or machakuykaspi have been published previously, but not paluwapu (“= snake stick”), which we learned from the canelo-kichwas cultures of Pastaza that we worked with [21]. We found that when a snake bite occurs, they take the bark, cook it for about one minute, and then drink the resulting liquid in a single dose.

### Table 1. Medicinal uses given to the species retrieved from our fieldwork prospections and literature review.

| Organ/System                | Part Used       | Formulation | Traditional Knowledge | Ethnic Group-Province (Country)          | Reference |
|-----------------------------|-----------------|-------------|-----------------------|-------------------------------------------|-----------|
| Circulatory system          | leaves          | decoction   | hypertension          | Amerindian NorthWest District (Guiana)    | [26]      |
| Digestive system            | bark stem       | cooking     | gases                 | Siona-Sucumbíos (Ecuador)                 | [23]      |
|                             | inner bark      | finely grate| treat cough           | Secoya-Sucumbíos (Ecuador)                | [23]      |
|                             | leaves          | decoction   | cold and shortness of breath | Amerindian (French Guiana)               | [26]      |
|                             | fruit           | suck        | snot in babies        | Amerindian NorthWest District (Guiana)    | [26]      |
| Respiratory system          | leaves          | decoction   | whooping cough         | Amerindian NorthWest District (Guiana)    | [26]      |
| Musculature and skeleton    | leaves          | crush the leaves and rub the body with them | rheumatism, sprains, muscle aches, bruises | Amerindian NorthWest District (Guiana)    | [26]      |
| Nervous system and mental illness | leaves          | baths with the decoction of the leaves | madness and psychiatric disorders | Yanesha (Perú) | [27]      |
|                             | bark            | decoction   | headache              | Secoya-Sucumbíos (Ecuador)                |           |
|                             | flowers         | infusions   | indeterminate conditions | Kichwa del Oriente-Orellana (Ecuador) | [23]      |
|                             | fruit           | infusion    | dizziness             | Wao-Orellana (Ecuador)                    |           |
|                             | leaves          | decoction   | fever                 | Amerindian NorthWest District (Guiana)    | [26]      |
| Symptoms and states of undefined origin | leaves | apply directly in the affected place | spider bite, to decrease inflammation and prevent gangrene | East Kichwa-Napo and Orellana (Ecuador) | [23]      |
|                             | fruit           | cooking     |                        |                                           |           |
|                             | plant           | cooking     |                        |                                           |           |
|                             | bark            | cooking     |                        |                                           |           |
|                             | root            | cooking     |                        |                                           |           |
| Poisoning                   | bark            | scraped and in water | infusion   | snake bites, to decrease inflammation and prevent gangrene | East Kichwa, Shuar-Napo, Orellana, Pastaza, Sucumbíos (Ecuador), Piaroa (Venezuela) | [23,26] |
|                             | brown           | infusion    |                        |                                           |           |
|                             | stem            | juice       |                        |                                           |           |
|                             | fruit           | juice       |                        |                                           |           |
|                             | young leaves    | chewed      |                        |                                           |           |
|                             | leaves          | apply directly in the affected place |                        |                                           |           |

2.2. Chemical and Activity Prospection: Results of the Bibliographic Review

The main component of the extract was quercetrin, a 3α-L-rhamnoside of quercetin. The genine has a chemical structure based on a C6-C3-C6 carbon skeleton, with a chromene ring bearing a second aromatic ring at position 2. Therefore, it is a flavonoid, specifically a flavonol (Figure 2). This is a chemical group in which antiophidic properties are known [28].
The literature review performed is summarized in the following tables. It is known that snake venom comprises peptides and proteins that act as cytotoxins, neurotoxins, hemotoxins or myotoxins. The 21 molecular targets of snakebite poisonings, retrieved from our bibliographic research, are shown in Table 2.

**Table 2.** Toxins from Ecuadorian (1–2), Latin American (3–13) or non-American (14–21) snakes, and the corresponding Protein Data Base Identifier (PDB ID).

| Toxin | PDB ID | Reference |
|-------|--------|-----------|
| MT-I—Basic phospholipase a2 myotoxin iii | 5TFV |
| LAAO—L-amino acid oxidase from Bothrops atrox | 5TS5 |
| PLA2—Phospholipase A2: Piratoxin I (myotoxic Lys49-PLA2) from Bothrops piraiaj | 3CYL |
| PLA2—Phospholipase A2: BthTX-I—Bothrostxin I from Bothrops jararacussu venom/ | 3CXL |
| PLA2—Phospholipase A2: Myotoxin (MJTX-I) from Bothrops moojeni | 6CE2 |
| PLA2—Phospholipase A2: Bothrostxin I (BthTX-I) | 6DIK |
| svPLA2—Phospholipase A2: myotoxin II from Bothrops moojeni | 1XXS |
| LAAO—L-amino acid oxidase from the B. jararacussu venom | 4EOV |
| svPLA2—Acidic phospholipase A2 (BthA-I) from Bothrops jararacussu venom | 1Z76 |
| VRV-PL-V—Crotoxin B, the basic PLA2 from Crotalus durissus terrificus | 2QOG |
| PLA2—Piratoxin-II (Prtx-II) - a K49 PLA2 from Bothrops piraiaj | 1QLL |
| Bothropasin, the Main Hemorrhagic Factor from Bothrops jararaca venom | 3DML |
| SVMP—P-I snake venom metalloproteinase BaP1 | 2W12 |
| NNH1—L-amino acid oxidase from venom of Naja atra | 52G2 |
| LAAO—L-amino acid oxidase from Vipera ammodytes venom | 3KVE |
| PDE I—Phosphodiesterase (PDE) from Taiwan cobra (Naja atra atra) venom | 5GZ4 |
| VRV-PL-V—Phospholipase A2 from Australian King Brown Snake (Pseudechis australis) | 3V9M |
| NN-PL-I—Phospholipase A2 from Indian cobra (Naja naja) | 1PSH |
| NNH1—L-amino acid oxidase oxidation from Bothrops jararacussu venom | 1XXS |
| 2.3. Docking

The liaison energies of quercetrin with the studied targets are presented in Table 3. They have been colored by groups according to their similarity to the rest of the sequences (see Supplementary Material for details). They oscillate between −10.03 kcal/mol and −7.01 kcal/mol.

**Table 3.** The liaison energies of quercetrin with the PDB ID studied targets.

| PDB ID | Reference |
|--------|-----------|
| 1. 5TFV | MT-I—Basic phospholipase a2 myotoxin iii |
| 2. 5TS5 | LAAO—L-amino acid oxidase from Bothrops atrox |
| 3. 3CYL | PLA2—Phospholipase A2: Piratoxin I (myotoxic Lys49-PLA2) from Bothrops piraiaj |
| 4. 3CXL | PLA2—Phospholipase A2: BthTX-I—Bothrostxin I from Bothrops jararacussu venom/ |
| 5. 3GUE | N-terminal kinase domain of RSK2 with flavonoid glycoside quercetrin |
| 6. 6ICE | PLA2—Phospholipase A2: Myotoxin (MJTX-I) from Bothrops moojeni |
| 7. 6DIK | PLA2—Phospholipase A2: Bothrostxin I (BthTX-I) |
| 8. 1XXS | svPLA2—Phospholipase A2: myotoxin II from Bothrops moojeni |
| 9. 4EOV | LAAO—L-amino acid oxidase from the B. jararacussu venom |
| 10. 1Z76 | svPLA2—Acidic phospholipase A2 (BthA-I) from Bothrops jararacussu venom |
| 11. 2QOG | VRV-PL-V—Crotoxin B, the basic PLA2 from Crotalus durissus terrificus |
| 12. 5A4W | AtGSTF2 from Arabidopsis thaliana |
| 13. 1QLL | PLA2—Piratoxin-II (Prtx-II) - a K49 PLA2 from Bothrops piraiaj |
| 14. 3DML | Bothropasin, the Main Hemorrhagic Factor from Bothrops jararaca venom |
| 15. 2W12 | SVMP—P-I snake venom metalloproteinase BaP1 |

Figures 3–15 show the molecular models of the quercetrin binding with the targets of Table 3 (left) and the corresponding 2D interaction diagram generated with LigPlot + (right) [50], made with UCSF Chimera Software. Toxins are represented in golden yellow, quercetrin in blue, and original ligands in pink. The small squares (a) show the toxin-quercetrin complex in the most favorable arrangement. When it occupies the hollow of another ligand present in the structure of the target, it has been preserved (shown as thin sticks in pink) to allow a comparison. The augmented figures (b) show, in detail, the dispositions of the quercetrin molecule between the chains of the toxins.
Figure 3. 5TFV-quercetin complex. in the most favourable arrangement (a) and augmented (b) showing the disposition between the chain of toxins. Right: 2D interaction diagram.

Figure 4. 5TS5-quercetin complex. in the most favourable arrangement (a) and augmented (b) showing the disposition between the chain of toxins. Right: 2D interaction diagram.
Figure 5. 3CYL quercetin complex in the most favourable arrangement (a) and augmented (b) showing the disposition between the chain of toxins. Right: 2D interaction diagram.

Figure 6. 3CXI-quercetin complex in the most favourable arrangement (a) and augmented (b) showing the disposition between the chain of toxins. Right: 2D interaction diagram.

Figure 7. 6CE2-quercetin complex in the most favourable arrangement (a) and augmented (b) showing the disposition between the chain of toxins. Right: 2D interaction diagram.
Figure 8. 6DIK-quercetin complex in the most favourable arrangement (a) and augmented (b) showing the disposition between the chain of toxins. Right: 2D interaction diagram.

Figure 9. 1XXS-quercetin complex in the most favourable arrangement (a) and augmented (b) showing the disposition between the chain of toxins. Right: 2D interaction diagram.

Figure 10. 4E0V-quercetin complex in the most favourable arrangement (a) and augmented (b) showing the disposition between the chain of toxins. Right: 2D interaction diagram.
Figure 11. 1Z76-quercetrin complex in the most favourable arrangement (a) and augmented (b) showing the disposition between the chain of toxins. Right: 2D interaction diagram.

Figure 12. 2QOG-quercetrin complex in the most favourable arrangement (a) and augmented (b) showing the disposition between the chain of toxins. Right: 2D interaction diagram.

Figure 13. 1QLL-quercetrin complex in the most favourable arrangement (a) and augmented (b) showing the disposition between the chain of toxins. Right: 2D interaction diagram.
Figure 14. 3DSL-quercetin complex in the most favourable arrangement (a) and augmented (b) showing the disposition between the chain of toxins. Right: 2D interaction diagram.

Figure 15. 2W12-quercetin complex in the most favourable arrangement (a) and augmented (b) showing the disposition between the chain of toxins. Right: 2D interaction diagram.

3. Discussion

The traditional use of this plant for its antiophidic properties has been previously documented by the Shuar and the Napo-Rune people of neighboring provinces, although the method of application is different. In these cases, the plant is applied directly to the affected place, or they chew the young leaves and the fruits. They also prepare infusions, not with the bark, but with the root or the juice of the stem [23]. Published references on the activity of quercetin have indicated that it inhibits lipoxygenase svPLA2 [51] and hyaluronidase NNH1 [52], neutralizing the hemorrhagic venom of *Bothrops jararaca* [51–53], a Latin American snake.

In the docking tests that we carried out, the toxic snakes studied showed very high affinities with quercetin. There were formed complexes of energy comparable to the ones with original targets such as 4GEW or 5A4W. Detailed information about these protein homologies has been included in Appendix A (Table A1). Thus, reasonable binding free energy values of $-7$ to $-10$ kcal/mol were obtained. The most favorable values were for the venom of the Asian snake (Chinese cobra or Taiwan cobra) *Naja atra* (5Z2G, $-10.03$ kcal/mol) and the Latin American *Bothrops pirajai* (3CYL, $-9.71$ kcal/mol). Very good results were also found with the 5TFV of the Ecuadorian snakes *Bothrops asper,*
(ΔG −9.47 kcal/mol) and 5TS5 of Bothrops atrox (ΔG −9.49 kcal/mol). Therefore, quercetin can not only be used as an antitoxin for Ecuadorian venomous snakes, but for many others.

The action can be expected to be effective, especially because, in addition, the models have not presented unfavorable interactions according to SwissDock scoring terms. On many occasions, the quercetin molecule is placed in pockets that occupy other known ligands of the targets used in this study. This is the case for 3CYL and 3CXI (Figures 5b and 6b), which occupy the pocket of α-tocopherol, 5TS5 (Figure 4b), which is close to that of FAD, 6CE2 (Figure 7b), that occupies that of suramin (a known inhibitor), 1XXS (Figure 9b), that of two stearic acids, 1QLL (Figure 13b), that of tridecanoic acid, and 2W12 (Figure 15b), which occupies the peptidomimetic inhibitor site. All of this reinforces the validity of the results of the performed docking tests.

In the 5Z2G and 5TS5 L-amino acid oxidases, quercetin binds in a pocket adjacent to the FAD cofactor, while in the myotoxic homologues of PLA2, 3CYL and 5TFV, it joins in the hydrophobic channel formed when oligomerizing in the first one, similar to α-tocopherol.

These facts reinforce the validity of the traditional use reported. They will have to be corroborated in vitro, in vivo, and even with subsequent clinical trials. Nevertheless, this is encouraging evidence in the field of finding new solutions to this pathology.

4. Materials and Methods

4.1. Ethnobotanical Survey

All the information referring to the ethnobotanical study from which the data derives is available in Appendix A, which contains references to voucher specimens, authorizations and permissions. Table 1 summarizes the medicinal uses of the species retrieved from our fieldwork prospections and literature data.

4.2. Chemical and Activity Prospection: Bibliographic Review

A bibliographic review was carried out following the PRISMA Group method [54]. The databases accessed were Academic Search Complete, Agricola, Agris, BIOSIS, CABS, Cochrane, Cybertesis, Dialnet, Directory of Open Access Journals, Embase, Espacenet, Google Patents, Google Academics, Medline, PubMed, Science Direct, Scopus, Teseo, and ISI Web of Science. The selected citations were summarized, and a critical reading allowed us to develop the discussion.

4.3. Docking

The molecular docking method applied comprises the following procedures: ligand preparation, protein selection, docking, and analysis of the results. The energies produced after docking, interaction residues and interaction types were studied for the analyses following general procedure for molecular docking [55,56]. Docking was performed with the SwissDock Docking Web Service (Available online: http://www.loc.gov). A 3D quercetin virtual structure was built with Spartan®, Wavefunction Inc. Selection of targets was made based on a bibliographic review of natural bioactive compounds against snake bites [28,54]. A total of 21 venoms from snakes (targets) with known X-ray structures were tested (see Tables 2, 3 and Supplementary Material). Molecular structures were consulted in the Protein Data Bank (PDB), and the reference IDs were taken to include them in the Swiss Dock Program. The target + ligand set was considered stable when the values of the binding free energy were lower than −7 kcal/mol. This consideration is based on docking experiments with the known X-ray structures 4GUE and 5A4W complexes of quercetin resulting in binding energies values of −9.30 and −8.28 kcal/mol, respectively. Once the results of the docking were obtained, they were analyzed with UCSF Chimera.

5. Conclusions

The information obtained from the ethnobotanical investigations carried out in the Bobonaza Basin (Ecuador) allowed us to verify the good capacity in silico of quercetin, the active ingredient
obtained from Cordia nodosa. The binding energies of quercetrin with all the macromolecules (toxins from venoms of different snakes) were adequate, since they were all less than −7 kcal/mol.

The in silico docking evaluation combined with ethnobotanical information was very effective as a research method. It allowed us to select the appropriate active principle from the beginning, thus avoiding the tedious previous work of testing principle assets that have no references and therefore working blindly with molecules that would not couple to these toxins. The search for new bioproducts oriented from basic ethnobotanical knowledge is an investigation that could result in products with great therapeutic efficacies.

Supplementary Materials: The following are available online at www.mdpi.com/1420-3049/24/22/4160/s1, Table S1: Similarity matrix for the target proteins. Table S2: Heat map of sequence similarities as indicated in Table S1.

Author Contributions: Conceptualization T.R.-T. methodology, C.E.C.-M. and C.X.L.-Q.; validation J.B.-S.; formal analysis, R.P. and L.M.M.-C.; investigation, C.X.L.-Q.; data curation, C.E.C.-M. and C.X.L.-Q.; writing—original draft preparation, T.R.-T.; writing—review and editing, J.B.-S., R.P. and L.M.M.-C; visualization, L.M.M.-C. and R.P.; supervision, C.X.L.-Q.; project administration, T.R.-T.; funding acquisition, C.X.L.-Q. J.B.-S. and T.R.-T.

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Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

Abbreviations

| PDB ID | Toxin                                      |
|--------|--------------------------------------------|
| 4GUE   | N-terminal kinase domain of RSK2           |
| 5AAW   | AtGSTF2 from Arabidopsis thaliana          |
| 3CYL   | PLA2—Phospholipase A2: Piratoxin I (myotoxic Lys49-PLA2) from Bothrops pirajai |
| 5TFV   | MT-I—Basic phospholipase a2 myotoxin iii  |
| 5TS5   | LAAO—L-amino acid oxidase from Bothrops atrox |
| 3CXI   | PLA2—Phospholipase A2: BthTX-I—Bothrotoxin I from Bothrops jararacussu venom/ |
| 6CE2   | PLA2—Phospholipase A2: Myotoxin (MjTX-I) from Bothrops moojeni |
| 6DJK   | PLA2—Phospholipase A2: Bothrotoxin I (BthTX-I) |
| 1XXS   | svPLA2—Phospholipase A2: myotoxin II from Bothrops moojeni |
| 4E0V   | LAAO—L-amino acid oxidase from the Bothrops jararacussu venom |
| 1Z76   | svPLA2—Acidic phospholipase A2 (BthA-I) from Bothrops jararacussu |
| 2QOG   | VRV-PL-V—Crototoxin B, the basic PLA2 from Crotalus durissus terrificus |
| 1QLL   | PLA2—Piratoxin-II (Prx-II) - a K49 PLA2 from Bothrops pirajai |
| 3DSL   | Bothropasin, the Main Hemorrhagic Factor from Bothrops jararaca venom. |
| 2W12   | SVMP—P-I snake venom metalloproteinase BaP1 |
| 5Z2G   | NNH1—L-amino acid oxidase from venom of Naja atra |
| 3KVE   | LAAO—L-amino acid oxidase from Vipera ammodytes venom |
| 5GZ4   | PDE I—Phosphodiesterase (PDE) from Taiwan cobra (Naja atra atra) venom |
| 3V9M   | VRV-PL-V—Phospholipase ACII4 from Australian King Brown Snake (Pseudechis australis) |
| 1PSH   | NN-PL-I—Phospholipase A2 from indian cobra (Naja naja) |
| 1REO   | LAAO—L-amino acid oxidase from Agkistrodon Halys Pallas (Gloydius halys) |
| 5GZ5   | NNH1—Phosphodiesterase (PDE) from Taiwan cobra (Naja atra atra) |
| 1A3D   | PLA2—Phospholipase A2 (Pla2) from Naja naja |
Appendix A

The Kichwa community of Pakayaku (Bobonaza River, Pastaza, Ecuador) lies in a fairly isolated region where bio- and ethno-diversity studies are still lacking. One of us (CXLQ) was based in the Biological Station PindoMirador in the northern Bobonaza River basin (S1°27′09″-W 78°04′51″), and since 2008 was in charge of environmental monitoring and education programs involving the local population.

Permissions and Authorizations/Ethnobotanical Survey: Under the Protocole of Nagoye (CBD, 2010) collective written research consent was granted by Mrs. Luzmila Gayas, the community president of the Assembly of Pakayaku. Permissions for collecting and moving plant material (MAE-DPAP-2016-2243) was granted by the Ministry of Environment of Ecuador. Prior verbal individual consents were obtained from the persons taking part in our survey. Our investigation consisted of a series of planned residential visits and treks accompanied by Kichwa interpreters and the local inhabitants of Pakayaku.

Vouchers: Sheets at Herbarium Alberto José Paredes, Universidad Central de Ecuador, Quito (QAP). Ecuador, Pastaza: Pakayaku, banks of the Bobonaza River, sector of Yanalpa, chacra n° 6 belonging to Mr. Aparicio Aranda, two hours walking away from the community, 592 m, 01°39′03″ S, 077°34′36″ W, lowland evergreen forest, 23 January 2016, C. X. Luzuriaga-Q & R. Aranda (QAP 92888); sector of Aychatambo, banks of the Bobonaza River, upstream by canoe, twenty minutes away from the community, 425 m, 01°37′51.8″ S, 077°36′31.4″ W, lowland evergreen forest, 27 November 2015, C. X. Luzuriaga-Q & L. Gayas (QAP 92547). Material identified by C. Cerón Martínez.

Interviews: They were semi-structured and included a series of open questions aimed to encourage discussion. Knowledgeable elders of the Pakayaku community acted as informants and agreed to reveal their knowledge on the species and to be recorded. The informants answered freely on several topics, including the common name of Kichwa, part of the plant used, description of usage, harvest season, storage (if any), preparation of concoction, and the target of the treatment.

Informatization: After the fieldwork, the data were included into an MS Excel spreadsheet. All the uses recorded were classified according to the classification scheme proposed by [21], which is in turn based on the report of De la Torre and coworkers [23]. Cordia nodosa data are included in Table 1.
Table A1. Similarity matrix for the target proteins. In each cell the upper number represents the percentage of identity and the lower the percentage of similarity for the protein in that row. The number in parenthesis represent the corresponding amino-acid number after Smith-Waterman comparison of the sequences. The cells are colored according to the percentage of similarity: <25% yellow, 25 – 50% orange, 50-75% green and >75% light blue. Diagonal cells with 100% identity are colored in dark blue. The protein IDs in the upper row have been colored by groups according to their similarity to the rest of the sequences.

|       | 4GUE (305) | 5A4W (212) | 1QLL (121) | 1XXS (122) | 1Z76 (123) | 2QOG (122) | 2W12 (202) | 3CXI (121) | 3CYL (121) | 3DSL (419) | 4EUV (497) | 5TFV (122) | 5TS5 (484) | 6CE2 (121) | 6DIK (121) |
|-------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 4GUE (305) | 100%       | 2.95% (9)  | 1.64% (5)  | 2.95% (9)  | 1.64% (4)  | 8.20% (25) | 1.97% (6)  | 1.64% (5)  | 5.90% (18) | 8.52% (26) | 1.97% (6)  | 6.23% (19) | 1.31% (4)  | 1.97% (6)  | 2.95% (9)  |
| 5A4W (212) | 4.25% (9)  | 100%       | 2.36% (6)  | 2.83% (9)  | 3.77% (8)  | 6.60% (14) | 2.36% (5)  | 2.62% (13) | 4.25% (9)  | 19.81% (42) | 2.36% (5)  | 19.81% (42) | 5.19% (11) | 2.36% (5)  | 2.83% (6)  |
| 1QLL (121) | 4.13% (5)  | 4.13% (5)  | 100%       | 93.39% (113) | 48.76% (59) | 48.76% (59) | 4.96% (6)  | 98.35% (119) | 99.17% (120) | 14.05% (17) | 19.83% (124) | 60.33% (73) | 5.7% (7)   | 86.78% (105) | 99.17% (120) | 99.17% (120) |
| 1XXS (122) | 4.10% (5)  | 4.92% (6)  | 92.62% (113) | 100%       | 50.82% (62) | 49.18% (60) | 4.92% (6)  | 94.26% (115) | 92.62% (113) | 13.93% (17) | 9.84% (12)  | 59.84% (73) | 4.10% (5)  | 82.79% (101) | 93.44% (114) | 95.08% (116) |
|   | 4E0V   | 5TVF   | 5TS5   | 6CE2   | 6DIK   |
|---|--------|--------|--------|--------|--------|
|   | (497)  | (122)  | (484)  | (121)  | (121)  |
|   | 5.23%  | 4.92%  | 3.93%  | 3.31%  | 4.96%  |
|   | (26)   | (6)    | (19)   | (4)    | (6)    |
|   | 8.45%  | 4.10%  | 8.68%  | 9.09%  | 4.13%  |
|   | (42)   | (5)    | (42)   | (11)   | (5)    |
|   | 4.83%  | 59.84% | 15.50% | 86.78% | 99.17% |
|   | (24)   | (73)   | (50)%  | (105)  | (120)  |
|   | 2.41%  | 69.67% | 2.27%  | 89.26% | 94.21% |
|   | (12)   | (86)   | (11)   | (108)  | (116)  |
|   | 2.01%  | 71.31% | 2.48%  | 85.95% | 94.21% |
|   | (19)   | (87)   | (12)   | (104)  | (114)  |
|   | 2.41%  | 76.23% | 4.55%  | 66.12% | 49.59% |
|   | (12)   | (93)   | (22)   | (80)   | (60)   |
|   | 1.41%  | 63.93% | 2.27%  | 57.85% | 48.76% |
|   | (7)    | (78)   | (11)   | (70)   | (59)   |
|   | 4.83%  | 59.02% | 2.27%  | 21.49% | 4.96%  |
|   | (24)   | (72)   | (11)   | (26)   | (6)    |
|   | 4.83%  | 69.67% | 1.45%  | 89.26% | 99.17% |
|   | (42)   | (85)   | (7)    | (105)  | (120)  |
|   | 2.01%  | 97.93% | 1.45%  | 89.26% | 99.17% |
|   | (12)   | (477)  | (7)    | (120)  | (120)  |
|   | 3.22%  | 59.02% | 1.45%  | 89.26% | 99.17% |
|   | (16)   | (72)   | (7)    | (116)  | (120)  |
|   | 95.37% | 13.11% | 1.45%  | 89.26% | 99.17% |
|   | (474)  | (16)   | (7)    | (116)  | (120)  |
|   | 95.98% | 100%   | 1.45%  | 89.26% | 99.17% |
|   | (477)  | (28)   | (7)    | (116)  | (120)  |
|   | 5.63%  | 13.11% | 1.45%  | 89.26% | 99.17% |
|   | (28)   | (16)   | (7)    | (116)  | (120)  |
|   | 4.23%  | 100%   | 1.45%  | 89.26% | 99.17% |
|   | (21)   | (28)   | (7)    | (116)  | (120)  |
|   | 8.65%  | 59.02% | 1.45%  | 89.26% | 99.17% |
|   | (43)   | (72)   | (7)    | (116)  | (120)  |
|   | 4.83%  | 98.55% | 1.45%  | 89.26% | 99.17% |
|   | (24)   | (474)  | (7)    | (116)  | (120)  |
|   | 98.55% | 100%   | 1.45%  | 89.26% | 99.17% |
|   | (477)  | (28)   | (7)    | (116)  | (120)  |
|   | 100%   | 13.11% | 1.45%  | 89.26% | 99.17% |
|   | (16)   | (28)   | (7)    | (116)  | (120)  |
|   | 69.67% | 100%   | 1.45%  | 89.26% | 99.17% |
|   | (85)   | (28)   | (7)    | (116)  | (120)  |
|   | 66.39% | 100%   | 1.45%  | 89.26% | 99.17% |
|   | (81)   | (28)   | (7)    | (116)  | (120)  |

### Notes
- **4E0V**
- **5TVF**
- **5TS5**
- **6CE2**
- **6DIK**
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