Pharmacogn. Res.
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ORIGINAL ARTICLE

Pharmacognostic, Phytochemical, and Anti-Inflammatory Effects of *Corynaea crassa*: A Comparative Study of Plants from Ecuador and Peru

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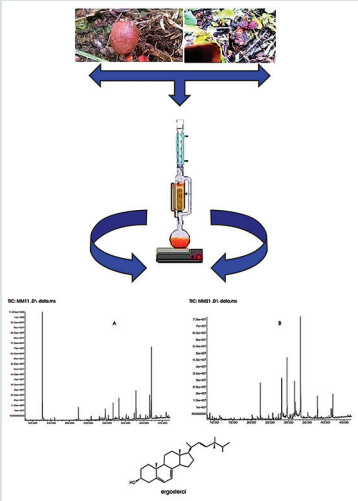
ABSTRACT

Background: *Corynaea crassa* Hook. F (Balanophoraceae), is a hemiparasitic plant that grows on the roots of other species, commonly known as "*huanarpo male*" and traditionally used as an anti-inflammatory and aphrodisiac. **Objective:** The objective of this work was to carry out a comparative pharmacognostic, physicochemical, and pharmacological study between extracts obtained from plants that grow in Peru and Ecuador. **Materials and Methods:** Macro and micromorphological analysis, physicochemical characteristics, and phytochemical screening were determined according to established standards. Successive extraction was carried out with solvents of increasing polarity, and the composition of the ethyl acetate extract was performed using a gas chromatograph Agilent connected to the mass spectrometer (GC-MS). The anti-inflammatory activity was determined by the aqueous and alcoholic extracts of the plants by the carrageenan test. **Results:** The macro- and micromorphological characteristics did not show differences, the physical-chemical properties presented some differences attributable to the ecological conditions of the places of origin, the phytochemical screening exposed a complex chemical composition. In the ethyl acetate extract obtained, safrole and squalene were identified as major components for the Ecuadorian species and hexadecanoic and octadecanoic acids for the Peruvian species. The anti-inflammatory effect of aqueous and hydroalcoholic extracts was demonstrated on the carrageenan-induced acute inflammation model in female Wistar albino rats. **Conclusion:** The extracts showed a similar anti-inflammatory behavior, although less than the indomethacin used as a positive control. This work brings novel results to the pharmacognostic, chemical and pharmacological properties of the species *C. crassa*.

Key words: Anti-inflammatory activity, ethyl acetate extract, gas chromatograph agilent connected to mass spectrometer, pharmacognosy, phytochemistry

SUMMARY

A comparative study was carried out on the species that grows in Peru and Ecuador and that is used as an aphrodisiac and anti-inflammatory in traditional medicine. The species did not present macro and micromorphological differences, but in some physicochemical parameters and in the chemical composition that may be due to geographical ecological conditions or to the plants that grow in its environment, since it is a parasitic plant. The anti-inflammatory activity of the hydroalcoholic extracts was similar, although less than the positive control. The importance of this study lies in the fact that for the species there are few reports found in the literature.



Abbreviations Used: GC-MS: Gas chromatography coupled to mass spectrometry; MDMA: 3,4-methylenedioxymethamphetamine; COX: Cyclooxygenase; PGE2: Prostaglandin E2.

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DOI: 10.4103/pr.pr_42_20

Access this article online
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INTRODUCTION

The use of plants for therapeutic purposes dates to the origins of man, and even in the 21st century, they are still the only source of choice for many people in the world, although a relative few have been scientifically studied. In particular, *Corynaea* is a monotypic genus of parasitic plants from Balanophoraceae family. The only known species, *Corynaea crassa* Hook. f., is a hemiparasite plant that grows on the roots of other species, commonly known as "*huanarpo male*" and conventionally used as an aphrodisiac in man and could modulate fertility. The species is originally

Cite this article as: Lopez-Barrera AJ, Gutiérrez-Gaitén YI, Miranda-Martínez M, Choez-Guaranda IA, Ruíz-Reyes SG, Scull-Lizama R. Pharmacognostic, phytochemical, and anti-inflammatory effects of *Corynaea crassa*: A comparative study of plants from ecuador and peru. Phcog Res 2020;12:394-402.

Submitted: 01-May-2020 **Revised:** 25-Jun-2020
Accepted: 15-Jul-2020 **Published:** 23-Jan-2021

from tropical America and the natural distribution reaches from Costa Rica to Bolivia, in general, at altitudes from 1250 to 3600 m.[1]

Many aspects of the biology and medical uses of *C. crassa* are poorly known, due largely to their underground life habit and the seasonal appearance of the inflorescence. In addition, because this species is entirely dependent on other plants for its survival, as well as the conversion of many areas of cloud forest into agriculture and pastures makes the species vulnerable. Parasitic plants may also be limited by the additional suite of factors of host availability. That is, host quality, host resistance to parasitism, and parasite preference,[2] are factors that contribute to the limited study of this plant.

During the last decade, Bussmann *et al.*[3] have reported that ethanolic extracts from *C. crassa* collected in Peru had shown inhibitory activity against *Staphylococcus aureus*[3] and exhibited toxicity in the brine-shrimp lethality assay. However, the water extract was found to be non-toxic at a concentration of 10,000 μg/mL in the same lethality assay.[3] On the other hand, Malca *et al.*[4] reported the presence of β-sitosterol and triterpenoids lupenone, amirone, lupeol, and α-amyrin in plants that were also collected in Peru.

Then, in order to provide key factors to drug standardization as a process to assay biological applications of plant-derived products, herein, we describe the pharmacognostic characteristics and chemical profiles of *C. crassa* from Ecuador and Peru. In addition, the anti-inflammatory effect in an animal model was also studied.

MATERIALS AND METHODS

Plant material

In the present work, plants of *C. crassa* were studied from two different countries. First one was collected in La Libertad province, Santiago de Chuco Department Agasmarca, Peru (08°07′53″S, 78°03′23″E, 2900 m elevation) labeled as sample Pe and from Yanachoca Reservation in North of Pichincha province, Ecuador (00°05′S, 78°33′E, 3700 m elevation) and labeled as Ec. One specimen of each collection was identified at the herbarium of Natural Science Faculty from Guayaquil University and deposited under the voucher specimen of 13.115 and 13.116, respectively. In each case, the complete plant was used, which was washed with water. The samples were dried in an oven AISET model VLD-6000 (China) with controlled temperature, at 40°C ± 2°C, over 7 days, and subsequently fragmented in a knife mill for processing and analysis.

Aqueous and hydroalcoholic extracts from *Corynaea crassa*

The aqueous extract was prepared 1 h before the pharmacological experiment by decoction, heating 20 g of drug with 100 mL of boiling water for 20 min. It was filtered hot, and the percentage of total solids was determined according to Miranda and Cuellar.[5] Aqueous extracts from Peru and Ecuador were namely AE-Pe and AE-Ec, respectively.

Hydroalcoholic extracts were made by maceration from 20 g of dried and ground drug in 100 mL of 80% ethanol. The extraction was left for 7 days, stirring the extracts almost one time per day. Once the extraction was finished, it was filtered, concentrated to 50 mL, and the percentage of total solids was determined.[5] Hydroalcoholic extracts from Peru and Ecuador were named as HE-Pe and HE-Ec, respectively

Animals

Female Wistar albino rats were obtained from the National Center to produce Laboratory Animals (CENPALAB, Havana, Cuba) with their corresponding quality certificates that guaranteed their health. The weight of the rats ranged from 180 to 200 g. The room temperature was 20°C ± 3°C, relative humidity: 30 – 70% ±5%, light/dark cycle: 12/12 h. Water and food were supplied "*ad libitum*." The food was withdrawn 24 h. before the start of the trial and they were only allowed access to water.

Pharmacognostic studies

The analysis of macromorphologic characteristics of both specimens was carried out, including the size of piece, shape, and intern/extern surface.[5] In the case of micromorphologic study, the obtained power from each specimen was used, which were treated with 1% sodium hypochlorite solution for 5 min and then rinsed with copious running water and stained with safranin 1% and fixed with glycerine gelatin; the histochemistry reactions were included to determine the presence of oil (Sudan III solution at 5% in ethanol 70%) and starch (Lugol solution with iodine at 1% and 2% of potassium iodide in water). To identify these internal anatomic characteristics optical microscopy (NOVEL, China) at ×10 was used coupled with a digital camera HDCE-50B (Alltion (Wuzhou) Co. Ltd., Guangxi, China).

Physicochemical analysis

The physicochemical analysis was carried out on the powdered sample according to previously reported standard methods.[5,6] The moisture content (azeotropic method), water-extractable substances, 30%, 50%, 80%, and 98% ethanol and extractable substances in ethyl acetate and hexane were determined.

Phytochemical analysis

The detection of secondary metabolites through a phytochemical screening was carried out.[5] The powdered materials were extracted by exhaustion with petroleum ether, ethanol, and water. Finally, corresponding assays to determine fats or oil, volatile oil, reducing sugars, resins, saponins, sterols, triterpenes, phenols/tannins, flavonoids, anthocyanins, free amino acids or amines, alkaloids, coumarins, quinones mucilage, and catechins were performed.[5]

Preparation of extracts and gas chromatography mass spectrometry equipment analysis

Forty grams of previously obtained power of each specimen were used to obtain extracts by maceration with 400 mL of different solvents, including hexane, dichloromethane, and ethyl acetate. The ethyl acetate extract was concentrated by rotary evaporation in an ECO-R20, Vela Quin S.A, (Mexico), at a temperature of 50°C and 30 rpm. Then, chemical analysis was performed in a gas chromatography mass spectrometry equipment (GC-MS) Agilent Technologies (7890A GC system and 5975C inert XL MSD with triple-axis detector). A capillary column DB-5MS (30 m × 0.25 mm) with phenyl dimethylpolysiloxane was used as stationary phase (0.25 μ film thickness) and helium as the carrier gas (1.0 mL/min). The injection of 1 μL of ethyl acetate samples was done with the splitless mode at 250°C. The oven temperature was started at 70°C for 2 min, then it was increased to 285°C at 5°C/min, and it was maintained at 285°C for 2 min. The compounds identification was made by comparison of mass spectra based on Wiley 9th and NIST 2011 MS libraries. An electron ionization of 70 eV at 230°C was used in the ion source, and the data were collected with the full scan mode (50–550 amu) in the quadrupole mass analyzer.

Determination of anti-inflammatory effect

The normal volumes of the right leg of the rats were measured using a digital plethysmometer (Panlab, Spain). Then, the mice were randomly divided into six different groups of six animals each, weighed as a group and received the following treatments.[7] Group A – 0.5 mL of AE-Pe

at 400 mg/kg of by oral route, Group B - 0.5 mL of AE-Ec at 400 mg/kg by the oral route, Group C – 0.5 mL of HE-Pe at 400 mg/kg of by oral route, Group D - 0.5 mL of HE-Ec at 400 mg/kg of by oral route, Group E – 10 mg/kg of Indomethacin (Sigma Aldrich) by oral route and Group F – 0.5 mL of 0.9% sodium chloride solution. All treatments were carried out using an intragastric cannula and after 30 min, 3% aqueous carrageenan solution (inflammation-inducing agent) was administered in the right plantar aponeurosis of all animals. The volumes of the inflamed leg were measured 1, 2, 3, and 5 h after carrageenan administration. The difference between the initial value and subsequent readings for each study time showed the volume of edema. The percentages of inflammation inhibition were calculated by the following expression.[8]

% Inhibition = Vc – Vt/Vc × 100

Where:

Vc = Mean value of edema volume of animals in the negative control group.

Vt = Mean value of the edema volume of the animals in the group treated with the test substance.

At the end of the test, the animals were sacrificed using a saturated atmosphere of ether, always considering the refinement techniques currently proposed for testing experimental animals. All procedures and handling of animals were performed following the ethical principles for the use of laboratory animals recommended in the International Guidelines.[9]

Statistical analysis

The results corresponding to quality control and chromatographic analysis were processed to calculate average values and standard deviations. The Statgraphics® Plus program, version 5.0, was used, carrying out a normality test (Kolmogorov–Smirnov), subsequently an analysis of variance through ANOVA-1 and for the comparison of the means, the Student's *t*-test was used. The data obtained in the pharmacological study were processed using the SPSS Statistical Package for Windows version 8.0 (IBM-SPSS Statistical Package for Windows version 8.0. Chicago. U. S). Data were expressed as arithmetic mean/standard deviation. One-way ANOVA analysis was used to determine if there was a statistically significant difference for the variable evaluated and then Kruskal–Wallis was applied, followed by the Friedman test. In all cases, the level of significance set was $P < 0.05$.

RESULTS

Pharmacognostic studies

In the macromorphological analysis carried out on the species from the two collection sites [Figure 1], it was found that the plant presents reddish-brown or brown coloration (A); the upper end has a rounded and ovoid shape, of spongy-fibrous consistency (B and C), followed by an elongated piece, slightly grooved on its external surface (D) and fibrous groove (E) on its internal surface. In the lower part, the haustorial root (F) is shown, protruding, rough, which, when dried, fragments leaving fibers. Plant length measurements showed the same dimensions ($P < 0.05$), with 14.0 ± 1.4 cm and 11.8 ± 2.1 cm for Ecuador and Peru, respectively. Figure 2 shows a prototype of the macro morphological characteristics of *C. crassa*.

In the microscopic study of the powdered drugs [Figure 3], xylem vessels of the scalariform type (A and B) were observed. It was possible to visualize epidermal cells with a sinusoidal contour, together with the stomata classified as anomocytic (C and D). In the samples Ec, the contour of the epidermal cells was more pronounced. We also observed cells of the fundamental parenchyma (E and F) with small granules of starch, which suggests that it could be an amiliferous reserve parenchyma (accumulates starches). Sclerid cells (G and H), which are part of the protective tissue, were found in the samples from both origins; this group is usually called idioblastic because they are cells that can be isolated or in small groups.

The samples were subjected to some histochemical reactions [Figure 4]. Oil bags (A and B) could be seen with great clarity, thanks to the tests carried out with Sudan III, a reagent that dyes red oils or fatty compounds in different shades. Starch grains (C and D) were observed, which showed an intense violet-blue color compared to the Lugol reagent and were more abundant in the samples from Pe. In both cases, the starch grains are of the lenticular type.

Physicochemical analysis

Some of the parameters that were evaluated to the powered drugs from Ecuador and Peru are Table 1.

Phytochemical screening

Qualitative differences were not observed between both specimens tested, in which several classes of metabolites were identified [Table 2]. However, differences in the color intensities were observed in some metabolites assayed.

Gas chromatography mass spectrometry equipment analysis of ethyl acetate extract

The chromatograms obtained of ethyl acetate extract obtained after the degreasing of the drugs with hexane and dichloromethane by GC-MS of both specimens studied are presented in Figure 5. There are notable differences between the chromatographic profiles of the species from different origins. For the species native to Ecuador, two chromatographic

Figure 1: *Corynaea crassa* Hook. f. in natural habitat. (a) Peru; (b) Ecuador. Photographs taken by the authors during the collection of the plant

Table 1: Physicochemical parameters of the powdered drugs from Ecuador and Peru

Parameters (%)	Results $\bar{X}$±SD	
	Ecuador	Peru
Moisture content	8.64±0.03[a]	8.36±0.10[a]
Water-soluble extractive	19.18±0.04[b]	19.22±0.07[b]
Alcohol-soluble extractive at 30%	21.75±0.06[c]	22.55±0.04[d]
Alcohol-soluble extractive at 50%	29.68±0.18[e]	29.63±0.20[e]
Alcohol-soluble extractive at 80%	44.76±0.11[f]	30.79±0.14[g]
Alcohol-soluble extractive at 98%	13.69±0.24[h]	10.57±0.06[i]
Ethyl acetate-soluble extractive	2.14±0.04[j]	4.38±0.06[k]
Hexane- soluble extractive	2.00±0.04[l]	3.18±0.02[m]
Total ash content	7.81±0.09[n]	3.74±0. 12[o]
Water-soluble ash	0.69±0.02[p]	2.83±0.03[q]
Acid-insoluble ash	5.37±0.02[r]	0.57±0.03[s]

Legend: X/SD=Average value of determinations±SD. Different letters represent statistical differences (P<0.05) among studied drugs. SD: Standard deviation

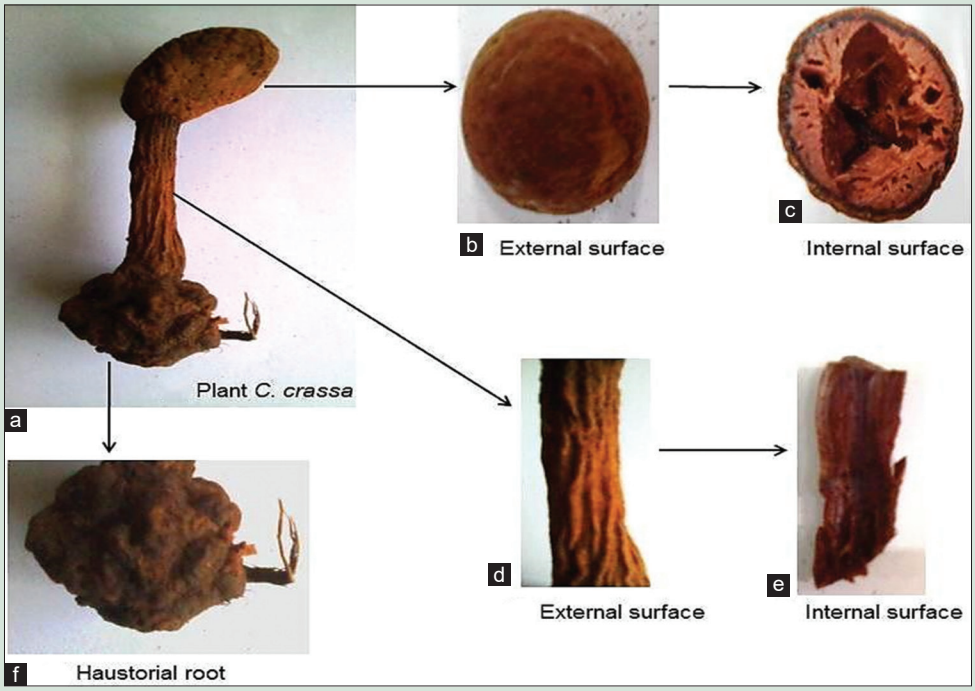

Figure 2: Representative Macro morphological details from *C. crassa* species. Photographs taken by the authors during pharmacognostic analysis of samples from Ecuador. (a) Plant *Corynaea crassa*. (b) External surface. (c) Internal surface. (d) External surface. (e) Internal surface. (f) Haustorial root

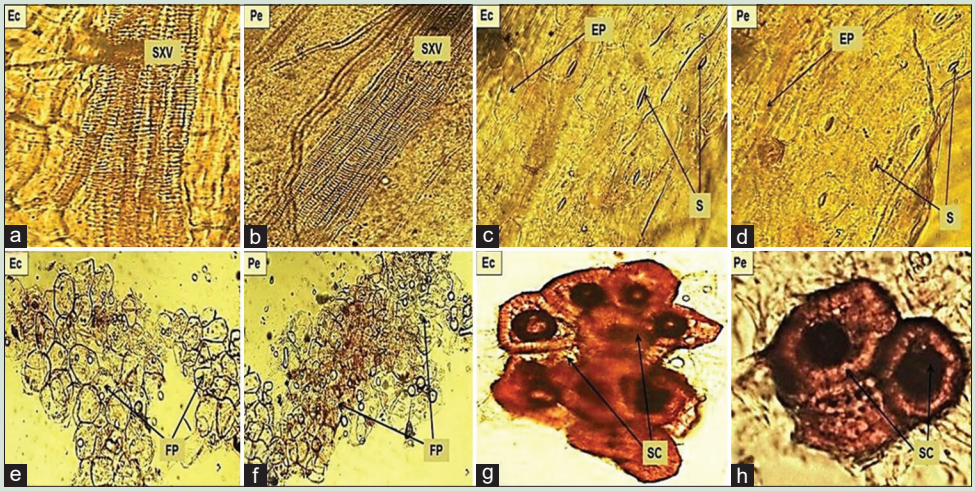

Figure 3: Micromorphological details of the powdered drugs *Corynaea crassa* from Ecuador and Peru. Ec: Ecuador; Pe: Peru; SXV: scaleriform xylem vessels (a, b); EP: epidermal cells; S: stomata (c, d); FP: fundamental parenchyma (e, f); SC: sclera cells (g, h). Photographs were taken by the authors with an optical microscope at ×10

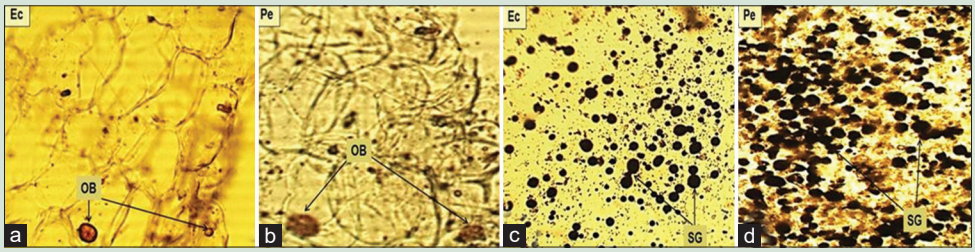

Figure 4: Histochemical reactions performed to the powdered drugs of *Corynaea crassa*. (I-J): Test Sudan (a, b); Test Lugol (c, d); Ec: Ecuador; Pe: Peru; OB oil bags; SG: starch grains. Photographs were taken by the authors with an optical microscope at × 10

peaks of relative intensity are distinguished, one around 13 min and another less intense around 42 min, the rest of the signals showed low intensity. Structures could be assigned by comparing their mass spectra with those of the team library to a total of 21 compounds, which are shown in Table 3.

For the extract obtained from the plant of Peruvian origin, a compound of high relative abundance was found, represented by a chromatographic peak around 35 min and four peaks of medium intensity around 22, 29, 30, and 32 min. The identification of the compounds by comparison of their spectra with those of the equipment library is presented in Table 4.

Determination of anti-inflammatory effect

The anti-inflammatory activity of aqueous and hydroalcoholic extracts of the species *C. crassa* was measured on the first signs that appear during acute inflammation (mainly edema during the first five hours), by the carrageenan plantar edema test, which is used currently for the evaluation of anti-inflammatory drugs. As shown in Table 5, except for the negative control (treated with NaCl), the volume of edema was decreased over time. All groups showed the highest values of edema at the first and second hours after the administration of carrageenan. From the third hour, a significant decrease in edema volumes occurs until the 50 h, which the lowest value was obtained.

The values represent the average of the edema/standard deviation volumes (n = 6); Differences letters in a column indicate significant differences $P < 0.05$ (Kruskal–Wallis y Friedman)

AE-Pe: Aqueous extract from C. *crassa* collected in Peru, AE-Ec: Aqueous extract from C. *crassa* collected in Ecuador, HE-Pe: Hydroalcoholic extract from C. *crassa* collected in Peru, HE-Pe: Hydroalcoholic extract from C. *crassa* collected in Ecuador.

When applying the Kruskal–Wallis test, it was found that treated animals with studied extracts showed significant differences ($P < 0.05$) among respect to positive (Indomethacin), but lower edema ($P < 0.05$) compared with negative control (treated with NaCl). When using the Friedman test (for the same treatment), it was observed that the volume of edema was significant ($P < 0.05$) when the different hours were compared, mainly, the first hour with the last hour. Although the indomethacin-treated group showed the highest inhibition rates mainly at 3 and 5 hr, both extracts from *C. crassa* collected in Peru and Ecuador showed a significantly reduce ($P < 0.05$) carrageenan-induced edema in the rat's leg.

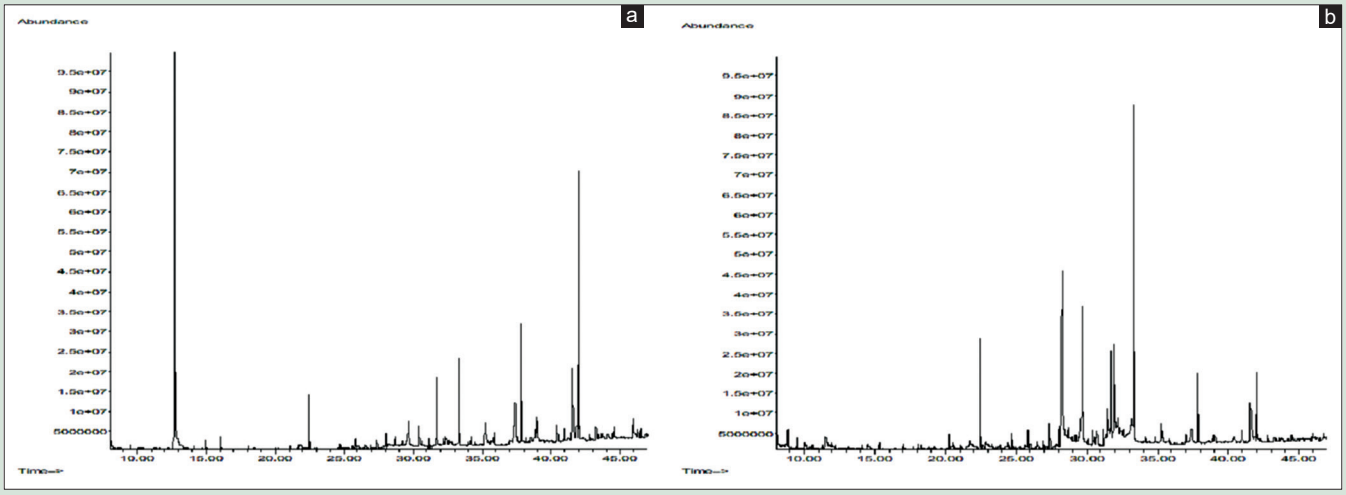

Figure 5: Analytical gaseous chromatogram of ethyl acetate extract from *Corynaea crassa* collected in Ecuador (a) and Peru (b)

Table 2: Phytochemical screening of powdered drugs from *Corynaea crassa* collected in Ecuador and Peru

Name of test	Constituents groups	Extracts					
		Petroleum ether		Alcohol		Water	
		Ec	Pe	Ec	Pe	Ec	Pe
Sudan III	Fats or oil	+	++	ND	ND	ND	ND
Dragendorff	Alkaloids	–	–	+	+	±	±
Mayer	Alkaloids	–	–	+	+	±	±
Wagner	Alkaloids	–	–	+	+	±	±
Mucilage test	Mucilage	ND	ND	ND	ND	–	–
Baljet	Coumarins/lactones	–	–	+	+	ND	ND
Liebermann-Burchard	Triterpenes/steroids	+	+	+	+	ND	ND
Foam test	Saponins	ND	ND	++	+	+	+
Resin test	Resins	ND	ND	–	–	ND	ND
Ninhydrin	Amino acids	ND	ND	±	±	ND	ND
Fehling test	Reducing sugars	ND	ND	+	+	++	++
Ferric chloride test	Phenols/tannins	ND	ND	++ Dark green	++ Dark red	+ Dark green	+ Dark red
Anthocyanins	HCl conc./pentanol	ND	ND	++	+	ND	ND
Böntrager	Quinones	ND	ND	++	+	ND	ND
Shinoda (Mg-HCl)	Flavonoids	ND	ND	++	+	++	++
Catechin test	Catechins	ND	ND	+	+	ND	ND

Ec: Ecuador; Pe: Peru; -: Negative; +: Positive; ++: Highly positive; ±: Indefinite test; ND: Not done

Table 3: Chemical composition of ethyl acetate extract from *Corynaea crassa* collected in Ecuador

Peak number	RT	Compounds	Formula	Molecular weight (g/mol)	Content %/SD
1	12.709	Safrole [5-(2-propenyl)-1,3-benzodioxole]	$C_{10}H_{10}O_2$	162.19	43.58/0.93
2	14.924	α-Copaene	$C_{15}H_{24}$	204.36	0.63/0.01
3	16.043	β-Caryophyllene	$C_{15}H_{24}$	204.36	1.00/0.01
4	16.943	α-Humulene	$C_{15}H_{24}$	204.35	0.04/0.00
5	18.007	α-Muurolene	$C_{15}H_{24}$	204.36	0.04/0.00
6	18.479	δ-Cadinene	$C_{15}H_{24}$	204.35	0.08/0.00
7	20.006	Caryophyllene oxide	$C_{15}H_{24}O$	220.36	0.16/0.01
8	24.693	1-Octadecene	$C_{18}H_{36}$	252.48	0.49/0.00
9	26.527	1-Nonadecene	$C_{18}H_{36}$	252.48	0.65/0.01
10	27.374	Methyl palmitate	$C_{17}H_{34}O_2$	270.45	0.85/0.20
11	28.198	Palmitic acid	$C_{16}H_{32}O_2$	256.40	2.09/0.26
12	30.416	Docosanol	$C_{22}H_{46}O$	326.61	2.54/0.16
13	30.667	(*E*)- Methyl oleate	$C_{19}H_{36}O_2$	296.49	0.85/0.19
14	31.168	Methyl Stearate	$C_{19}H_{38}O_2$	298.51	0.61/0.17
15	32.386	1-Docosene	$C_{22}H_{44}$	308.59	1.03/0.03
16	34.203	Tricosane	$C_{23}H_{48}$	324.63	0.73/0.03
17	35.213	(*Z*)-9-Octadecenamide	$C_{18}H_{35}NO$	281.47	4.67/0.06
18	40.447	Eicosane	$C_{24}H_{50}$	338.65	1.68/0.04
19	41.541	(*Z*)-13-Docosenamide	$C_{22}H_{43}NO$	337.59	11.75/0.95
20	41.992	Squalene	$C_{30}H_{50}$	410.73	22.95/0.91
21	45.965	Ergosterol	$C_{27}H_{46}O$	386.66	3.55/0.41

RT: Retention time; SD: Standard deviation

Table 4: Chemical composition of ethyl acetate extract from *Corynaea crassa* collected in Peru

Peak number	RT (min)	Compouds	Formula	Molecular weight (g/mol)	Content %/SD
1	8.856	Camphor	$C_{10}H_{16}O$	152.23	2.08/0.08
2	15.355	(*E*)-2-tetradecene	$C_{14}H_{28}$	196.378	0.81/0.02
3	20.257	1-hexadecene	$C_{16}H_{32}$	224.432	1.87/0.03
4	24.687	1-octadecene	$C_{18}H_{36}$	252.486	1.77/0.05
5	27.368	Methyl palmitate	$C_{17}H_{34}O_2$	270.457	2.61/0.14
6	28.248	Palmitic acid	$C_{16}H_{32}O_2$	256,400	40.18/1.28
7	30.521	(*Z, Z*)-Methyl linoleate	$C_{19}H_{34}O_2$	294.479	1.24/0.04
8	30.655	(*E*)-Methyl oleate	$C_{19}H_{36}O_2$	296.488	1.48/0.04
9	31.162	Methyl stearate	$C_{19}H_{38}O_2$	298.511	1.80/0.01
10	31.445	(*E*)-Oleic acid	$C_{18}H_{34}O_2$	282.470	10.04/0.56
11	31.916	Stearic acid	$C_{18}H_{36}O_2$	284.480	19.82/1.39
12	35.235	(*Z*)-9-Octadecenamide	$C_{18}H_{35}NO$	281.477	6.57/0.35
13	38.973	Heptacosane	$C_{27}H_{56}$	282.550	0.88/0.02
14	41.982	Squalene	$C_{30}H_{50}$	410.730	7.39/0.16
15	45.973	Ergosterol	$C_{27}H_{46}O$	386.664	1.45/0.00

RT: Retention time; SD: Standard deviation

Table 5: Average volume of plantar edema over time of Wistar albino rats treated with studied products and carrageenan solution (inflammation inducing agent)

Groups	Edema volume±SD (mL)*			
	1 h	2 h	3 h	5 h
A (AE-Pe)	0.74±0.03[a]	0.72v±0.02[a]	0.65±0.05[a]	0.60±0.03[a]
B (AE-Ec)	0.73±0.06[a]	0.70±0.04[a]	0.64±0.03[a]	0.59±0.04[a]
C (HE-Pe)	0.75±0.04[a]	0.71±0.04[a]	0.64±0.04[a]	0.56±0.05[a]
D (HE-Ec)	0.75±0.03[a]	0.70±0.04[a]	0.63±0.05[a]	0.57±0.03[a]
E (Indomethacin)	0.62±0.03[b]	0.57±0.06[b]	0.48±0.02[b]	0.35±0.04[b]
F (NaCl 0.9%)	0.80±0.02[c]	0.83±0.02[c]	0.96±0.02[c]	1.03±0.03[c]

SD: Standar desviation; different letters indicate significant differences for $P<0,05$, n=3

DISCUSSION

Although medicinal plants have been used for many centuries, only a relatively small number of species have been studied for possible medical applications.[10] To perform the effective integration of plants in a medical system, researchers and professionals must be trained in their correct handling, for which it is necessary to carry out studies that allow converting empirical knowledge into arguments based on evidence.[11]

Corynaea crassa has conventionally been used for its aphrodisiac property. However, scientific information about the plant is scarce, so it is very important to carry out pharmacognostic and phytochemical studies that help to establish its quality.

The macroscopic and microscopic studies of plant drugs are important tools for the interpretation of similarities and differences between them. In the present study, differences were observed in the size of the plants, due to different ecological factors that could have influenced the development of the same.

The fundamental peculiarity of this species is its classification within hemiparasitic plants[1,12] due precisely to the presence of modified roots called haustoria. These plants have chlorophyll and partially synthesize the elements necessary for their nutrition; they are usually fixed to the xylem, tissue that carries water and inorganic nutrients.

The microscopic analysis showed no structural changes between the powdered drugs of both countries, which could suggest that it is the same species. The

micromorphological results of *C. crassa* have not been previously reported, so they constitute an interesting contribution to the study of the plant.

In the determination of the physical-chemical parameters, moisture content was observed within the required range (8%–14%). The largest soluble extractives were obtained for the hydroalcoholic mixture at 80%, being higher in the sample from Ecuador. The previous results suggest that the drugs have a high composition in metabolites of medium polarity.

Ash values are used to determine the identity and purity of plant drugs and indicate the presence of various impurities such as carbonates, oxalates, and silicates. Water-soluble ashes are used to estimate the amount of inorganic compound present and those insoluble in acid are mainly related to silica.[13,14]

Some pharmacopoeias have a total ash index of up to 5%[6,15] and another such as the Chinese Pharmacopoeia, which refers to 15%,[16] although the values can vary significantly depending on the plant material and collection site. The value obtained for both drugs was different, being higher for the one coming from Ecuador; nevertheless, they are framed in the limit established by the current pharmacopoeia, although it would be advisable to carry out more in-depth analyzes by quantifying metals in the plant with the use of atomic absorption spectrophotometry.

The amount of ash soluble in water and insoluble in 10% hydrochloric acid are also parameters that help to assess the purity of the drug. The water-soluble ashes are indicative of the presence of alkaline and alkaline earth metals, while those insoluble in acid may be related to the presence of heavy metals. In the last determination, the value exceeds 2% (limit established by the norms and Pharmacopoeias) for the sample from Ecuador, for which studies are suggested to know the chemical composition of said ashes.

Some statistically significant differences were found between the two samples, which may be attributable to the characteristics of the soil, in which the plant was developed, where the availability of nutrients may vary from one place to another.

The presence of triterpenoids and steroids detected by phytochemical screening agrees with that reported by Malca *et al*.[4] for the species, which isolated from hexane extracts of tubers and roots, β-sitosterol, lupenone, amirone, lupeol, and amirine. It has been reported that triterpenoids have shown several pharmacological effects such as anti-inflammatory, anti-ulcer, antibacterial, antiviral (including anti-HIV), hepatoprotective, immunomodulatory, hypolipidemic and to reduce cholesterol, anticoagulant, anticancer, etc.[17] Sterols have been investigated as one of the possible safe alternative methods to reduce plasma cholesterol levels and LDL cholesterol. On the other hand, evidence has been discussed of the beneficial effects of plant sterols in disorders such as cutaneous xanthomatosis, colon cancer, and prostate hyperplasia.[18,19]

The alcoholic extract [Table 3] responded positively to almost all phytochemical assays. The concentrations of phenolic compounds (flavonoids and tannins), in general, were surprising, which showed very positive results for both collections. These compounds were detected in previous studies carried out on extracts of the species.[4]

Phenolic compounds have demonstrated antioxidant activity, hepatoprotective, are anti-inflammatory, antitumor, antiviral, prevent coronary heart disease,[20,21] have analgesic properties[22-24] among other activities.

Other compounds detected in the hydroalcoholic extract were the alkaloids. They are particularly interesting substances due to their multiple pharmacological activities. They have activity on the central nervous system, where they are depressants or stimulants; they act on the autonomic nervous system with sympathomimetic or sympatholytic, parasympatholytic, anticholinergic, or ganglioplegic effects.[25] Others may act as potent analgesic agents, hypertensive agents, amebicides, virucides,[26] antitussives, and antispasmodics.[14,27] However, the abundant presence of lactonic and phenolic compounds may have resulted in a false positive for Baljet and $FeCl_3$ tests, respectively.

It is important to point out that there were no differences in the qualitative chemical composition determined by phytochemical screening in the batches evaluated. However, the color intensities for some tests, very clearly demonstrate the presence of compounds of phenolic nature, reducing substances, triterpenoids and steroids, as well as abundant saponins in the aqueous extract.

In the GC-MS analysis of the ethyl acetate extract of the drug from Ecuador, the major component turned out to be safrole, a constituent compound of several essential oils such as basil (*Ocimum basilicum* L), cinnamon (*Cinnamomum zeylanicum* J. Presl), nutmeg (*Myristica fragrans* Houtt.), pepper (*Piper nigrum* L.) and the sassafras root (*Sassafras albidum* (Nutt.) Nees), where it acts as a biological insecticide.[28,29] It is part of the list of controlled substances by the authorities due to its use as a precursor in the synthesis of drug use as MDMA[30] and its carcinogenic and cytotoxic effects.[31] However, it is useful in the synthesis of compounds with high biological activity.[32]

The other compound with relative abundance (11.64%) turned out to be squalene, acyclic triterpene very abundant in the vegetable and animal kingdom, and biogenetic precursor of steroids and tetracyclic and pentacyclic triterpenoids, which is attributed a powerful antioxidant action.[14]

The presence of various sesquiterpenoids is part of this extract, although in the low percentage of abundance, as well as of various free and esterified fatty acids, hydrocarbons and fatty acid amides, is striking in this extract.

Most components of the extract of Peruvian origin could not be identified by comparison of their spectra with that of the team's library Wiley 9th and NIST (2011). Of the 15 compounds identified, the most abundant were hexadecanoic acid (palmitic), octadecanoic acid (stearic acid) and 9-octadecenoic acid (oleic acid). Squalene, cholesterol was also found in this extract and camphor, a natural terpenoid, was found as a distinctive component, with a limited presence in nature.

From the quantitative point of view, differences are observed, standing out the Peruvian species for containing higher concentrations of fatty compounds and Ecuadorian triterpenes and steroids, aspects observed in the phytochemical screening.

Being the parasitic species of the roots of other plants, their chemical composition can vary according to the host they parasitize. Tupac *et al*.[1] in a study conducted on the species that grows in Costa Rica and found it parasitizing species of different families such as Papaveracea, Asteracea, Cucurbitacea and Rubiacea, which could explain the differences found in the chemical composition of those from Peru and Ecuador.

The carrageenan plantar edema test used to quantify the anti-inflammatory effect of products, constitute a reproducible and simple assay. Two of the most characteristic parameters of inflammation are edema and plasma extravasation by inducing an acute inflammation located in the animal's leg after the administration of carrageenan in the plantar aponeurosis of the mouse or rat.[33] Indomethacin is frequently used as a positive control in this experimental method, as it inhibits cyclooxygenases (COX) and therefore inhibits the formation and release of prostaglandins that in the second phase (between 3 and 4 h) acquire their maximum manifestation, mainly PGE2.[34]

The results of the study are in correspondence with studies carried out by Siddalingappa[35] to aqueous extracts of *Tinospora cordifolia* (Lour.) Merr., where they show that the highest inhibition rates are achieved after the third hour. A similar study conducted by Sivakumar *et al*.[36] to

an extract of the *Chloroxylon sweitenia* DC. leaves, using Indomethacin as a reference drug, also showed that maximum edema inhibition was achieved from the third hour of the test. In accordance with these studies is the research developed by Kumari *et al*.[37] to the species *Sarcostemma secamone* L. who demonstrated that from the third hour, the lowest volume of edema of the groups treated with hydroalcoholic extracts at different doses and the positive control (indomethacin) is achieved.

As the model tested in the present investigation is related to the activation of mast cells, the release of cynins, mediators such as histamine, derivatives of arachidonic acid and reactive oxygen species, the evaluated extracts could contain molecules with modulation capacity on said targets.[38] It has been shown that tannins, flavonoids, and saponins are well known for their ability to inhibit pain perception and have anti-inflammatory properties due to inhibition of enzymes involved in inflammation, especially the metabolic pathway of arachidonic acid and synthesis of prostaglandins.[39-41] Flavonoids are present in any part of the plant and have an important role in inflammatory processes. Many (genistein, apigenin, kaempferol, myricetin, among others), are inhibitors of COX and nitric oxide, which is a pleiotropic mediator of inflammation. Others, such as rutin and quercetin, inhibit the metabolism of arachidonic acid, thus preventing the increase in prostaglandins. In the carrageenan-induced edema test, these flavonoids have shown high anti-inflammatory activity.[42,43]

Triterpenoids and saponins (steroidal of the type hespirostane or furostane and the triterpene ones with oleanane, ursane and dammarane nucleus) also contribute to the anti-inflammatory effect, due to the inhibition of prostaglandin synthetase, reducing the level of prostaglandins in the inflammatory process.[44] Some of these compounds were found in the phytochemical analysis and/or in the chromatographic analysis, which supports the results obtained in this test.

CONCLUSION

The micromorphological and physicochemical results reported in this study are described for the first time for the species. The chemical components reported, although known, have not been reported previously, so they constitute a new report for it. Data provided herein contribute to the standardization of *C. crassa* as a medicinal plant-derived product. The anti-inflammatory activity of aqueous and hydroalcoholic extracts of *C. crassa* on the carrageenan-induced acute inflammation model was demonstrated. The extracts showed similar anti-inflammatory behavior, although less than the indomethacin used as a positive control.

Acknowledgements

To PhD. Lianet Monzote-Fidalgo and PhD. William Setzer for their collaboration in the revision of these work.

Biotechnological Research Center of Ecuador, ESPOL Polytechnic University, for the support provided in chromatographic analyzes.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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