Short communication

Caracterización fitoquímica preliminar de Heliotropium curassavicum y H. veronicifolium de Tucumán con interés farmacológico en producción caprina

Preliminary phytochemical characterization of Heliotropium curassavicum and H. veronicifolium from Tucumán with pharmacological interest in goat production

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Abstract

In Argentina, goat farming represents an important agricultural activity, being Tucumán a province with productive areas represented by Trancas, Tafí del Valle and Graneros departments. Among biotic diseases that affect this activity, infectious diarrhoea stands out caused by Escherichia coli. Emergent bacterial resistance to antibiotics mainly used in livestock production affects their profitability and leads researchers to consider alternative therapies based on metabolites from plants. Boraginaceae plant family produces a large amount of secondary metabolites, such as alkaloids, and phenolic compounds with well-known antibacterial properties, standing out Heliotropium genus. In our laboratory, preliminary studies on extracts of three species of Heliotropium from Tucumán demonstrated antimicrobial activity against bacteria related with infectious diarrhea in goats and food-borne pathogen. In this work, chemical composition of H. curassavicum (from Tapia and Santa Maria areas) and H. veronicifolium (from Tapia area) extracts, was determined with the aim of characterizing them and inferring presence of compounds families with antimicrobial potential. The extractions were carried out with ethyl ether, isopropanol-water and methylene chloride. After a comparative analysis considering plant species and collection area, some variations were found. Polar compounds showed the highest yields and FT-IR reveal similar functional group profile in all extracts related with phenols, tannins, flavonoids, triterpenes, steroids, and alkaloids. The results obtained will allow a further deeper study of antimicrobial activity of fractions enriched in phenolic compounds and alkaloids in accordance with the extensive bibliographic evidence supporting their biological activities.

Key words: *Boraginaceae*; Goat farming; *Heliotropium curassavicum*; *Heliotropium veronicifolium*; Phytobiotics

Resumen

En Argentina, la ganadería caprina representa una actividad agrícola importante, siendo Tucumán una provincia con áreas productivas en los departamentos de Trancas, Tafí del Valle y Graneros. Entre las enfermedades bióticas que afectan esta actividad destaca la diarrea infecciosa causada principalmente por Escherichia coli. La resistencia bacteriana emergente a los antibióticos utilizados en producción ganadera afecta su rentabilidad y lleva a los investigadores a considerar terapias alternativas basadas en metabolitos de plantas. La familia vegetal Boraginaceae produce numerosos metabolitos secundarios, como alcaloides y compuestos fenólicos con reconocidas propiedades antibacterianas, destacándose el género Heliotropium. En nuestro laboratorio, estudios preliminares sobre extractos de tres especies de Heliotropium de Tucumán demostraron actividad antimicrobiana contra bacterias relacionadas con diarrea infecciosa en cabritos y patógenos transmitidos por alimentos. En este trabajo, se determinó la composición química de extractos de H. curassavicum (recolectado en zonas de Tapia y Santa María) y H. veronicifolium (Tapia), con el objetivo de caracterizarlos e inferir la presencia de familias de compuestos con potencialidad antimicrobiana. Las extracciones se realizaron con éter etílico, isopropanol-agua y cloruro de metileno, y se encontraron variaciones, al realizar análisis comparativo considerando especies de plantas y área de recolección. Los compuestos polares mostraron los rendimientos más altos y el FT-IR reveló un perfil de grupos funcionales similar en todos los extractos asociados con fenoles, taninos, flavonoides, triterpenos, esteroides y alcaloides. Los resultados obtenidos permitirán aportar al estudio más profundo de las fracciones extraídas enriquecidas en compuestos fenólicos y alcaloides asociados a actividad antimicrobiana debido a la amplia evidencia bibliográfica que respalda sus actividades biológicas.

Palabras clave: *Boraginaceae*; Cría de caprinos; *Heliotropium curassavicum*; *Heliotropium veronicifolium*; Fitobióticos.

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Development of goat production in Argentina has 4,280,903 heads of goats (FAOSTAT, 2021) for meat consumption, mainly in semi-arid areas of Argentinean Northwest. Thus, Tucumán contributes with 15% of national production and shows productive areas basins in Trancas (26°04'06.9" S, 65°19'40.6" W), Tafí del Valle (26°56'58.4"S, 65°40'36.4"W) and Graneros (27°38'06.5"S, 65°02'30.7"W) Departments (UEDP, 2020).

Infectious gastroenteritis (IG) is a significant condition that can impact 'creole' goat and sheep livestock production, causing illness in newborns (INTA, 2011). Its incidence remains relatively at low levels but is critically dependent on multifactorial sanitary conditions: intrinsic factors of animal (physiological and immune status) and extrinsic factors (feeding, rearing conditions, overcrowding, and excessive heat or humidity). Baby goats and lambs dehydrate quickly and pass away if they are not treated. IG presents a non-discriminatory pathogenic profile, affecting small ruminant populations (goats and sheep) indiscriminately, without demonstrating breed-specific predilection (Osman et al. 2013). Among the most common microorganisms causing IG are E. coli and Salmonella spp. (Mishra et al., 2019; Navruzov, 2024). In this context, phytochemicals emerge as an alternative to conventional antibiotics for disease treatment, offering a natural therapeutic approach.

In *Boraginaceae* family secondary metabolites such as phenolic compounds and alkaloids with antioxidant and antimicrobial activity would be found (Ozntamar-Pouloglou et al., 2023; Singh and Sharma, 2015; Dresler et al., 2017).

An important genus within *Boraginaceae* family is *Heliotropium*. In Tucumán, *Heliotropium* species were reported in Tafí del Valle, Trancas and Burruyacú (Di Fulvio et al., 2016), among which *H. curassavicum* was found in the first two regions and H. veronicifolium in the second one (Danesi et al., 2020).

In studies of *Heliotropium* species, phenols and alkaloids were detected as main constituents (Mughal et al., 2010; Fayed, 2021) and were also found to exhibit antimicrobial activity against a wide range of pathogens (Singh et al., 2002; Mughal et al., 2010). Preliminary antimicrobial activity on *E. coli* strains isolated from goats and *Listeria monocytogenes* was demonstrated in our laboratory. Thus, a significant bacterial growth inhibition was evidenced, and because of that an effectively reducing of chemical preservative concentrations in processed meat by approximately 50% (Danesi, 2021; Santillan, 2022). These background guides the research to conduct a deeper study of the chemical composition aimed at developing a phytobiotic.

For the present work, *H. curassavicum* (HC) was collected from Tucumán province in Trancas (HCT) (locality of Tapia, 795 m.a.s.l.; 26° 54' 3" S, 65° 30' 23" W) and Tafí del Valle (HCA) (locality of Amaicha del Valle, 1850 m.a.s.l.; 26° 39'10" S, 66° 3'23''' W), while *H. veronicifolium* (HV) was only found and collected in Trancas (Di Fulvio and Ariza Espinar, 2016) (HVT).

Plant material of both HC (from both locations) and HV was dried at room temperature and protected from sunlight, then aerial parts were recovered and grounded.

Two solid-liquid extractions of *H. curassavicum* and *H. veronicifolium* were carried out with each solvent or solvent mixture, firstly with ethyl ether (EE), for low polarity compounds; isopropanol-water (65-35%) (IW), for middle and high polarity compounds and finally, IW extract was subjected to liquid-liquid extraction with methylene chloride, to obtain a sub-extract of intermediate polarity compounds (SMC). Data from three independent replications and expressed as mean ± standard error were registered. An ANOVA assessment to compare extraction yield means was performed and Fisher Test was applied to identify differences between media values ($p \leq 0.05$). Factorial designs were conducted to examine how the factors extract type and plant species interacted in their influence on extractive yield. In all cases for performance analysis, distribution and uniformity of data were analyzed and adjust with varConstPower if it corresponded. For Statistical analysis using InfoStat software were performed (Di Rienzo et al., 2018).

Table 1 shows extractive yield for HCT. There was a significant effect of the extraction method over yield (F= 838; DF= 2; p <0.0001; CV= 4.98). Total plant material processed, an 8.72% was recovered spread in the different extracts. The amount recovered of IW extract was 38.08±0.15 g (5.45%) and was significantly higher than 22.95±0.05 g in EE (3.28%), while the SMC subextract represented only 0.36% (2.52±0.10 g).

Extract	Final weight (g)	Percentage yield	Aggregate yield
Ethyl Eter	22.95±0.05^{b}	3.28%	3.28%
Isopropanol-water	38.08±0.15^{c}	5.45%	8.72%
Methylene Chloride	2.52±0.10^{a}	0.36%	-

Table 1. Performance of *H. curassavicum extracts* from Tapia. The values shown represent the mean value of three independent trials ± standard error. $^{a-c}$: Different letters indicate significant differences (p ≤ 0.05) according to Fisher's Test (p ≤ 0.05). Direction of arrow represents cumulative yield value considering total weight of dry plant material.

Extract	Final weight (g)	Percentage yield	Aggregate yield
Ethyl Eter	22.09±0.07^{c}	3.15%	3.15%
Isopropanol-water	18.48±0.15^{b}	2.64%	5.79%
Methylene Chloride	5.2±0.08^{a}	0.74%	-

Table 2. Performance of *H. curassavicum* extracts from Amaicha del Valle The values shown represent the mean value of three independent trials ± standard error. $^{a-c:}$ Different letters indicate significant differences (p ≤ 0.05) according to Fisher's Test (p ≤ 0.05). Direction of arrow represents cumulative yield value considering total weight of dry plant material.cumulative yield value considering total weight of dry plant material.

Table 2 shows extractive yield for HCT. There was a significant effect of the extraction method over yield (F = 1935.64; DF= 2; p <0.0001; CV= 42.3). Low polarity compounds (EE) showed the highest extraction yield (22.09±0.07 g, 3.15%) compared to high-polarity compounds in IW (18.48±0.15 g, 2.64%) and medium-polarity compounds (SMC extract) which showed 28.13% (5.2±0.08 g; 0.74%) of total yield. Complete extractive process showed a 5.79% of yield in dry weight.

Table 3 shows extractive yield for HCT. There was a significant effect of the extraction method over yield (F = 1907.98; DF= 2; p <0.0001; CV= 2.28). The highest yield value (24.75±0.18 g, 3.53%) for IW extract was showed compared to EE (10.45±0.20 g, 1.49%) and SMC (1.35%, 9.43±0.09 g).

Figure 1 shows extraction yield with each solvent respect to type of plant (HC or HV) in Tapia collection area. It was observed that the interaction plant species*type of extract significantly influenced the results (F= 321.49; DF= 2; p <0.0001; CV: 4.32), being the highest IW yield, mainly for HCT (IWm= 0.05 ± 0.01) followed by IW HVT (IWm= 0.04 ± 0.01). Then, yield for EE was 0.03 ± 0.01 and 0.01 ± 0.01 for HCT and HVT respectively. Finally, the lowest recovery was in SCM.

A similar analysis was performed for extraction yield of H. curassavicum from Amaicha and Tapia (Figure 2). For second time, influence of interaction collection area*extract type was observed (F= 213.63; DF= 2; p <0.0001; CV: 4.29). The highest yield corresponded to IW HCT extract (IWm= 0.05 ± 0.01), followed by the EE extracts of HCT and HCA which did not present significant differences between them (0.03 ± 0.01). The lowest yields corresponded to SMC extracts (<0.01 ± 1. 10-4). In addition, FTIR technique was conducted in extracts to analyze presence of different functional groups associated with representative molecules based on the literature consulted for Boraginaceae family. Different absorption bands and chemical groups are shown in Table 4, in which a similar band patterns for Heliotropium species under study, highlighting presence of chemical groups associated with the structure of phenolic compounds and alkaloids (non-displaying IR spectrum) can be observed.

Results of this work allowed us to study the extractive yield and relative abundance of compounds of different polarities, as well as

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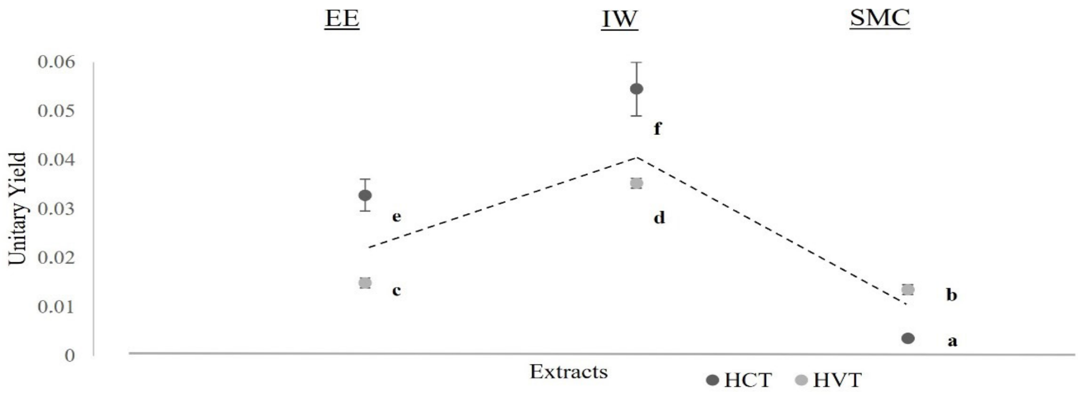

Figure 1. EE: ethyl ether, SMC: sub-extract with methylene chloride, IW: isopropanol-water. a-b: different letters indicate significant differences in medium values for interaction plant species*type of extract considering Tapia collection area. Dotted line indicates a trend between medium yield of each extract for Heliotropium species.

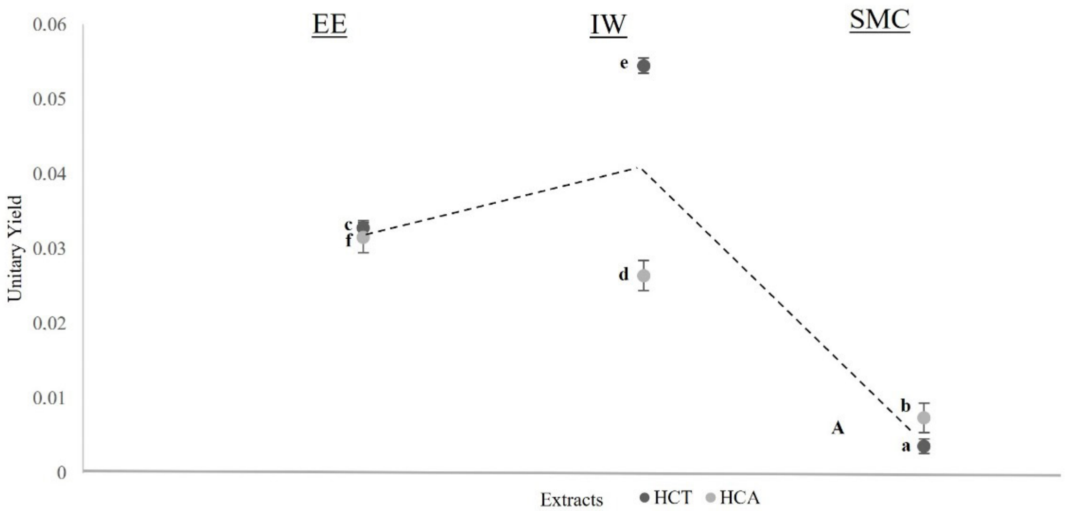

Figure 2. EE: ethyl ether, SMC: sub-extract with methylene chloride, IW: isopropanol-water. a-b: different letters indicate significant differences in medium values for interaction collection area*extract type considering H. curassavicum. Dotted line indicates a trend between medium yield of each extract for both collection area (Tapia and Amaicha del Valle).

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Table 3. Performance of *H. veronicifolium* extracts from Tapia. The values shown represent the mean value of three independent trials ± standard error. [a-c]: Different letters indicate significant differences ($p \leq 0.05$) according to Fisher's Test ($p \leq 0.05$). Direction of arrow represents cumulative yield value considering total weight of dry plant material.

Absorption bands and chemical groups	Extract EE (cm-1)	Extract IW (cm-1)	Sub-extract SMC (cm-1)
ν OH (inter- or intramolecular)	3,437.20	3,410	3,453.80
ν t diffuse and broad t. N-H amines	3,200	Masked by the wide band of OH	3255.4
ν t sym. and asym. CH3 two or three strectching bands	3,934.3 & 2,869.3	2,930.3 & 2,880	2,909.3 & 2856
ν C=O ketone or aldehyde	1,775.20	1,636.40	1,648.10
C=C conjugated alkene of the benzene ring	1,558.10	1,555	1,558.30
ν asym C-O ether forming ring or aryl ether	1,370	1,398.60	1,376.00
ν sym C-O ether forming ring or aryl ether	1,036	1,045.70	1,039.60
ν asym C-O-C aryl or cyclic ether	915.8	910	905.4
ν out-of-plane deformed =C - H benzene ring	756.6	750.1	746.6

Table 4. ν: vibrational wave frequency; t: tension band; asym: asymmetric; sym: symmetric

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Results of this work allowed us to study the extractive yield and relative abundance of compounds of different polarities, as well as

to identify functional groups and families of compounds in Heliotropium species from Tucumán, highlighting the high-polarity compounds, mainly in plants from Tapia. This would lead us to report a phytochemical profile rich in phenolic compounds and alkaloids that could be associated with antimicrobial activity previously observed by our laboratory group and would allow us to intensify actions related to the preliminary design of a phytobiotic product for livestock production.

Aknowledgments

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