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Obtaining of Polar Organic Extracts of *Agave angustifolia* and its Evaluation Antibacterial and Molluscicide

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Abstract: The *Agave angustifolia* is a plant native to Mexico, commonly used for the production of mezcal and as an ornamental. It is well known that agaves, have active substances, a product of secondary metabolism like a steroidal saponins, which can be toxic to some types of pathogenic microorganisms. The present work describes the selective extraction of alcoholic phase fiber *Agave angustifolia*, their biological evaluation against bacterial strains in order to determine the effectiveness as inhibitors of bacterial growth and further it is isolated phytocompounds molluscicide evaluation as determining the LD_{50} and LD_{90} by an empirical Probit regression analysis.

In the molluscicide evaluation the LD50 is 0.015g/20ml±0.003 and LD90 corresponds to 0.214g/20mL±0.056. Regarding its antimicrobial activity has the following zones of inhibition (mm) and inhibition percentage (%IR) based on the strain tested: *S. epidermidis* 15mm/20%, *S. saprophyticus* 15mm/23.5%, *S. aureus* 28mm/17% and *Pseudomona aeruginosa* 10mm/20.9%. This Polar extracts showed molluscicidal effects on a populations of *Fossaria obrussa*. Furthermore quantitatively established high antimicrobial activity against strains of environmental origin.

Keywords: *Agave angustifolia, polar extracts, molluscicide, antimicrobial*

INTRODUCTION

The search for new molecules, has taken a different route where the science of ethnobotany and ethnopharmacognosy are used as a guide to direct the chemical towards different sources and kinds of compounds. It is in this context that the flora of our country and its diversity has an important role to play by providing a huge range of products that can be evaluated against different medical needs[1].

Today, there are a lot of herbal medicine widely demonstrated in clinical use as: anticancer, antibacterial, antifungal, antihypertensive, retroviral, immunostimulants, antispasmodics, and molluscicides [2, 3].

Currently are being sought new molecules as a therapeutic alternative to face medical needs, such as vector-borne parasitic diseases. An example is the snail *Fossaria obrussa*, vector of *Fasciola hepatica* affecting humans producing fasciolosis [4, 5]. As it has been demonstrated that the application of synthetic molluscicides seriously affects the habitat of these vectors, acting as residual biocides that eliminate flora and fauna associated with snails that you want to delete. In consequence the interest is has increased by the study of extracts or products isolated from plants, in the hope that they may be cheap, effective, less polluting and which may be applied with simple techniques[6].

On the other side is the ineffectiveness of treatments against infections with the use of common antibiotics. This is the cause for such diseases emerge and re-emerge with pathogenic resistance against multiple drugs; such is the case of *Pseudomonas* spp and *Staphylococcus* spp, among others[7]. This is why it has increased interest in the study of natural agents that inhibit bacterial growth and have molluscicide effect, hoping to be more effective, cheaper and less polluting[8].There are more than 50 compound molluscicides which have been isolated from plants including saponins, terpenoids, naphthoquinones and flavonoids, tannins[9]. Among the alternatives being explored is considered the development of these and their application without damage to the environment that are easy to obtain, that the cost does not disturb the economy of communities affected by parasitic infections[10].

Some Agavaceae have been tested as a biological and chemical control of parasitic and bacterial diseases[4], but it is necessary to assess the toxic damage to ensure that the application of these products do not alter the environment of the organisms that inhabit the bodies of water. A total of 166 plant species included in the genus Agave worldwide, 125 are native to Mexico; therefore, it is to this country as the center of origin of the genre[11]. The *Agave angustifolia* is a plant native to Mexico, commonly used for the production of mezcal and as an ornamental. It is well known that agaves, have active substances, a product of secondary metabolism, which can be toxic to some types of pathogenic microorganisms [8, 12-16].

The present work describes the selective extraction of alcoholic phase fiber *Agave angustifolia*, their biological evaluation against bacterial strains in order to determine the effectiveness as inhibitors of bacterial growth and further its isolated phytocompounds molluscicide evaluation as determining the LD50 and LD90 by an empirical Probit regression analysis.

METHODS

Samples of *Agave angustifolia* were obtained in the town of Santa Catarina Minas, corresponding to the State of Oaxaca. It is located in the central part of the State, in the coordinates 16 ° 47´ of 38´ 96 ° of North latitude and longitude West, at an altitude of 1,560 meters above sea level. There were brought the necessary samples of agaves to the laboratory of Parasitology and Vector of the school of Biology of the University Autónoma

de Puebla. The identification of the used Agave was corroborated and stalks or leaves are placed in a dryer for 8 weeks, to avoid moisture and fungus formation. Once dry the leaves, fiber is subjected to extraction method Soxhlet using ethanol as a solvent. After filtration, the crude extract is concentrated up to two-thirds of its initial volume and becomes filtered to remove non-soluble residue. It leads to dryness by distillation under reduced pressure and prepare solutions problem from 1 g of solute in 20 ml of water and from there further dilutions are made.

Biological material: The mollusk collection was performed manually during the months of July to October 2012, canals and bodies of water in the community of Huililco, located to the South of the State of Puebla, in the coordinates 18° North 47´latitud 98 ° 27´longitud West, at an altitude of 1600msnm. Once collected, are transported and were identified and adapted to its massive breeding[17].

Molluscicides bioassays. A standard solution was prepared with freeze dried Agave product and sterile at the rate of 1 g/20mL.A distilled water starting from this were dilutions in proportions of 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128. We assessed total 560 molluscs of 14 days of hatching, divided into seven treatments of 80 bodies and control group, in 4 repetitions that were carried out on different dates. The surveillance was carried out every 2 hours during 24 hours, 12 hours and finally to 36 hours. The observation was carried out under stereoscopic microscope, placing cardiac and physical molluscum activity until they cease. Molluscs dead at this time were counted and survivors assessed them until his death.

Determination of the lethal dose. It was determined the value of the lethal dose to 50% and 90% of the toxicity of the agave on gastropods through empirical *Probit* regression, using the program[18] Sigma Plot 9.0. This analysis is based on quantifying probability of vulnerability of animal populations exposed to toxic agents via any route less inhalation, and gives a confidence interval.

Antibacterial assay. For the evaluation of antimicrobial activity, were taken into account the specifications of the Clinical and Laboratory Standards Institute (CLSI, 2011). The tests were carried out in triplicate. The alcoholic extract of *Agave angustifolia* was tested for antimicrobial activity by agar well-diffusion method against pathogenic bacteria *Pseudomona aeruginosa* (Gram-negative), and two bacteria Gram-positive: *Staphylococcus epidermidis* and *Staphylococcus saprophyticus* environmental origin, and one strain of *S. aureus* ATCC 25923 as a control. Mueller Hinton agar was used, 50 ul was added to each well, and the plates were incubated at 37 ° C for 18-24 hrs.

RESULTS

Obtained 3.2g of the crude extract (CE), were observed as a dark brown oil, dense, charactestic sweet smell. It valued the presence of saponins steroidal giving all the qualitative tests as positive[7].

The spectrum of FT-IR shows characteristic absorption peaks corresponding to saponins steroidal at 3394 cm^{-1} the vibration of bond O-H, at 2946 cm^{-1} assigned to stretching bond C_{sp3}-H, at 1608 cm^{-1} the vibration valence of the bond C=C, at 1462 cm^{-1} corresponding to the bending vibration of C-H bond, at 1392 cm^{-1} bending vibration of the O- H bond and 1234 cm^{-1} assigned to the vibration of C-O-C bond ether group.

Molluscicides activity. In the first instance used the crude extract (CE) of A. *angustifolia*, to verify its toxicity, facilitating collection and biodegradability to conserve the natural properties of the plant. This extract was shown to be effective on experimental individuals of *F. cubensis*, observed that in all concentrations evaluated presented lethal toxic effects. According to the analysis of empirical regression *Probit* on the set of repetitions performed, determine the LD_{50} corresponds to a dilution of 0.015 g / 20 mL (+ 0.003) equivalent to 7. 5 g/L

with the concentration of 1:64, and in the case of the LD_{90} corresponds to 0.214 g / 20 mL (+ 0.056) with equivalent to 107.5g/L and concentrations 1:4 and 1:8.

Table 1: Molluscicides bioassays *Agave angustifolia vs Fossaria obrussa*

Sample	25X 10^{-3} g/ml	12.5X 10^{-3} g/ml	6.25X 10^{-3} g/ml	3.12 X 10^{-3} g/ml	1.56 X 10^{-3} g/ml	7.8 X 10^{-4} g/ml	3.9 X 10^{-4} g/ml	1.93 X 10^{-4} g/ml	Control
1	1	8	12	14	17	19	18	20	20
2	0	10	13	15	18	18	17	20	20
3	1	9	15	14	16	18	17	19	20
4	0	11	12	13	17	17	18	20	20
Survivors (average)	0.5	9.5	13	14	17	18	17.5	19.75	20

Table 2: Average results of the antimicrobial activity against different microorganisms extracts of environmental origin.

MICROORGANISIUM	Average inhibition halos of different extracts in millimeters (mm)		Inhibition percentage (%IR)
	EXTRACTS		
	E1	E2	
Pseudomonas aeruginosa	10	17	20.9%
**Staphylococcus aureus* ATCC 25923	28	21	17%
***Staphylococcus saprophyticus*	20	10	23.5%
***Staphylococcus saprophyticus*	12	18	
***Staphilococcus epidermidis*	15	17	20%
***Staphilococcus epidermidis*	15	20	

* Cepa sensible a meticilina.
****** Cepa bacteriana aislada en diferentes zonas de la Ciudad de Puebla.
E1. N-1; **E2.** N-1 Et-OH

Antibacterial assay. The results of the antimicrobial activity can be observed in the measurement of the inhibition halos and inhibicion percentage (%IR) (Table 2.) based on the strain tested; *S. epidermidis* 15mm/20%, *S. saprophyticus* 15mm/23.5%, *S. aureus* 28mm/17% and *Pseudomona aeruginosa* 10mm/20.9%.

CONCLUSIONS

Polar organic extracts were obtained with soxhlet equipment. The polar extract of *Agave angustifolia* showed molluscicidal effects on a population of *Fossaria obrussa* under laboratory conditions. The results of our tests at different concentrations allowed us to identify LD_{50} and LD_{90} by an empirical *Probit* regression analysis and

established quantitatively the effect of polar extract *Agave angustifolia* fiber, demonstrating the high antimicrobial activity.

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