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Original Article

IN-VITRO Evaluation of The Antifungal Activity of The Extract of Bocconia Frutescens L. Against the Fungus Trichophyton Mentagrophytes

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SUMMARY

Trichophyton mentagrophytes is considered the main producer of a fairly widespread dermatomycosis called tina pedis that currently affects 79% of the world's population; although there is treatment for this disease many people turn to traditional medicine to treat it; among it has been used *Bocconia frutescens* for its antimycotic capacity. For this reason, in this research was evaluated the ability antifungal in vitro ethanolic extract of *Bocconia frutescens* against the fungus *Trichophyton mentagrophytes*, also identified the secondary metabolites of the plant and evaluated the toxicity of this extract.

Ethanolic extracts of leaves and petioles (EEH, EEP) were obtained, which were subjected to preliminary phytochemical analysis where the presence of secondary metabolites such as flavonoids, tannins, coumarins, sapo-ninas, among others, was observed; cytotoxic analysis was performed by nauplii of *Artemia salina.* It was determined that the EEH and EEP presented significant differences in the degree of toxicity, since the concentration needed to achieve a mortality of 50% of nauplii was 0.698 mg / mL of extract, while for EEP it was 1.007 mg / mL, as the EEH presented greater toxicity, antifungal activity tests were performed; these showed significant differences between the concentrations used for EEH, EEP and positive control (C+). The EEH presented a higher percentage of inhibition, with 70%, while the EEP presented an inhibition of 63%. This research aims to be a scientific basis to project *Bocconia frutescens* as a pharmacological product for the treatment of *tina ped^s.*

Keywords: *Tinea pedis, Bocconia frutescens L., secondary metabolites, extracts, antifungal activity*

1. INTRODUCTION

Trichophyton mentagrophytes (T *mentagrophytes*), is the main producer fungus of one of the most widespread surface mycoses called *tinea pedis* or at-leta foot (1), this is one of the most important health problems today, this fungus is distributed worldwide, so it is cosmopolitan. Its incidence has increased, since in the 40s it was isolated by 23% (2); It currently affects 79% of the population at some point in life, without distinction of race, sex, age (1). According to Angulo *et al.* (2008) (2) *T. mentagro-phytes* is a hyaline fungus, which can be identified macroscopically since its colonies can be observed that are cottony white and consist of hard, have wine-red pigment, which is visible on the back of the colony, microscopically lateral microconidia are observed in the form of tears. Showers and swimming pools are usually the reservoirs and transmitters of athlete's foot, there are also factors that predispose infection by this fungus, these can be, endogenous such as vascular diseases, diabetes, and immunopathies; On the other hand there are exogens that are due to poor perspiration and heat, accumulation of moisture and hyperhidrosis, as well as the habit of walking barefoot in public places (1). One of the current sources of treatment is through the intake or administration of synthetic antimycotics that, although highly effective, show the presence of notable side effects (1). The effects may be due to the dose of the antifungal since the maximum concentration is reached one to two hours after administration, for example concentrations of Ke-toconazole that can be 200 to 400 mg or between 600 and 1,200 mg per day, can cause endocrinological effects, which are due to the interference that the drug causes in the adrenal and gonadal steroidogenesis, likewise it has been shown that they interfere in the synthesis of testosterone in the Leydig cells of the testiculo, these two phenomena can alter the ratio andro-genos / estrogenos and contribute to the appearance of gyneco-mastia and other antiandrogenic effects during treatment, also appear digestive effects: nausea, vomiting, diarrhea, abdominal pain, among others; neurological: headaches, insomnia, drowsiness, pareste-sias; hematologic: eosinophilia, thrombocytopenia; Cutaneous: pruritus, photoinduced dermatoses, the most important of all is its hepatotoxic potential that can manifest itself in two ways: one symptomatic in the form of toxic hepatitis and the other clinical asymptomatic form is the most frequent, since it appears in 12% of cases and occurs with the elevation of liver enzymes (3,4). Due to the presence of these side effects and to avoid the use of synthetic antimycotics, the other highly used source of treatment is traditional medicine, which has

very good acceptance by the population, and also has great economic importance due to its low costs (5). As reported by Lasso (2010) (6) one of the plants is *Bocconia frutescens* L., (*B. frutescens)* commonly known as "trumpet" or "healer", this has been widely used because it has great antimycotic capacity, it has also been implementedu Use in traditional medicine for the treatment against various fungi. This plant belongs to the family Papaveraceae (7). It can be found on roadsides, from Mexico to South America along mountain ranges, in Colombia it is distributed in the three mountain ranges, from 1500 to about 3200 meters of altitude (6-8, 9,10). It can be identified by its shrubs, since they are branched and their stems can measure from 2 to 6 m long, their leaves can present oblong-obovate forms (7). This work contributed to the knowledge of plants with antimycotic capacity such as *B. frutescens*, against pathogenic microorganisms such as *T. mentagro-phytes*, producer of *tinea pedis*, as well as contributed to the investigation of the main functional phytochemical groups with therapeutic and antimycotic application by detecting some of the secondary metabolites present in *B. frutescens*, finally traditional knowledge was reinforced through socialization with the community, all this provides scientific basesIt is necessary to project *B. frutescens* as a pharmacological product for the treatment of tinea pedis.

2. PATIENTS AND METHODS

The plants of *B. frutescens L.*were obtained in the municipality of Pijao, Quindio (4° 19'' 58' North and 75° 42'' 20' West Central Mountain Range), these were taxonomically determined and deposited in the herbarium of the University of Quindio. The plants of *B. frutescens* L were collected between 6:00 and 8:00 am and were taken to the laboratory of the GECAVYME research group, after the plant material was selected by separating into leaves and petioles (parts of the plant used in the research), was washed and dried at room temperature to remove excess moisture and taken to an oven at 40 ° C until obtaining a dry weight subsequently check each part of the Plant was carried out a grinding process until obtaining a fine powder (11) (with some modifications).

OBTAINING AND PREPARATION OF DRY EXTRACTIONS OF THE PLANT

Dry ethanolic extracts of leaves (574.56 g) and petioles (527.06 g) were obtained by leaching for 8 days, using 500 mL of 96% ethanol, this was constantly circulated. The chlorophylls of the ethanolic extract were separated by a li-quido-liquid extraction with ethanol-water (1:7), filtered by filter paper, with the help of a vacuum pump and finally the

solution was concentrated by rotaevapo-racion, finally this mother solution was stored at 4 ° C, until its use in the tests (12,13). At the time of the test, solutions of concentrations 0.1, 0.2, 0.5 and 1mg/mL were prepared (11).

PRELIMINARY FITOQORMICA GAIT

Ethanolic extracts obtained from leaflets (EEH) and petioles (EEP) were evaluated for the identification of tannins, flavonoids, quinones, alkaloids, saponins, triterpenoids, cardiotonics, alkaloids, coumarins, sugars and deoxysugars according to the phytochemical identification method described by Bilbao (1997) (12) and Sanabria (1998) (13).

TOXICITY TESTS

Determination of mean lethal concentration (LC_{50})

Artemia salina eggs were hatched, using 3.8% sea salt solution (14). 24 hours later they were fed and when they reached the stage of nauplii they were exposed to the different concentrations (1mg/mL 0.5mg/mL 0.2mg/mL 0.1mg/mL) of ethanolic extracts (EEH and EEP) (15,16, 17). Dead nauplii were counted after 24 hours of exposure, mortality percentages were calculated and compared with controls. The value of the LC50 was calculated by probit analysis (statistical package S.P.S.S. V.20.0), with 95% confidence intervals, consisting of a logistic regression. For each fraction a test was made, in triplicate.

DETERMINATION OF ANTIFUN-GICA ACTIVITY BY THE METHOD OF DIFFUSION ON AGAR

The strains of *T. mentagrophytes* were obtained at the biological research center of Antioquia (C.I.B), antifungal activity tests were performed. According to the methodology described by Segovia and Suarez (2010) (18), with modifications, for this first a reactivation of the fungus was carried out by pep-tonada water, then three Petri dishes were used with sabouraud agar and the fungus was sown in them, they were vatted for two hours and during that time samples of both extracts and positive control (keto-conazole) were prepared in different concentrations. We proceeded to carry out the assembly of the test this consists of the three vatted boxes that were named asu box n° 1 for the ethanolic extract of leaves (EEH), box n° 2 for the ethanolic extract of petioles (EEP), the box n° 3 for the positive control (C +), each box was made orderly perforations, In each of them, the corresponding positive extract or control sample and a certain concentration were deposited. (C1: 1mg/mL, C2: 0.5 mg/mL, C3: 0.2 mg/mL, and C4: 0.1mg/mL), it should be clarified that boxes n°1 and n°2 pose^an perforation for negative control (distilled water) (Figure 1). The 3 boxes were incubated for 24 hours at 37 ° C and post-teriormente results

were observed, for the analysis of these were used the tests of Kruskal Wallis, Wilcoxon and descriptive statistics, also percentages and ranges of inhibition were found for all tests was established a degree of significance when $p < 0.05$. (S.Ptag.S.S. V. 20.0). This assembly was carried out in triplicate in three different times.

SOCIALIZATION WITH EDUCATIONAL INSTITUTIONS

Three socializations of results were carried out in educational institutions, two in the municipality of Pijao Quindio and one in Armenia Quindio.

3. RESULTS

PHYTOQmMICA PRELIMINARY MARCH

Thanks to the series of tests carried out it was possible to show that EEH and EEP presented se-cundarios metabolites such as tannins, flavonoids, some quinones, deoxysugar saponins, sugars, cardio-tonics, among others (Table 1), both extracts (EEH and EEP) showed sensitivity to ultraviolet light in thin plate chromatography tests, in one of them faint red colors were observed, faint green and strong blue for EEH (line 2) and the same colors including faint violet for EEP (line 1) (Figure 2), this indicated the presence of coumarins, steroids and terpene lactones, and therefore the positivity of this test. The test to determine the presence

Table 1. Preliminary phytochemical analysis or march of ethanolic extracts of leaves and petioles (EEH and EEP).

PHYTO-CHEMICAL TEST		ETHANOLIC EXTRACT OF LEAVES (EEH)	ETHANOLIC EXTRACT OF PETIOLES (EEP)
Tannins	Gelatin - salt	++	++
	Ferric trichloride	+++	++
	Lead acetate	+++	++
Flavonoids	Shinoda	++	+
	Leucoanthocyani-	-	+
	Dinas		
	Rosenhein	-	-
Quinones	Behaviour		
	vs. acid	++	+
	Confirmation with		
	sodium hydroxide	++	+
	Borntrager- Kraus	-	-
Saponins	Foam	+++	++
	Haemolysis	++	+
Steroids and/or Triterpenes		+	+
Sugars	Molish	+++	+
	Tollens	+	+
Alkaloids	Valser	-	-
	Mayer	-	-
	Bouchart	-	-
	Draguendorff	-	-
Cardiotonics	Antrona	-	-
	Baljet	++	+
	Kedde	-	-
Deoxysugars	Keller - Killiani	+	-
Coumarins	Simple for cuma-	++	+
	Rinas		
Steroid Coumarins and Terpene Lactones		+++	+++

TOXICITY TESTS

• Determination of mean lethal concentration

(^{CL}5")

Before the evaluation of toxicity of the extracts etano-licos leaves and petioles by analysis probit consisting of a regression log^stica, it was evidenced that EEH obtained a mean lethal concentration of 0.698 mg / mL and EEP a mean lethal concentration of 1.007 mg / mL, both with confidence interval of 95% and with a P value of 0.0001, so that both were re-sultados significant, this also showed that EEH obtained a higher degree of toxicity since the concentration necessary to reach a mortality of 50% of nauplii was lower compared to EEP.

DETERMINATION OF ANTIFUN-GICA ACTIVITY BY THE METHOD OF DIFFUSION ON AGAR

The Kruskal Wallis test showed significant differences between the four concentrations evaluated (1mg / mL, 0.5 mg / mL, 0.2 mg / mL, 0.1mg / mL) in the leaf extract (EEH) with a p = 0.024, there were also significant differences in the concentrations evaluated (1mg / mL, 0.5 mg / mL, 0.2 mg / mL, 0.1mg / mL) for the petiole extract (EEP) with a p = 0.031 and the same happened for the positive control (C +) with a p = 0.015. The Wilcoxon test determined that EEH compared with C + showed the existence of significant differences with a p = 0.002, as when comparing the EEP with the C + that obtained the same value (p = 0.002), when comparing the EEH with the EEP was obtained p = 0.137, which showed that between the two extracts there were no significant differences in its use. Finally descriptive statistics were made where it was observed that the EEH showed a halo of inhibi ti on average of 9.59 mm, and presented a range of inhibition ranging from a halo of 7.4mm to-ta one of 11.6mm; the EEP showed a halo of inhibi ti on average of 8.49mm presenting a ran-go of inhibition ranging from a halo of 6.9mm to 10.0mm; the C + obtained a halo of average inhibition of 13.7mm, with an inhibitory range ranging from a halo of 11.6mm to 15.6mm. The percentage of in-hibicion of EEH compared to C+ was 70%, and that of EEP compared to control was 62%.

SOCIALIZATION WITH EDUCATIONAL INSTITUTIONS

Finally for the socialization of the project 3 conferences were held, two of them, in the municipality of Pijao Quindio, in the Educational Institution "Institute Pijao" and in the Educational Institute "Santa Teresita", the last one was carried out in the city of Armenia Quin-dio, in the educational institution "Normal Superior del Quindio" headquarters Rojas

Pinilla. In the three conferences the results achieved in this project were socialized, as well as the ethnobotanical richness of the plant and the use of medicinal plants was encouraged.

4. DISCUSSION

PRELIMINARY PHYTOQOMICA MARCH

Secondary metabolites such as coumarins, saponi-nas and triterpenes may influence inhibitory effects on the growth of pathogenic microorganisms, (bacteria - fungi), (19, 20, 21, 22, 23, 24, 25). Others such as flavonoids have pharmacological effects in the human body such as antihepatotoxic potential, antimicrobial, fungitoxic and insecticidal activity (26). Tannins bring benefits due to their bacteriostatic and fungal activity (27). So the previous results on obtaining these secondary metabolites in both extracts (EEH and EEP) agree with what was reported by Lasso (2010) (6) on the effectiveness of B. frutescens against the inhibition of the growth of microorganisms such as *T. mentagrophytes* pro-ductor of *tina pedis,* these results also show that extracts of *B. frutescens* (EEH and EEP) when used topically can counteract side effects such as the hepatotoxic potential produced by synthetic drugs of non-generalized oral use.

In the phytoqmmico analysis performed the results for the alkaloid test were negative for both extracts of B. frutescens (EEH and EEP), although Diaz - Molina (2000) (28) carried out a study where he found that among the secondary metabolites of B. frutescens are the alkaloids, in addition he could also determine that these are produced in defense of the plant, When conducting a phytochemical study to ethanolic extracts of some parts of this plant such as leaves and bark, I verify and isolate 7 of them, this complex of alkaloids I call it as Bocconina. against this Pinol et al (1993) (29); Orozco et al (2002) (30) and Vivanco et al (2005) (31), argue that it is important to take into account that the biosynthesis of these secondary metabolites is usually restricted to specific developmental stages or periods of stress, Some plant cells mainly of leaves produce important secondary metabolites in the interactions of the plant with the environment (protection against predators, pathogens or environmental stress) or that are related to the reproductive machinery of the plant, therefore, under imposed environmental conditions of stress (light, aeration, soil pH, de-predation by animals, humidity, pathogens, among others) different chemical compounds may be produced; as well as increasing or decreasing the amount of secondary metabolites (such as alkaloids) present in plants of the same species located in different sites and with different conditions, therefore the expression of different metabolic pathways is given.

Sepulveda et al. (2003) (32) demonstrated that alkaloids have inhibitory effects on the growth of pathogenic microorganisms since they have the ability to intercalate with DNA, and thus stop protein synthesis, induce apoptosis and inhibit enzymes of carbohydrate metabolism, also Sanchez (2000) (7) argues that the alkaloids present in the Papaveraceae family have antimicrobial biological activity. The existence of these compounds (alkaloids) in some parts of B. frutescens demonstrates once again what has already been reported on the effectiveness of the use of this plant in skin diseases caused by fungi (6).

The EEP showed fewer positive tests of secondary metabolites compared to EEH, the reason for this could be that the leaves are structures where it is carried out in addition to primary metabolism (photosynthesis-sis), a type of metabolism called secondary, as a result of this compounds such as ta-ninos, coumarins, alkaloids, triterpenes and flavonoids, among others, are produced, while the petioles are structures of support and anchoring of the leaf, They also help transport the product of primary metabolism from the leaf to the stem (32). Therefore, there was a greater variety of secondary metabolites in the leaf extract (EEH), than in the petiole extract (EEP). The results obtained in this preliminary phytochemical march may explain the biological activity of *B. frutescens* on pathogenic micro-organisms described by many authors, and may also offer the opportunity to use them for the management of diseases such as tina pedis.

TOXICITY TESTS

• Determination of mean lethal concentration

(^{CL}5")

This higher degree of toxicity in the leaf extract (EEH) may be due to what was mentioned by Taiz *et al.* (2006) (33) that secondary metabolites are manufactured and accumulated more in leaves than in plant petioles. These have biological and / or toxic activities, as in this case that the secondary metabolites present in EEH exerted an effect of greater toxicity on nau-plios of the crustacean *Artemia salina* than those present in EEP. With regard to this Diaz - Molina (2000) (27), through the realization of a study concluded that ethanolic extracts of *B. frutescens* have potentially toxic effects on the crustacean *Artemia salina* and tumor lines. Therefore Castro *et al.* (2006) (21) I name it as a biocidal plant. This could explain the use of this plant in traditional medicine, since thanks to this biocidal effect the growth of pathogenic microorganisms can be inhibited, among which bacteria and fungi

stand out. The components responsible for the inhibitory effect of *T. mentagrophytes* according to Vivanco *et al.* (2005) (30) are the secondary metabolites that are present in *B. frutescens,* the concentration of these depends on the part of the plant where they are produced. For this reason the leaf extract presented a greater inhibition (70%), compared to the petiole extract (62%). Although so far no reports have been found on the antifungal activity of B. frutescens specifically against *T. mentagrophytes*, Lasso (2010) (6) re-lizo a work using extracts of leaves, barks and seeds of *B. frutescens* against a fungus of the same genus called *Trichophyton rubrum,* As a result he obtained a pronounced sensitivity of the fungus to all extracts of the plant, being the extract of leaves the one that inhibited the growth of this fungus by 50%. Another study by Bernal *et al* (2010) (23) on the extract of *B. frutescens* against another type of fungus called *Microsporum canis* mos-tro resulted in a considerable involvement of the fungus since its sporulation was stopped in this way its growth was inhibited by 60%. Martinez *et al* (2007) (34) studied B. *frutescens* extract against a fungus called Corticium salmonicolor and found that this extract inhibited 98% fungal growth. This corroborates the effectiveness of leaf extracts (EEH) and pe-cioles (EEP) that also caused an inhibition of *T. mentagrophytes* with considerable inhibition percentages such as 70% (EEH) and 62% (EEP). The extracts of B. frutescens have also demonstrated their wide inhibitory effect not only preventing the growth of pathogens such as fungi, but also according to what was found by Castro et al. (2006) (21) can also inhibit the growth of insects such as Hypothenemus hampei F. (coleoptero causing damage to the fruits of coffee (Coffea arabica)), since when evaluating the ethanolic extract of leaves they found an inhibition of its growth by 62.2%.

5. CONCLUSION

• Secondary metabolites such as tannins, flavonoids, sugars, triterpene saponins, quinones, coumarins, steroids, among others, could be evidenced in the extracts of *B. frutescen.*

• The ethanolic extract of EEH leaves obtained a higher degree of toxicity compared to EEP due to a higher concentration of secondary metabolites.

• Thanks to the secondary metabolites present in *B. frutescens* and more precisely the extract ethane-lico leaves (EEH) and its synergistic action could evidence an activity Antifungica representa-da in a percentage of inhibicion of the growth of *T. mentagrophytes* of 70% compared to the control.

Ethical Issues: All ethical issues were approved by the authors from the Iraqi Ministry of Health. Verbal and signed informed consents were obtained from all patients who included in the study during their first visit.

Conflict of interest: None

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