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ETHNOBOTANICAL STUDY AND COMPARISON OF ANTITRICHOPHYTIC ACTIVITY LEAVES OF *ASPILIA AFRICANA* (PERS.) CD ADAMS VAR. *AFRICANA*, *AGERATUM CONYZOIDES* L. AND *ACANTHOSPERMUM HISPIDUM* DC. ON THE *IN VITRO* GROWTH OF *TRICHOPHYTON MENTAGROPHYTES*

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Abstract :

At the end of an ethnobotanical survey carried out in the district of Abidjan, Aspilia africana var africana, Ageratum conyzoides and Acanthospermum hispidum, three plant species widely known with weeds, were selected among the most used plants in the treatment of microbial diseases especially fungal ones. Thus, to make our contribution to the fight against opportunistic dermatophytosis in high recrudescence in the AIDS patients, we tested on Sabouraud medium the ethanolic and aqueous extracts of each of the three plants on the in vitro growth of a strain of Trichophyton mentagrophytes. The tests were carried out according to the method of double dilution in tilting tubes. The obtained results show that the tested T. mentagrophytes strain was sensitive to all the studied plant extracts. However, the EF70 $\%_{Ac}$ extract has a better antifungal potential on T. mentagrophytes (MCF = 1,56 mg/mL and IC_{50} = 0,29 mg/mL). Otherwise, T. mentagrophytes was more sensitive to ethanolic extracts (EF70 % : MCF from 1,56 to 3,12 mg/mL and IC_{50} from 0,29 to 0,86 mg/mL) than aqueous extracts (ATE : MCF from 3,12 to 25 mg/mL and IC_{50} from 1,42 to 3,12 mg/mL ; ARF : MCF from 6,25 to 12,50 mg/mL and IC_{50} from 0,58 to 3,15 mg/mL). This work justifies the use in traditional environment of these weeds as anti-fungal ones. Thus, the 70% ethanolic extracts of A. africana, A. conyzoides and A. hispidum can serve as a basis for the development of phytomedicines against dermatophytosis.

Keywords : *Dermatophytosis, Aspilia africana, Ageratum conyzoides, Acanthospermum hispidum, Trichophyton mentagrophytes*

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INTRODUCTION:

The excessive increase in the prevalence of fungal infections over the last years, has profoundly transformed the focus on medical mycology. According to the [1], fungal infections are responsible for more than 17 million deaths worldwide each year, more than half of which come from Africa alone. In Côte d'Ivoire, a study of cutaneous mycosis revealed that 73.26 % of consultations in conventional medicine represented dermatological diseases, of which 61 % of cases are superficial mycosis [2]. Similarly, studies conducted from 1996 to 2000 also revealed that in all cases of dermatology consultations, 52.12 % of cutaneous mycosis were caused by yeasts and 44.78 % by dermatophytes. The latter are widespread filamentous fungi that affect all social classes and have an affinity for keratin. Depending on the location of the lesions on the skin, we can distinguish those of the glabrous skin, scalp ringworm and onyxis [3]. Cosmopolitan affection, the dermatophytosis are first epidermal, extensive, itchy and contagious. Heat and humidity, immunosuppressive diseases, diabetes, promiscuity with animals, hypersudation, obesity and certain drugs such as corticosteroids contribute to the unexpected arrival dermatophytosis [4]. When they are not treated, there is a multiplication and an extension of the lesions. The evolution of dermatophytosis is generally benign but chronic and the prognosis is always favorable. Among dermatophytes, the genus *Trichophyton* is the most frequently implicated (71.55 % of isolates) [5]. The increase of this epidemiological modification is due to several factors especially to the resistance of certain germs to the usual drugs [6,7,8]. Faced with this situation, several laboratories have undertaken researches for the development of new drugs [9]. It is within this framework that we undertook to evaluate *in vitro* and to compare the antitrichophytic activity of *Aspilia africana* (Pers.) C.D. var. *africana*, *Ageratum conyzoides* L. and *Acanthospermum hispidum* DC., three medicinal plants with multiple therapeutic properties selected at the end of an ethnobotanical survey in the district of Abidjan [10]. Numerous pharmacological, phytochemical and toxicological studies was carried out by researchers to verify and justify the traditional use of these three plants [11,12,13]. This study is part of the program of scientific valorization of the traditional pharmacopoeia of our laboratory. Its purpose is to search from our floristic inheritance, new antimicrobial molecules to better control the chemotherapeutic approach against infectious diseases.

MATERIAL AND METHODS

Material

Vegetal material

The plant material is made from the leaves of *Aspilia africana* (Pers.) C.D. Adams var *africana*, *Ageratum conyzoides* L. and of *Acanthospermum hispidum* DC., three Asteraceae collected in the district of Abidjan in August 2014. Their identification was performed at the National Floristic Centre (NFC) from the University Felix Houphouet-Boigny Abidjan-Cocody where samples are preserved.

Fungal germ

The microbial strain is a fungal germ (*Trichophyton mentagrophytes* var. *mentagrophytes*) provided by the Mycology Laboratory at Training and Research Unit of the Faculty of Medical Sciences of Félix Houphouët-Boigny, University of Côte d'Ivoire. This mould was isolated from the nails of the large left toe of a patient with chronic urticaria (onyxis).

Culture medium

Sabouraud medium (HIMEDIA/Ref : M1067-500G Lot 0000215703) was used for the culture of fungal germ.

Methods

Monographic study of *Aspilia africana*, *Ageratum conyzoides* and *Acanthospermum hispidum*

To allow a better recognition of these three plants in a natural environment, a complete and comparative monographic study was carried out. It takes into account the general appearance of the plants, the detailed description of the various organs as well as some therapeutic uses of each of the plants.

Preparation of plant extracts

The leaves of these three species were dried separately in the Laboratory for two weeks and reduced to a fine powder using an electric grinder type IKA Labortechnik (MFC type).

Preparation of aqueous total extracts (ATE) : the preparation of these extracts was performed using the method described by [8] which consists in macerating 100 g of plant powder of each species in 1L of sterile distilled water using a blender Blinder type 7 SEVEN STAR. The homogenates were filtered over hydrophilic cotton and then on filter paper Whatman 3 mm. The aqueous filtrate thus obtained are evaporated in an oven type Med Center Venticell at 50 °C to obtain powders that constitute the aqueous extracts (ATE_{Aa} for *Aspilia africana*, ATE_{Ac} for *Ageratum conyzoides* and ATE_{Ah} for *Acanthospermum hispidum*).

Preparation of ethanolic fractions 70 % (EF70 %) and aqueous residual fractions (ARF) : these fractions were obtained separately by dissolving 5 g

of each ATE in 100 mL of a ethanol 70 % solution and then homogenized. After decantation and filtration of the alcoholic fraction on hydrophilic cotton and on filter paper Whatman 3 mm, the filtrate collected is evaporated in an oven at 50 °C. The powder obtained constitutes the EF70 % extract (EF70 $\%_{Aa}$ or EF70 $\%_{Ac}$ or EF70 $\%_{Ah}$ according to the specy). Likewise the aqueous residual deposit was collected and evaporated in an oven at 50 °C. The powder obtained constitutes the ARF extract and is called ARF_{Aa} or ARF_{Ac} or ARF_{Ah} according to ATE.

Yield calculation

The yield is the amount of extract obtained from the plant powder. It is expressed as a percentage or without any unit. In practice, it is determined by the ratio of weight of the solids content after evaporation by the weight of the dry powder of the plant material used for the extraction, multiplied by 100. This gives the following formula :

$$Yd = (m \times 100) / M$$

(Yd : Extraction yield in percentage ; m : mass in grams of the dry extract ; M : mass in grams of the drug powder).

Antifungal tests

Preparation of medium culture and extracts incorporation : the culture medium was prepared according to the manufacturer's instructions while taking into account the quantity taken and the incorporation of different extracts prepared medium was made in tubes by the method of double dilution which leads to the obtaining of different concentration from 50 to 0.097 mg/mL according to geometrical connection of 1/2 reason. For each extract, a series of 12 test tubes was constituted with 10 experimental tubes and 2 control tubes of which one without constituting the plant extract of germs growth control and the over without extract and germ, used as a culture medium for sterility control light.

Preparation of the *inoculum* and sowing of the tubes : the inoculum preparation is made by homogenization of a young colony (72 hours) well isolated from *T. mentagrophytes* in 10 mL of sterile distilled water to give a suspension of 10^0 (10^6 cells/mL).

From this suspension, 1 mL was taken and mixed in 9 mL of sterile distilled water to form the suspension 10^{-1} corresponding to 10^5 cells/mL. The 10 experimental tubes and the growth control tube was inoculated with 10 µL of the suspension 10^{-1}.

Sterilization : the 12 tubes of each series were autoclaved (PBI STEMATIC III) at 121 °C for 15 min and then all the tubes were incubated at 30 °C for 10 days and then tilted with a small stick to the room temperature to permit cooling and solidification of the agar.

Colonies counting : after the incubation time, the colonies of *T. mentagrophytes* were counted by direct counting with a colonies counter pen type Geiger. The growth in experimental tubes was evaluated as a percentage of survival, calculated at 100 % survival in the control growth control tube. The calculation of the percentage of survival was done according to the following formula :

$$S = (n / N) \times 100$$

(S = Survival of *T. mentagrophytes* percentage ; n = number of colonies in the control tube ; N = Number of colonies in the test tube).

Required antifungal parameters : data processing has determined the following parameters antifungals :

-MIC (Minimum Inhibitory Concentration) : this is the concentration of extract in the tube for which there was no growth visible to the naked eye ;

-IC_{50} (Concentration for fifty percent inhibition) : is the concentration which gives 50 % inhibition. It is graphically determined from the sensitivity curve plot of each extract of *T. mentagrophytes* ;

-Fungicidal (**MCF** ou **FSC**) : after 10 days of incubation, the surface of the agar contained in test tubes having resisted the growth of the fungal isolate was taken slightly then inoculate on a new agar and incubated for 10 days at room temperature. Two cases are possible :

. presence of colonies of *T. mentagrophytes*, the extract is said fungistatic. Thus, it is determined the **FSC** (Fungistatic Concentration) ;

. absence of colonies of *T. mentagrophytes*, the extract is said fungicide. This observation identified the **MCF** (Minimum Concentration Fungicide) which gave 99.99 % inhibition compared to control growth control tube.

Results

Monographic study

Aspilia africana, *Ageratum conyzoides* and *Acanthospermum hispidum* are three plant species belonging to the Asteraceae family [14]. They are common to the Guineo-Congolese region and the Sudano-Zambian region [15]. They are plants widely known like pluvial weeds of crops and plantations and propagated by seed [16]. The general appearance of the three plants is shown in figure 1.

Aspilia africana var. *africana* *Ageratum conyzoides* *Acanthospermum hispidum*

Figure 1 : General appearance of the leaves and flower stalks of *Aspilia africana*, *Ageratum conyzoides* and *Acanthaspermum hispidum*

- **Compared botanical description**

General aspect

Called Zeu-nanh in Akyé (southern ethnic group of Côte d'Ivoire) and Soumadibrou in Malinké (northern ethnic group of Côte d'Ivoire), *A. africana* is a hardy and disordered grass of variable size (between 60 cm and 1.5 m) depending on rainfall and soil fertility. It is also found in fallow, especially in the forest area. As for *A. conyzoides*, it is an ephemeral, annual, aromatic, standing erect and finely pubescent herb up to 70 cm high. It is called Koun-gbéni in Malinké (northern ethnic group of Côte d'Ivoire) and N'métin-dou in Akyé (southern ethnic group of Côte d'Ivoire). On the other hand, *A. hispidum* called Saraka-weini in Malinké (northern ethnic group of Côte d'Ivoire) and Gnéakeyébêko in Bété (west-central ethnic group of Côte d'Ivoire), is an annual, erected, very ramified, bushy species, able to reach 60 cm high. It also grows in fields, pastures, along roads and on vacant lots.

Stem

A. africana has a stiff at the base, very branched and rather rough to the touch stem. As for *A. conyzoides*, it has a weak, branched and finely pubescent stem. On the other hand, the stem of *A. hispidum* is sublignous, cylindrical and covered with stiff white hairs.

Leaves and petiole

A. africana has opposite and ovate-lanceolate leaves, from 6 to 15 cm long and from 3 to 7 cm wide. They are rounded at the base, pubescent, characterized by three protruding ribs. The petiole is about 1 cm long. As for *A. conyzoides*, these leaves are opposite, oval, finely pubescent, from 8 cm long and 5 cm wide with a sharp apex and serrated margins. The petioles are 5 cm long. On the other hand, *A. hispidum* has simple, opposite and obovate leaves, from 6 to 8 cm long and from 2 to 4 cm wide. They are pointed, cuneiform, sessile at the base, finely serrated at the margins and pubescent on the both sides.

Inflorescence and flowers

The inflorescence of *A. africana* consists of terminal and solitary capitules on pubescents stems from 4 to 10 cm long. The flowers are composed of bright yellow florets. As for *A. conyzoides*, its inflorescence is a terminal corymb in clusters of 10 capitules of 7 mm section. The flowers are composed of tubular florets, often pale blue but sometimes white. On the other hand, the inflorescence of *A. hispidum* is a solitary capitula from 5 mm of inserted section at the knots and subtended by 5 calyx bracts. The flowers are formed of yellow or pale green florets surrounded by 2-spine involucral bracts.

Fruits

The fruits of *A. africana* are quadrangular akenes about 5 mm long, covered with hard and fine hairs. As for those of *A. conyzoides*, they are linear and black akenes, surrounded by 5 white and pointed scales. On the other hand, the fruits of *A. hispidum* are arranged akenes in star form, bristling of prickles with curved thorn, two prickles are longer than the others and in the horn form.

- **Therapeutic use**

Aspilia africana

The paste resulting from the kneading of fresh leaves is used in local application against pimples, ostegia and enema to treat stomach pains. The decoction of leafy branchs is used as a drink against cough, lung

infections, malaria and in bath against scabies. The paste resulting from the kneading of fresh leaves and flowers is used in local application against whitlow, shingles, dermal spots, furuncles. The extract resulting from the kneading of roots is used as drink against caused bleeding by tuberculosis. The extract resulting from the pulverizing of fresh leaves is used in nasal instillation against migraines, ocular instillation against eye pain and redness of eyes and local application to stop bleeding during wounds.

Ageratum conyzoides

The decoction of leafy and flowered branchs is used in bath against malaria and skin diseases. This same decoction is used in intimate bath to treat vaginal discharge, cervical pain and hemorrhoids. The paste resulting from the kneading of fresh leaves and flowers is used in local application against foots diseases, dartre, furuncles, hemorrhoids, for drink and purge against malaria, stomach aches, hemorrhoids, ovarian cyst and to protect the pregnancy.

Acanthospermum hispidum

The extract resulting from the pulverizing of fresh leaves or whole plant is prescribed in ocular instillation in the treatment of cephalgia, syncope faint and convulsions. The paste resulting from the kneading of fresh whole plant is indicated per os and externally use in the treatment of vomiting, stern gastralgia, angina, snake bites, icterus, zona, furuncles, whitlow and post-partum bleeding. The decoction of the whole plant is used per os and externally in the treatment of foot disease, urethritis, cough, epilepsy, constipation, eruptive fevers, malaria, scabies, vaginal discharge and fibroma.

Yield extractions

Maceration with the distilled water of the leaf powders of each plant species studied, gave a blackish powder denoted ATE (Aqueous Total Extract). Similarly, the partition of 5 g of each ATE dissolved in 100 mL of a 70 % ethanol solution gave an ethanolic extract denoted EF70 % and an aqueous residual extract denoted ARF. The yields of the obtained extracts for each plant are summarized in Table 1. *A. hispidum* exhibited the best yield with 17 % for the ATE extracts and 52.2 % for the EF70 % extracts. By contrast, for ARF extracts, *A. africana* had the highest yield with 62 %. Of the three types of obtained extracts, the aqueous residual extracts showed the highest yields. In general, it was observed that *A.hispidum* gave the best yields.

Table 1 : Values of yied of nine *Aspilia africana, Ageratum conyzoides* and *Acanthospermum hispidum* extracts

Plant extract	ATE Aa	ATE Ac	ATE Ah	EF70 % Aa	EF70 % Ac	EF70 % Ah	ARF Aa	ARF Ac	ARF Ah
Yield (%)	11	15	17	28.8	50,4	52.2	62	42,6	42.8

ATE : aqueous total extract ; E**F70 % :** ethanolic fraction 70 % ; **ARF :** aqueous residual fraction ; **Aa :** *Aspilia africana* ; **Ac :** *Ageratum conyzoides* ; **Ah :** *Acanthospermum hispidum*

Antifungal tests

After 10 days of incubation at 30 °C, the results of the antifungal tests carried out with extracts of *A. africana*, *A. conyzoides* and *A. hispidum* with *T. mentagrophytes* are summarized in figure 2 and 3 and in the table 2.

In all the experimental series and this for the nine extracts tested, it was observed, compared with the controls, after the 10 days of incubation at 30 °C, a progressive decrease in the colonies number of *T. mentagrophytes* with the increase of the concentration of the extracts in the experimental tubes (Figure 2).

EF70 % extracts gave the activity curves with the highest slopes compared to the other two types of extract : ATE and ARF. But the slope of the activity curve of the EF70 $\%_{Ac}$ extract was the strongest in the whole (Figure 3).

On this fungus, the EF70 $\%_{Ac}$ and EF70 $\%_{Ah}$ extracts had the best values of MIC and MCF (1.56 mg/mL each) but with a smaller IC_{50} value (0.29 mg/mL) for EF70 $\%_{Ac}$ extract. As for the ATE extracts, ATE_{Aa} had the best value of MCF (3.12 mg/mL) and IC_{50} (1.42 mg/mL). On the other hand, at the ARF extracts level, ARF_{Aa} had the best value of MCF (6.12 mg/mL) for an IC_{50} value of 3.15 mg/mL and ARF_{Ac} had the best IC_{50} value (0.58 mg/mL) for a MCF value of 12.50 mg/mL (Table 2). The nine plant extracts tested all showed fungicidal potencies on *T. mentagrophytes* and *A. hispidum* extracts showed the best antifungal activities on this germ as a whole (Table 2).

Figure 2 : Cultures of *Trichonphyton mentagrophytes* in the presence of vegetable extracts with various concentrations

A : aqueous total extract of *Aspilia africana* ; **B :** ethanolic fraction 70 % of *Ageratum conyzoides* ; **C** : ethanolic fraction 70 % of *Acanthospermum hispidum* ; **TS** : Control of sterility ; **TC** : control of growth

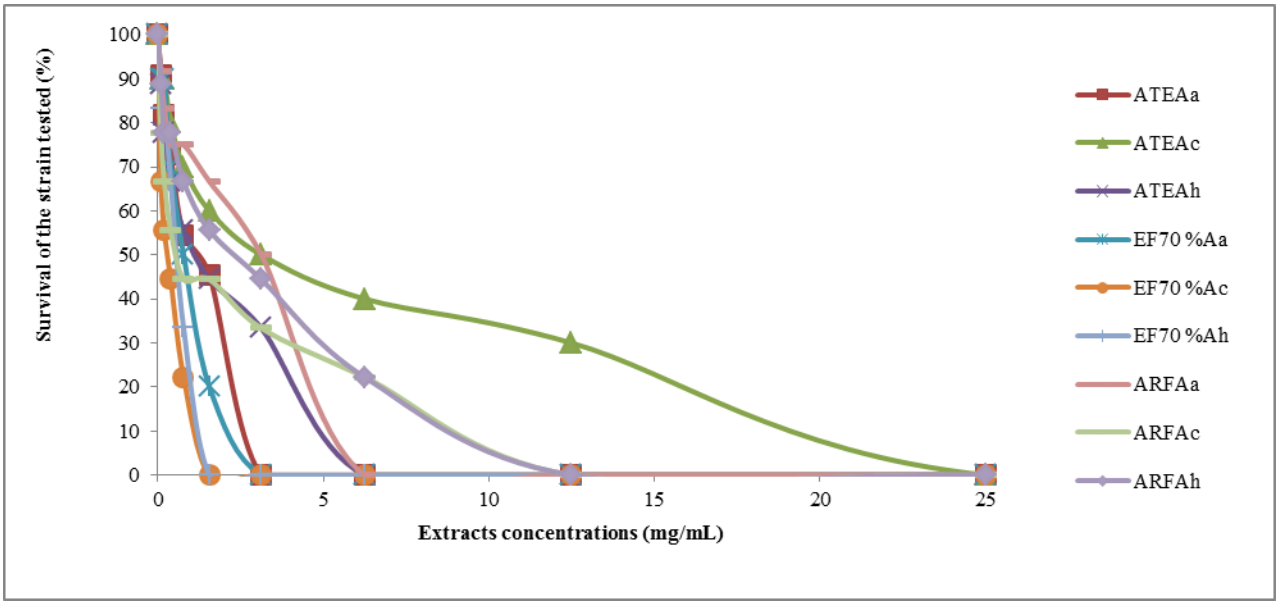

Figure 3 : Sensitivity of *Trichonphyton mentagrophytes* to the extracts of *Aspilia africana*, *Ageratum conyzoides* and *Acanthospermum hispidum*

Table 2 : Values of antifungal parameters of nine *Aspilia africana*, *Ageratum conyzoides* and *Acanthospermum hispidum* extracts at ten days of incubation at 30 °C

Plant extract / Antifungal parameters	ATE Aa	ATE Ac	ATE Ah	EF70 % Aa	EF70 % Ac	EF70 % Ah	ARF Aa	ARF Ac	ARF Ah
MIC (mg/mL)	3,12	25	6,25	3,12	1,56	1,56	6,25	12,50	12,50
IC_{50} (mg/mL)	1,42	3,10	1,45	0,86	0,29	0,61	3,15	0,58	2,35
MCF (mg/mL)	3,12	25	6,25	3,12	1,56	1,56	6,25	12,50	12,50
Fungicidal	Fce	Fce	Fce	Fce	Fce	Fce	Fce	Fce	Fce

Fce : Fungicide ; **Aa :** *Aspilia africana* ; **Ac :** *Ageratum conyzoides* ; **Ah :** *Acanthospermum hispidum*

DISCUSSION:

Botanically, *Aspilia africana*, *Ageratumconyzoides* and *Anthospermum hispidum* are three Asteraceae that have all the characteristics of this family. They are three ruderal species, characteristic of cattle grazing areas and three weeds of pastured plots after harvest. According [16], these are two species described as rainy weeds of crops and plantations. They are frequently used in the Ivorian pharmacopoeia [10].

On the microbiological level, the analysis of the results shows that all the extracts are active on *Trichophyton mentagrophytes*. In addition, *T. mentagrophytes* was sensitive to extracts according to a dose-response relationship. In accordance with MCF values, the EF70 % extracts are 2 or 8 times more active than the ARF extracts and 4 or 16 times more active than the ATE extracts used for their preparation. Similarly, the results also reveal that the MCF value of the EF70 $\%_{Ac}$ and EF70 $\%_{Ah}$ extracts is 1.56 mg/mL and that of EF70 $\%_{Aa}$ is 3.12 mg/mL. The comparison of the activities of these three ethanolic extracts shows that the EF70 $\%_{Ac}$ and EF70 $\%_{Ah}$ extracts are twice as active as EF70 $\%_{Aa}$. However, the ATE_{Aa} extract is 2 times more active than ATE_{Ah} and 8 times more active than ATE_{Ac}. In general, the results obtained demonstrate that the EF70% extracts from the ethanol / water partition significantly improve the efficiency of the ATE extracts which served as a basis for their preparation.

According to the activity level classification scale [17], the performances of *A. africana* are of "strong" level activity with the ATE and EF70 % extracts and of level "average" with ARF on *T. mentagrophytes*. On the other hand, *A. hispidum* and *A. conyzoides* have "high" level activities with their EF70 % and "medium" extracts with their ATE and ARF extract on *T. mentagrophytes*. From the analysis of all the results, it appears that the EF70 % extracts are the most active on *T. mentagrophytes*. These best activities have antifungal parameters of different values. The variability of the values reveals not only that the extracts are more or less active one than the other and that they do not all have the same potential for antifungal activity, but also that the sensitivity of a fungal germ varies according to the plant species [18]. On the other hand, the comparison of our results with those of [19] shows that EF70 $\%_{Ac}$ and EF70 $\%_{Ah}$ extracts are half as effective as soap made from the oils of *Mitracarpus scaber*, *Mareya micrantha* and *Cassia alata* on *T. Mentagrophytes*. This difference in activity can be explained by the fact that the action of soap is the combination of the activity of three plant species, contrary to our extracts which are tested separately and which each express the activity of a single species plant.

However, comparing the MCF values of the EF70 $\%_{Ac}$ and EF70 $\%_{Ah}$ extracts with that of the hydroethanolic extract of the stem bark of *Ficus platyphylla* (Moraceae) according to the work of [20], the three species *A. conyzoides*, *A. hispidum* and *F. platyphylla* have practically similar antifungal activities on *T. mentagrophytes*.

This slight difference in activity could be explained by the fact that in EF70 $\%_{Ac}$ and EF70 $\%_{Ah}$ extracts, certain chemical compounds such as tannins are found in trace form [10]. On the contrary, in the extract Ehy.Eth of *Ficus platyphylla*, the presence of tannins is clear [20]. According to [21], tannins for example, are known for their ability to inhibit the growth of many microorganisms including fungi.

CONCLUSION:

This study allowed us to show that all the studied extracts of *Aspilia africana*, *Ageratum conyzoides* and *Anthospermum hispidum* have a more or less pronounced antifungal activity on the *in vitro* growth of *Trichophyton mentagrophytes*. EF70 % extracts were the most active. Ethanol is the solvent that would allow a better concentration of active ingredients. The extraction method used, would be a way that would concentrate the active ingredients and improve the activity of ATE, traditionally used against opportunistic dermatophytosis. This work justifies the use in traditional environment of these weeds as anti-fungal. Thus, the 70 % ethanolic extracts of *A. africana*, *A. conyzoides* and *A. hispidum* can serve as a basis for the development of phytomedicines against dermatophytes.

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