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Antidermatophytic activity and adverse side effects of the methanolic extract from leaves of *Ageratum conyzoides* (Asteraceae)

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Abstract

Background: The incidence of dermatophytosis has increased in recent years in spite of the availability of antifungal drugs. Faced with all the above, medicinal plants could be an alternative. So, this work aimed at evaluating antidermatophytic activities of some medicinal plants used in Cameroon against dermatophytoses.

Methods: An ethnopharmacological survey was carried out by interview of traditional healers in six villages of Bafou locality. Nine plants were selected and methanol extracts were prepared therefrom. The *in vitro* antidermatophytic activities of these extracts were tested using microdilution method. The degree of dermal irritation of the extract from leaves of *Ageratum conyzoides* was determined in *Cavia porcellus* using the occluded dermal irritation test method. This extract was subjected to an acute dermal toxicity test using *C. porcellus* as animal model. For that, animals were randomly divided into four groups: Groups 1, 2 and 3 respectively received single doses of extract at 8000 mg/kg, 4250 mg/kg and 500 mg/kg body weight while control group received distilled water.

Results: Among the tested extract, that of leaves of *A. conyzoides* showed the best antidermatophytic activity (32≤MIC≤512 µg/mL). Dermal administration of the single dose of this extract led to skin irritation, weakness and less motor activities at the dose of 8000 mg/kg. The lethal dose fifty (LD_{50}) was defined as greater than 8000 mg/kg. In general, biochemical as well as hematological parameters of animals were normal.

Conclusions : These results show that *A. conyzoides* is the most effective against dermatophytes without adverse side effects at reasonable doses.

Keywords: Dermatophytes; *Ageratum conyzoides*; irritation; acute toxicity

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Background

Dermatoses are skin infection, and by extension that of the nails or hairs, they are usually caused by dermatophytes [1]. The dermatophytes are filamentous fungi that parasitize the skin as well as the integuments of man and animals. They live at the expense of keratin from the stratum corneum of the epidermis and integuments. Dermatophytes penetrate and lyse keratin mechanically, or by intermediate keratinolytic enzymes, keratinases. They are responsible for superficial fungal infections such as epidermomycoses of the glabrous skin; scalp ringworm and hairs; nail lesions or onyxis [2]. These infections generate reactions from the host that are highly variable (discrete or severe) depending on the parasites species, the anatomical location of the lesions, the intrinsic factors to the host and the environment [3]. They can also be responsible for allergic manifestations [4], and finally, exceptionally, invade deep tissues [2]. The incidence of dermatophytosis has increased in recent decades, especially in immunocompromised patients [5]. They affect about 20-40% of the world's population, mainly children of school age [6]. In some areas of Cameroon, the prevalence of dermatophytosis in children is about 31% [7]. They are not life threatening, but constitute a public health problem because of their prevalence, recidivism and the limited number of effective antifungals some of which presenting adverse side effects [8] without forgetting the emergence of the resistance of certain strains to available drugs.

In view of all the above, it appears necessary to search for new effective substances against dermatophytes without adverse side effects. Medicinal plants to which nearly 80% of African rural populations rely for first aid would be a good source of this type of substance [9]. The majority of therapies involve the exploitation of the active principle of medicinal plants [10, 11, 12]. In addition, these plants are an invaluable resource for the pharmaceutical industry because of their rich bioactive chemical molecules [13]. This scientific research on medicinal plants may contribute to the production of improved traditional medicines, at a cost accessible to a greater number of the population. This study aimed at evaluating the antidermatophytic activities of some plants of Cameroonian medicinal flora used against dermatophytic infections in traditional medicine.

Methods

Plant material and preparation of extracts

At the end of an ethnopharmacological survey conducted in six villages (Batseng'la, Baletet, Baghonto, Bawouwoua, Fokamezo and Tsinbeu) of the locality of Bafou, Department of Menoua, in the West Cameroon Region. The collected data were registered in a Microsoft Excel worksheet. The value of use, a method of quantification which shows the relative importance of a plant species, was calculated according to the following formula: UV = (n/N) x 100, where UV = value of use of a species, n = number of quotation of a species and N = full number of quotation. Following the application of this formula, nine of these plants that showed the highest value of use were chosen for antidermatophytic studies. The plants were harvested in January 2017 in the same locality. These plants were identified and compared to voucher specimen at the Cameroon National Herbarium (Yaounde, Cameroon). The plant materials were dried at room temperature and then crushed. 200 g of powder resulting from each plant material were macerated in 600 ml of methanol. The mixture was then stirred twice a day. After 48 hours, this mixture was filtered using Whatman paper N^0 1. The filtrate was concentrated at 65 °C in a rotary evaporator (Buchi R200). The extract was collected in a vial and then left in an oven at 40 °C for complete evaporation of the solvent. It was then kept under 4°C until further use.

Animals

The acute dermal toxicity test was performed using female *Cavia porcellus* (guinea pigs) aged three months (400 ± 50 g) bred at the animal house of the Department of Biochemistry, University of Dschang, Cameroon. The animals received *food ad libitum* and a dietary supplement rich in protein, vitamins and calcium. They were exposed to room temperature (22 ± 2 ^{0}C). The test was conducted in accordance with acceptable international standards for the evaluation of the safety and efficacy of medicinal plants [14, 15]. All studies involving animals were conducted according to the ethical guidelines of the Committee for Control and Supervision of Experiments on Animals (Registration no. 173/CPCSEA, dated 28 January, 2000), Government of India, on the use of animals for scientific research.

Phytochemical screening of crude extracts

Phytochemical screening was performed according to the method described by Brunetton (1999) [16]. Plant extracts were screened for various classes of secondary metabolites including flavonoids, alkaloids, triterpenes and steroids, polyphenols, saponins, tannins, anthraquinones and anthocyanins. Gas chromatography coupled to mass spectrophotometry was used to analyze the leaves extract of *A. conyzoides.*

Evaluation of in vitro anti-dermatophytic activities

The dermatophytes used in this study were *Microsporum gypseum*, *Trichophyton violaceum* and *Epidermophiton floccosum*. These isolates were obtained from Yaounde Pasteur Centre. The microdilution method [5] was used to determine the minimal inhibitory concentrations (MICs) and minimal fungicidal concentrations (MFC) of the tested substances using the 96-well microplates. These plates were prepared by introducing into each well 100 µl of Sabouraud Dextrose Broth (SDB). A volume of 100 µl of each test substance (extract) was added to the first wells of the plate followed by a two fold serial dilution. A volume of 100 µl of the standard inocula (5×10^3 CFU / ml) was added to each well to obtain approximately 2.5×10^3 CFU/mL, for a total volume of 200 µl. For each experiment, the sterility test (broth and aqueous solution of DMSO 5% (v/v) and the negative control (broth plus inoculum) were included. The content of each well was homogenized and the microplates were covered with sterile covers and incubated at 28 ^{0}C for 5 days. After incubation, fungal growth in each well was monitored by observing and comparing turbidities in the test wells and positive as well as negative controls. The minimum inhibitory concentration (MIC) was the lowest concentration that prevented any growth of the microorganism.

The fungicidal minimum concentration values were determined by transferring 50 µl of the preparations which prevented any visible growth of the microorganism during the determination of the MIC into 150 µl of broth. These preparations were then incubated as indicated above. The growth of fungus in each well was determined as specified above. The minimum fungicidal concentration (MFC) was the lowest concentration of the test substance that prevented the visible growth of fungus in the subculture. Each experiment was repeated trice.

Skin irritation test

The evaluation of the degree of dermal irritation was made on *C. porcellus* using the extract of *A. conyzoides* [17]. For this, twelve guinea pigs (six males and six females) were used and each animal served as its own control. They were divided into two groups (1 and 2) of six animals (three males and three females) treated with an aqueous paste of the extract (group 1) or an extract-based ointment (group 2). On day 0, the hairs were shaved at two sites of about 6 cm^2 at the dorsal portion of each animal. The right site served as negative control and the left site was the test site. Each animal was kept in a cage for 24 hours.

On day 1, 0.5 g of the extract moistened with distilled water was applied to Group 1 animal test site and the skin was covered with compress and a non-irritating adhesive plaster. The control sites were treated with distilled water and covered as indicated above. Group 2 animals were treated with the extract-based ointment in the same manner as indicated above. Palm kernel oil was used as an excipient and was applied to the control sites for Group 2 animals. After 24 h of exposure, the covers were removed and the test sites rinsed with distilled water and dried. The animals were examined for the presence of erythema and edema according to Draize dermal irritation score system (0: no erythema and no edema; 1: barely perceptible edema and erythema; 2: well-defined erythema or mild edema; 3: moderate to severe erythema or moderate edema; 4: severe erythema or edema) at increasing intervals of 1, 24, 48, and 72 h [18]. The Primary Irritation Index (PII) was calculated by dividing the sum of the edema and erythema scores of the intervals by the number of intervals (4). The extract was then classified according to the Draize (1959) [18] classification method based on PII as a mildly irritant (PII<2); moderately irritatant (2≤PII≤5), severely irritatant (PII>5).

Acute dermal toxicity

Twenty three-month-old female guinea pigs weighing between 350-450 g were used. They were randomly divided into four groups (1, 2, 3 and 4) of five animals each. The animals were individualized in cages at the animal house of the Department of Biochemistry of University of Dschang. The OECD (1987) [15] method was used to evaluate acute dermal toxicity. Single administration of a 0.5; 4.25 and 8 g/kg body weight, in a final volume of 1 ml was made on the smooth skin (9 cm^2 representing about 10% of the body surface) of animals of groups 2, 3 and 4 respectively. Animals of the control group were administered distilled water. The animals were observed daily for 14 days for the detection of a possible change in behaviour. This observation included assessment of the skin, hair and eyes, effects on respiration, salivation, diarrhea, urine and the effect on the central nervous system (tremor and convulsion, change of activity, gait and posture, reaction to the pinch, bizarre or stereotyped behaviour).

On the fourteenth day of the test, the animals were anesthetized with chloroform vapors, then dissected and the blood of each animal was collected by cardiac puncture using a syringe and intoduced into two tubes, one of which contained EDTA (anticoagulant) and the other without anticoagulant. The blood contained in the tubes with anticoagulant was used directly for the determination of the hematological parameters while the one collected without anticoagulant was used for serum preparation.

The organs (liver, kidneys, spleen, lungs, heart) were removed, freed of fats for the detection of a possible change in color and morphology, then washed and weighed. Part of each organ was milled to obtain the homogenate for the determination of protein contents. Body mass and amount of food ingested were recorded daily for 14 days. The total serum and tissue protein level was determined according to the Biuret method [19] while the total serum creatinine level was determined according to the method described by Newman and Prince [20].

Statistical analysis

The results of the various tests expressed as mean ± standard deviation were subjected to analysis of variance (ANOVA). Differences between averages where they existed were separated using the Waller-Duncan test at the 5% probability level. SPSS version 20 for Windows was used for this purpose.

Results

Ethnopharmacological survey

The listed plant species grouped by families are presented in Table 1. It appears that 19 plant species belonging to 15 botanical families are used by the traditional healers of the Bafou locality for the treatment of dermatophytosis. Three families contain at least two listed plant species: Asteraceae (3), Rutaceae (2) and Zingiberaceae (2). Of the listed species, fifteen are used in combination with other plant species while four are used alone. The most cited species used alone are in decreasing order: *Commelina benghalensis* (10 times), *Ageratum conyzoides* (trice), *Zingiber officinale* (trice), *Piper nigrum* (trice), *Cymbopogon citratus* (twice), *Emilia coccinea* (twice), *Nicotiana tabacum* (twice), *Thymus vulgaris* (twice) and *Citrus maxima* (twice). These nine plants were selected for *in vitro* antidermatophytic test based on the results of the calculated value of use. Table 1 also shows that four administration routes are concerned (oral, anal, topical and inhalation routes). The parts of the plant used in the different preparations are variable and are used fresh or dry. The methods of preparation identified are: decoction, maceration, infusion and powder. These different modes often depend on the part of the plant used, but the frequencies in the ethnopharmacological uses are variable.

Phytochemical screening of crude extracts

In order to highlight the different classes of secondary metabolites, a phytochemical screening was carried out on all the tested extracts. The overall results is recorded in Table 2 below. It appears from this table that the extract from leaves of *A. conyzoides* contained all the tested secondary metabolites. All extracts possessed flavonoids, polyphenols and tannins while other classes of compounds were selectively distributed in the different extracts.

Composition of leave extract of Ageratum conyzoides

The chromatogram of methanol extract from leaves of *A. conyzoides* is presented in Figure 1. From that figure, it appears that compounds are present in varying proportions in the extract. At retention time 23.93, the highest peak was observed representing the major compound (6,7-Dimethyl-2,2-dimethyl-2H-1-benzopyran). The other compounds are present in relatively small proportion. Table 3 below shows the various compounds (nine) derived from the GC-MS analysis of the extract from leaves of *A. conyzoides*. The identified compounds are mostly alkane and compounds with benzene ring.

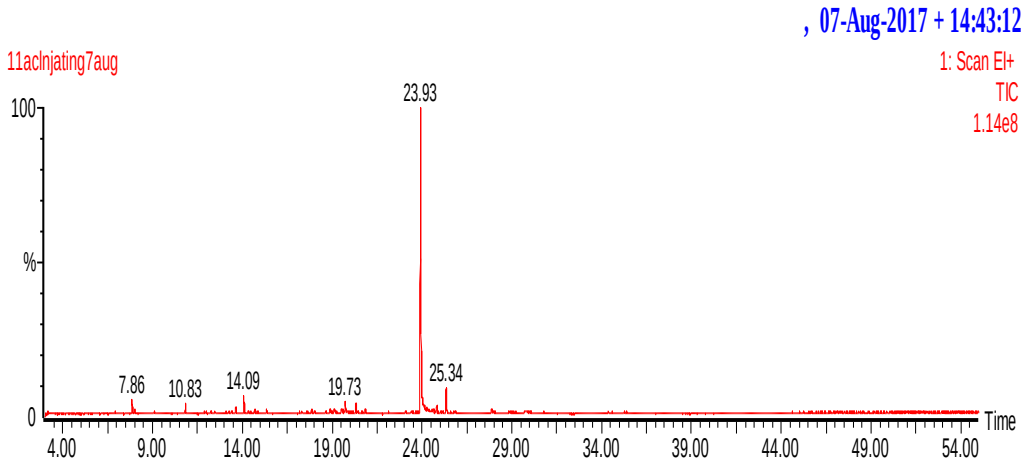

Figure 1. GC-MS chromatogram of extract from leaves of *A. conyzoides*.

In vitro anti-dermatophytic activities of crude extracts

The evaluation of anti-dermatophytic activity of various crude extracts was carried out by determining the MIC and the MFC of each extract. In order to determine whether different extracts showed fungistatic or fungicidal effects, the MFC/MIC ratios were calculated (Table 4). From that table, it appears that all tested plant extracts have anti-dermatophytic activity varying from one fungal isolate to another. The anti-dermatophytic activities (MIC) of the various extracts are generally between 32 and 2048 µg/mL. Except the extract of *C. bengalensis* that inhibited 66.66% of the tested microorganisms, all the tested extracts inhibited 100% of the tested dermatophytes. The most active extract was that from leaves of *A. conyzoides* with MIC ranging from 32 to 512 µg/mL.The most sensitive strain was *T. violaceum* susceptible to all the tested extracts. On that dermatophyte, almost all the tested extracts showed fungicidal effect as the MFC/MIC ratios were less than or equal to 2.

Skin irritation test

After 24 hours of exposure to the leaves extract of *A. conyzoides* (0.5 g), followed by rinsing and observation for 72 hours, the test and control sites showed neither edema nor erythema and therefore, IIP was 0 for animals of both groups.

Acute dermal toxicity

Effect of leaves extract of *Ageratum conyzoides* on the physical and behavioural parameters of guinea pigs

The various changes that occurred in this study are shown in Table 5. It appears that the animals treated at doses of 4250 mg/kg and 500 mg/kg exhibited no behavioural deviance after 48 hours following administration of the extract; likewise they remained normal during the 12 days of observation that followed treatment. In animals treated with 8000 mg/kg, however, there is irritation of the skin, a reduction in motor activity and, strength although there is no death during the experiment.

Effect of the leaves extract of A. conyzoides on guinea pig food consumption, weight growth, macroscopic features and relative weight of organs during the experimentation period

The administration of *A. conyzoides* leaves extract led to an increase in food consumption and body weight in the animals that received the extract compared to the control group throughout the test.

In order to detect possible pathological changes in the organs, animals were sacrificed 14 days following administration of extract. Macroscopic examination of the liver, heart, kidneys, spleen and lung revealed no abnormalities in treated guinea pigs compared to control. At all doses, the extract showed no statistically significant effect (P>0.05) on the relative mass of organs compared to control animals (Table 6).

Effect of leaves extract of A. conyzoides on tissue protein content, serum content and serum creatinine

The following Table 7 analysis shows that there is no significant difference in liver, heart, kidney, spleen and serum protein content compared to control. The protein content in the lungs increased with the doses of extract and this increase is significant at the dose of 8000 mg/kg. No significant difference (P>0.05) was observed on creatinine content compared with control.

Effect of leaves extract of A. conyzoides on hematological parameters of guinea pigs

Administration of *A. conyzoides* leaves extract did not influence hematological parameters as no significant differences (P>0.05) were observed compared to control (Table 8).

Discussion

At the end of survey, 16 people, mostly illiterate, male (11) and female (5) were surveyed. Male predominance is explained by the fact that the virtues of plants are ancestral knowledge that is passed down from generation to generation and generally to the successor, most often male [21]. In the study area, 19 species belonging to 15 families have been identified. The most represented family was Asteraceae (15.78%). This family is among those that provide the most plants to the African pharmacopoeia [22]. It would be part, in number of species, of the most important of the flora of Bafou. Ethnopharmacological surveys have been conducted in several parts of the world, particularly in Africa. Dibong *et al*. [23] counted in the markets of Douala-Cameroon, 30 species of medicinal plants belonging to 25 families dominated by Magnoliopsida. Tra-Bi *et al*. (2008) [24] inventoried on the Abidjan (Ivory Coast) markets, 25 species of plants used against diabetes, with a predominance of Euphorbiaceae and Asteraceae. The route of administration indicated by the therapists was the topical route, which would imply that the topical route would be the one adapted for the treatment of dermatophytic infections.

The plant extracts used in this study showed a difference in their composition in secondary metabolites. This could be explained by the difference

in the pedoclimatic conditions of the harvesting sites [16]. The tested plant extracts showed difference in their activities. This could be ascribed to the difference in phytochemical composition observed between the different tested plant extracts. Indeed, many previous studies have had to credit secondary metabolites of plants with enormous antifungal potential [25, 26]. Substances isolated from plant extracts such as berberine (alkaloid); Gallic acid (triterpene) and mangiferin (flavonoid) have shown interesting antimicrobial activity [27, 28]. It is also important to note that the activity of a plant extract does not only depend on the presence of secondary metabolites alone, but also on their types, their quantity and possible interactions with other constituents. Thus, the observed activity of the extract from *A. conyzoides* leaves could be due to the major compound known as 6,7-Dimethyl-2,2-dimethyl-2H-1-benzopyran or to the interaction between the different compounds it contains because according to several studies [29, 30, 31], the synergistic interactions between the different compounds can be at the origin of a much more pronounced activity. In addition, the difference in the genetic constitution of each strain [32] or the difference in the mechanisms of action of active principle (s) of extracts can also explain the variation observed in the activity of extracts [33].

At the end of the dermal irritation test, independently of the group, no change was observed on the test sites compared to the control sites after exposure to 0.5 g of leaves extract of *A. conyzoides*. These results suggest that although palm kernel oil is a good vehicle and can therefore be used as an excipient in drugs used for the treatment of dermatophytosis, it is not essential for dermal anti-dermatophytic treatment using less than 0.5 g of extract from leaves of *A. conyzoides*. These results were in contradiction with those found in the literature [25]. Indeed, using the extract of dichloromethane-methanol (1: 1 v/v) from *Polyscias fulva* bark, this latter showed that the use of palm kernel oil as a vehicle was preferable to water. This contradiction can be explained by the differences in the plant species and solvents used for extraction.

The reduction in activity and reactivity observed in guinea pigs receiving the highest dose (8000 mg/kg) compared to other groups has already been observed by Njateng *et al* (2010) [34] with the essential oil of *Ageratum houtonianum.* This result suggests that the methanolic extract of *A. conyzoides* exerts a sedative and depressive effect on the central nervous system [35] at high doses. The reduction of reaction to the pinch may be due to the action of the methanolic extract of *A. conyzoides* on the nociceptors, the inhibition of the production of algogenic substances (histamines, prostaglandins) or the inhibition of transmission of the message related to pain in the central nervous system [36]. The observed irritation effect at highest dose of extract showed that above at high dose, the topical use of *A. conyzoides* extract without a vehicle (Palm kernel oil) is risky. The absence of death throughout the test indicates that the LD_{50} of methanolic extract of *A. conyzoides* leaves is greater than 8000 mg/kg. This was confirmed by the absence of particular signs of toxicity after macroscopic examination of the various organs. Also, no significant difference ($P > 0.05$) was observed between the relative body weights of the treated animals compared to those of the controls. In the majority of cases, except for the brain, the change in weight of an organ is proportional to body weight [37], as was the case in this study.

Creatinine results from the metabolism of creatinine in skeletal striated muscles. Under normal physiological conditions, it is filtered by the kidneys and eliminated in the urine. An increase in serum concentration is indicative of renal dysfunction [38]. According to Katheen and James (1992) [39], measuring serum creatinine level can also help to assess muscle mass. The results obtained in this study showed no significant difference in creatinine content in the animals receiving the extract compared to control. This suggests that the extract from leaves of *A. conyzoides* would not affect renal function but would have induced an increase in body weight compared to control after dermal administration.

Regarding the tissue protein content, no significant difference was observed except for the lung protein content which significantly increased at the 8000 mg/kg dose. Endogenous proteins not only guarantee the transport of xenobiotics from the blood to organs, but also their biotransformation in the liver to activate, excrete or detoxify [40]. In this study, an increase in protein level was noted, this may be due to an increase in hepatic protein synthesis required for xenobiotic metabolism [41]. The absence of a significant difference observed on the haematological parameters could be due to the fact that the methanolic extract of the leaves of *A. conyzoides* does not exert any significant action on the synthesis of red blood cells, its components, white blood cells, lymphocytes, granulocytes and monocytes.

Table 1. Classification of plants according to their families, scientific, vernacular or common names.

Scientific name (Family)	Vernacular (Yemba)/ Common name	Part used	Voucher number	Mode of preparation/ Administration route	Associated plants (Vernacular or common name)	Other traditional uses
*Cymbopogon citratus (*Poaceae)	Fumbagrass/ Citronnelle	Leaves	C : Dang D N^{o} 202 H:18628 SFR/CAM	D, I, M/O, T	/	Folk remedy for coughs, elephantiasis, flu, gingivitis, headache, leprosy, malaria, ophthalmic, pneumonia and vascular disorders. Mixed with pepper, it is a home therapy for menstrual troubles and nausea. It helps to detoxify the liver, pancreas, kidney, bladder and the digestive tract. It cuts down uric acid, cholesterol, excess fats and other toxins in the body while stimulating digestion, blood circulation, and lactation [42].
*Ageratum conyzoides (*Asteraceae)	Mvengngangcia/Roi des herbes	Stems, leaves, roots	C : Leuwenberg A. J. M. N° 7505 H : 21155/SRF-CAM		Mveng nguim, mveng lapin	In Cameroon and Congo, it is traditionally used to treat fever, rheumatism, headache, and colic [43, 44].
*Erigeron floribundus(*Asteraceae)	Mvengnguim	Stems, leaves, roots	C : Letouziy R. N^{O} 4658 H: 5619/SFR/CAM	P/T	Mveng lapin, Mveng ngang cia	Treatment of skin disorders [24].
*Emilia coccinea (*Asteraceae)	Mveng lapin	Stems, leaves, roots	C : Mpon. Benoit N^{o}446 H : 19052/SFR/CAM		Mveng ngang cia, Mveng nguim	Treatment of diarrhea, jaundice [45].
*Rubus fellatae (*Rosaceae)	Lekeukeu/Framboisier	Buds and fruits	C : Leuwenberg A. J. M. N° 8150 H : 44163/HNC	Pt, G/T	/	Hypogycaemic, anti inflammatory, against angina and diarrhea [46, 47].
Commelina benghalensis (Commelinaceae)	Lewouwou	Sap	C : Letouziy R. N^{O} 2945 H: 3079/SFRK/CAM	Pr/T	Aloe	The plant is used to fight against inflammation of the conjunctiva, psychosis, epilepsy, nose blockage in children [48].
*Aloe vera (*Aloeaceae)	Aloe vera/Aloe	Leaves	No reference sample	M/O, T	Citronnier	Used externally to treat wound, burns and eczema.commonly used to treat first and second degree burns, as well as sun burns and poison, infections, and eczema. Its juice is used gainst ulcerative colitis, an inflammatory bowel disease, coughs, wounds, ulcers, gastritis, diabetes, cancer, headaches, arthritis, immune-system deficiencies when taken orally [49].
*Alium sativum (*Liliaceae)	Ail	Bulb (pod)	C : Westphal N° 10019 H : 44810/HNC	P, D, I, M/O, T	Capucine, tamarin	It has been used around the world to cure many diseases, including hypertension, infections, and snake bites, and some cultures have used it to ward off evil spirits, for reducing cholesterol levels and cardio-vascular risk, as well as for its antineoplastic and antimicrobial properties [50].
*Cinnamomum verum (*Lauraceae)	Cannelle	Leaves, bark	No reference sample	P, D, I, M/O, T	Gingembre	It has been traditionally used as tooth powder and to treat toothaches, dental problems, oral microbiota,

						and bad breath [51].
*Curcuma longa (*zingiberaceae)	Curcuma longa	Rhizome	C : BiholongN° 563 H: 42173/HNC	P, D, I, M/O, T, A	Poivre noir	It is used as Anticancer, anti-inflammatory, against microbial infections, diabetes, arthritic, muscular disorders, biliary disorders, anorexia, cough, diabetic wounds, hepatic disorders and sinusitis [52].
*Zingiber officinale (*zingiberaceae)	Ginger /Gingembre	Rhizome	C : Westphal N° 10107 H : 43146/HNC	P, D, I, M/O, T, A	Poivre noir	Ginger has been used to treat a wide range of ailments including dyspepsia, diarrhoea, nausea, asthma, respiratory disorders, toothache, gingivitis and arthritis [53].
*Syzygium aromaticum (*Myrtaceae)	Clou de girofle	Fruit	No reference sample	P, D, I, M/O, T	*Curcuma longa*	It can increase blood circulation. It relieve the stomach pain, nausea, vomiting the pain in chronic rheumatism, toothache and lumbago [54].
*Symphytum officinale (*Boraginaceae)	Consoude/ Comfrey	Leaves, bark	No reference sample	M/A	Plantain, géranium	Comfrey root has been used as a traditional medicinal plant for the treatment of painful muscle and joint complaints [55].
*Nicotiana tabacum (*Solanaceae)	Tabac	Leaves, root	No reference sample	P, M/O, T	Olivier, neem	It is used in the treatment of Alzheimer disease, Parkinson disease, depression and anxiety, schizophrenia, attention deficit hyperactivity disorder (ADHD), pain, and obesity: [56].
*Thymus vulgaris (*Lamiaceae)	Thym	Leaves, stem	C : Dang D N^{o} 516 H:25746/ SFR/CAM	P, M/T	Capucine, renouée	It is incredibly useful in cases of assorted intestinal infections and infestations caused by gram-positive and gram-negative bacterium, fungi or hookworms, ascarids. It may also improve liver functioning, and act as an appetite stimulant. Used as a gargle, it is helpful in the treatment of laryngitis and inflammation [57].
*Citrus maxima (*Rutaceae*)*	Pamplemouse	Seed, pericarp	Sn 25860/SRFC	P, D, I, M/O, T, A, I	Citronnier, ail	It has been used as a folk medicine in many countries as antimicrobial, antioxidant, larvicidal, hepatoprotective, anticancer, antiplatelet, antidiabetic and anti-inflammatory [58].
*Citrus limon (*Rutaceae*)*	Citronier	Pericarp	C : Gayum H. N° 1 H : 65106/HNC	P, D, I, M/O,T, A, I	Pamplemousse	It is used as Anti-infective, anti-nausea, tonic, anti-cholesterolaemic, lipolytic, hypoglycaemic, ulcer protector. [59]
*Piper nigrum (*Piperaceae)	Poivre noir	Seeds	C : Dang D N^{o} 297 H: 25818/SFR/CAM	P, D, I, M/O, T	/	In traditional Chinese medicine, black pepper has been used for the treatment of epilepsy [60]. The use of black pepper is traditionally well-known in the treatment of a variety of gastrointestinal disorders, and to improve digestion [61].
*Azadirachta indica (*Meliaceae)	Neem	Bark, leaves	H : 4447/SRFK/ CAM	P, D, I, M/O, T	/	All parts of neem trees including leaves, seeds, roots, bark and flowers are used to cure stomach ulcers, jaundice and to overcome a variety of infectious and parasitic diseases, ranging from leprosy, chicken pox, and malaria [62].

G : Frozen ; D : Decoction ; I : Infusion ; M : Maceration ; P : Powder ; Pt : Paw ; Pr : Pressing ; O : Oral : T : Topical : A : Anal : In : Inhalation : C : Collector identification ; H :Registration number to the Herbarium ; sn : Without number ; HNC : Cameroun National Herbarium ; SRFC : Society of Forest Reserves of Cameroun

Table 2. Qualitative phytochemical composition of the different crude extracts

Phytochemical classes	**Crude extracts**									
	PN	**NT**	**TV**	**EC**	**ZO**	**CM**	**AC_L**	**AC_S**	**CC**	**CB**
Alkaloids	+	-	-	-	-	-	+	-	+	+
Polyphenols	+	+	+	+	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+	+	+	+	+
Anthraquinones	+	-	-	+	+	+	+	+	+	+
Anthocyanins	-	-	+	+	+	-	+	+	-	-
Tannins	+	+	+	+	+	+	+	+	+	+
Triterpenes	+	-	+	+	-	-	+		+	-
Steroids	+	-	+	+	-	-	+	+	+	-
Saponins	-	+	+	+	+	-	+	+	-	+

(-) = Absent ; (+) = Present ; PN = *Piper nigrum* ; NT = *Nicotiana tabacum* ; TV = *Thymus vulgaris*; EC = *Emilia coccinea*; ZO = *Zingiber officanale*; CM = *Citrus maxima* ; AC_L = *Ageratum conyzoides* (Leaves) ; AC_S = *Ageratum conyzoides* (Stem) ; CC = *Cymbopogon citratus* ; CB = *Commelina benghalensis*

Table 3. Main compounds obtained after analysis of *A. conyzoides* leaves extract by GC-MS

Compound's name	**Formula**	**Molar mass**	**Registration number (CAS)**
3-Ethyl-3-methylnonadecan	$C_{22}H_{46}$	310	900360-46-6
Hentriacontan	$C_{31}H_{64}$	436	630-04-6
Precocen I	$C_{13}H_{18}O$	190	17598-02-6
2,6-Bis (1,1-dimethylethyl)- Phenol	$C_{14}H_{22}O$	206	128-39-2
6,7-Dimethyl-2,2-dimethyl-2H-1-benzopyran	$C_{13}H_{16}O_3$	220	644-06-4
3-methylpentacosan	$C_{26}H_{54}$	366	6902-54-1
Ethanone, 1-(7-hydroxy-5-methoxy-2,2-dimethyl-2H-1-benzopyran-6-	$C_{14}H_{16}O_4$	248	529-70-4
Benzoic acid, 4benzyloxy-3-(1-methoxyethoxy)-butan-2-yl ester	$C_{21}H_{26}O_5$	358	900196-06-9
3,5-bis (1,1-dimethylethyl)- Phenol	$C_{14}H_{22}O$	206	1138-52-9

Table 4. Minimum inhibitory and minimum fungicidal concentrations of plant extracts

Extract		**Strains**		
		T. violaceum	***M. gypseum***	***E. floccosum***
P. nigrum	MIC	64	512	1024
	MFC	512	1024	2048
	MFC/MIC	8	2	2
N. tabacum	MIC	256	1024	2048
	MFC	256	1024	/
	MFC/MIC	1	1	/
T. vulgaris	MIC	256	512	1024
	MFC	256	1024	/
	MFC/MIC	1	2	/
E. coccinea	MIC	256	512	2048
	MFC	256	512	/
	MFC/MIC	1	1	/
Z. officinale	MIC	32	2048	1024
	MFC	32	/	/
	MFC/MIC	1	/	/
C. maxima	MIC	512	1024	2048
	MFC	/	1024	/
	MFC/MIC	/	1	/
A. conyzoides (leaves)	MIC	32	32	512
	MFC	32	32	512
	MFC/MIC	1	1	1
A. conyzoides (stem)	MIC	32	512	2048
	MFC	32	1024	/
	MFC/MIC	1	2	/
C. citratus	MIC	256	2048	2048
	MFC	256	/	/
	MFC/MIC	1	/	/
C. benghalensis	MIC	128	/	2048
	MFC	128	/	/
	MFC/MIC	/	/	/
Griseofulvin	MIC	16	256	32
	MFC	16	512	128
	MFC/MIC	1	2	4

MIC: Minimum Inhibitory Concentration, MFC: Minimum Fungicidal Concentration

Table 5. Physical and Behavioural parameters of guinea pigs after single doses administration of the extract of *A. conyzoides.*

Study parameters	Doses (in mg/kg)			
	8000	**4250**	**500**	**0**
Reaction to noise	N	N	N	N
Reaction to the pinch	D	N	N	N
Locomotion	D	N	N	N
Stool appearance	G	G	G	G
Convulsions	A	A	A	A
Sleep	A	A	A	A
Salivation	A	A	A	A
Appearance of the skin	I	N	N	N
Hair appearance	N	N	N	N
Strength	D	N	N	N
Mortality after 14 days	0	0	0	0
Number of pigs used	5	5	5	5

N = Normal; D = Slightly diminished; G = Granular; A = Absent ; I = irritation

Table 6. Effect of leaves extract of *A. conyzoides* on the relative weight of organs of animals according to dose

Dose (mg/kg)	Organs (g)				
	Liver	**Heart**	**Lung**	**Kidney**	**Spleen**
0	2.44±0.23^{a}	0.37±0.04^{a}	0.79±0.05^{a}	0.74±0.06^{a}	0.16±0.02^{a}
500	2.54±0.55^{a}	0.31±0.04^{a}	0.77±0.09^{a}	0.70±0.06^{a}	0.15±0.01^{a}
4250	2.60±0.47^{a}	0.32±0.04^{a}	0.88±0.07^{a}	0.77±0.10^{a}	0.18±0.06^{a}
8000	2.70±0.38^{a}	0.35±0.03^{a}	0.90±0.13^{a}	0.79±0.16^{a}	0.32±0.37^{a}

The table values are presented as mean ± standard deviation of 5 repetitions. In the same column and for the same parameter, the value bearing the different letters are significantly different (p<0,05).

Table 7. Effect of the leaves extract of *A. conyzoides* on the content of tissue protein, serum and serum creatinine

Dose (mg/kg)	Tissue and serum protein content (g/dl) and serum creatinine (mg/dl)						
	Liver	**Heart**	**Lung**	**Kidney**	**Spleen**	**serum**	**Serum creatinine**
0	1.25±0.20^{a}	0.54±0.02^{a}	0.67±0.08^{a}	0.82±0.46^{a}	0.91±0.06$^{a.\ b}$	4.57±0.29^{a}	1.42±0.54^{a}
500	1.10±0.36^{a}	0.46±0.16^{a}	0.73±0.15^{a}	0.58±0.31^{a}	0.88±0.09^{a}	4.17±0.11^{a}	1.04±0.43^{a}
4250	0.95±0.18^{a}	0.68±0.40^{a}	0.87±0.32$^{a.\ b}$	0.74±0.17^{a}	0.79±0.11^{a}	4.33±0.13^{a}	1.42±0.84^{a}
8000	0.94±0.35^{a}	0.52±0.19^{a}	1.17±0.40^{b}	0.69±0.13^{a}	1.09±0.16^{b}	4.27±0.41^{a}	2.42±2.11^{a}

The table values are presented as mean ± standard deviation of 5 repetitions. In the same column, the value bearing the different letters are significantly different (p<0,05).

Table 8. Effect of the leaves extract of *A. conyzoides* on the hematological parameters of *C. porcellus*

parameters	Doses (mg/kg)			
	0	**500**	**4250**	**8000**
White blood cells(X 10^3/µl)	7.78±2.47[a]	8.34±1.18[a]	8.48±1.73[a]	8.58±3.15[a]
Lymphocytes (%)	50.80±7.38[a]	52.02±5.81[a]	51.34±5.43[a]	46.48±3.81[a]
Monocytes (%)	11.56±2.56[a]	11.10±2.87[a]	9.88±2.38[a]	10.78±2.99[a]
Granulocytes (%)	37.64±7.77[a]	36.88±4.38[a]	38.78±5.86[a]	42.74±1.96[a]
Platelets(X 10^3/µl)	75.40±19.35[a]	82.20±14.75[a]	68.40±12.89[a]	85.60±11.05[a]
APV(fl)	7.84±0.70[a]	7.46±0.48[a.]	7.72±0.51[a]	6.86±0.34[a]
Red blood cells (X 10^6/µl)	5.32±0.43[a]	5.17±0.42[a]	5.20±0.50[a]	5.23±0.37[a]
Hemoglobin(g/dl)	14.70±1.20[a]	14.44±1.20[a]	14.60±1.55[a]	14.36±0.92[a]
Hematocrit(%)	32.34±2.58[a]	31.54±2.47[a]	32.50±3.16[a]	32.50±2.77[a]
ACV(fl)	60.80±1.02[a]	61.02±0.84[a]	62.62±1.82[a]	62.10±1.53[a]
MCHC(pg)	27.54±0.59[a]	27.84±0.23[a]	28.04±0.39[a]	27.42±1.15[a]
ACCH (g/dl)	45.40±0.24[a]	45.72±0.44[a]	44.86±1.03[a]	44.26±2.53[a]

The table values are presented as mean ± standard deviation of 5 repetitions. In the same line, the value bearing the different letters are significantly different (p<0,05). ACV = Average Corpuscular Volume, MCHC = Mean Corpuscular Hemoglobin Content, ACCH = Average Corpuscular Concentration of Hemoglobin, APV = Average Platelet Volume.

Conclusions

The methanolic extracts from the tested plants showed antidermatophytic activities with that from the leaves of *Ageratum conyzoides* being the most active and may not be toxic when used topically at reasonable doses.

Authors' Contribution

A-JAD was the main investigator and LCY helped in the biological assays. GSSN designated the study, supervised the assays and revised the manuscript. All authors read and agreed on the final version of the manuscript,

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Conflict of interest

The authors declare that there is no conflict of interest

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