Chapter

Developments and Perspectives in Bryophyte Biotechnology in Sub-Saharan Africa

Kenneth Yongabi Anchang and Henrik Toft Simonsen

Abstract

The work described here covers an examination of new bioproducts based on sub-Saharan bryophytes. The work includes in vitro testing of extracts from moss and liverworts against plant pathogenic microbes causing food decay and field crop losses. Additionally, we have shown specific antimicrobial activities of *Marchantia debilis* and **moss** against *Erwinia spp* and *Pseudomonas spp*. The extracts were also tested against aflatoxin-producing fungi isolated from food crops such as maize and peanuts. The efficacy of the extracts on clinical dermatological fungal isolates like *Dermatophilus congolensis* has not been reported. This led to the production of an antifungal solution of bryophyte extracts, which was tested in vivo on animals with skin diseases caused by *Dermatophilosis*. Around 99.5% of the animals were treated. The antifungal solution for treatments has been labeled **Bryosol**, while the disinfectants solution is labeled Bryo-disinfectants and the crop-fungicide is labeled Bryo-fungicides. A mini field pilot trial with Bryo-fungicide showed that crops infected with pathogenic fungi were treated. The results provide the first attempt to demonstrate the use of bioproducts for organic treatment of agricultural crops and diseases in animals based on sub-Saharan bryophytes.

Keywords: moss, Marchantia, Bryosol, disinfectants, antifungal, bioproducts, foods, *Dermatophilosis*

1. Introduction

Bryophytes are non-vascular plants, which are constituted of mosses, liverworts, and hornworts [1]. Although not usually seen to have any importance, bryophytes have recently been used as bioindicators of pollution and are often used for decorations. However, the medicinal value of bryophytes is huge with a panoply of bioactive compounds isolated from bryophytes, especially liverworts [1, 2]. Bioactive compounds have been isolated from liverworts from Asia, Europe, and South Africa. For example, Allison in 1975 identified a number of bioactive compounds from liverworts in New Zealand. Volatile constituents have been identified in liverworts like *Tritomaria polita*, *Marsupella emarginata*, *M. aquatic*, and *M. alpine* [3].

It has been seen that bryophytes are rich in diverse phytochemicals such as sesquiterpenoids, norsesquiterpenoids, anthocyanidins, riccionidins etc. with interesting biological activities, such as antimicrobial, antifungal, insect-repellent, molluscicidal, cardiotonic activity, and fragrance compounds among others [1]. Bryophytes

are very common across the world, particularly in wet areas like Cameroon. The ecology of Cameroon is rich in algae and lichens; bryophytes in Cameroon are part of the Congo forest and the highlands from Mount Cameroon via the Atlantika Mountains to the Mandara Mountains collectively constituting the Congo forest from Nigeria, ranges from 1400 to 4000 m, and harbors a rich biodiversity of both lower and higher plants. A survey of bryophytes in Cameroon revealed many unidentified species with familiar dormant species such as *Marchantia spp* [2].

Phenanthrenes and other phenolics have been isolated from in vitro cultures of *Marchantia polymorpha*. Recently, extensive report was published on the biology and constituents and chemistry and organic natural products of bryophytes [1, 4], though this lacks data on bryophytes from West and Central Africa, especially Cameroon. Screening of bryophytes and lower plants for biologically active compounds from Cameroon is of great importance considering that Cameroon is centrally placed in Africa and harbors all the ecological and geographical characteristics widespread across Africa. The search for bioactive compounds from plants in the past 30 years in Africa has concentrated on higher plants with little or no interest on bryophytes, which is the same in the rest of the world [2]. The prevalence of many plant and animal pathogenic diseases is growing along with drug resistance strains. This generates huge losses in agricultural yield and productivity across Africa. Treatment and management are expensive for many African farmers and therefore a cheap alternative, preferably organic, is needed [3].

Bioactive compounds from bryophytes could bridge this gap. Here, we show that new drug leads could be identified from bryophytes from Cameroon to address plant pathogenic diseases and animal diseases like *Dermatophilosis* infection in cattle.

2. Dermatophilosis in animals

Dermatophilosis is caused by the bacterium *Dermatophilus congolensis*, which is an aerobic actinomycete (facultatively anaerobic) and usually affects animals and occasionally humans [5].

Dermatophilosis is distributed worldwide, prevailing in tropical areas, and related to humid environments and other factors, such as poor veterinary services, coinfection with a number of bacterial infections, especially in animals with compromised immune systems, and poor hygiene conditions in favor of its occurrence and spread.

In Africa and many other places, the impact caused by animal diseases continues to negatively affect the local economy. Dermatophilosis is a tick-borne disease of ruminants and other animals [5] and affects all parts of the body of the animal. In Nigeria and Cameroon, Dermatophilosis accounts for about 75% of morbidity in herds and about 12% in cattle. Mortality rate has been reported to be quite high due to the resulting toxemia and general debility [5]. Dermatophilosis is an intractable disease and highly contagious, spreading from cattle to man (zoonotic).

The common and orthodox treatment for dermatophilosis is through the use of classical antibiotics like lamstreptocide, charmil, and terramycin long acting (TLA), 1% potassium aluminum sulphate dip, and co-biotic (penicillin and dihydrostreptomycin). Apart from the toxicity of some of these drugs, some of them contain heavy metals, which on accumulation could cause tumors and cancers in both man and animals [3, 6]. The use of organophosphate dips has also been reported to have a negative effect on the environment, and it has been observed to cause systemic damages on internal organs of both animals and humans.

3. Plant pathogenic fungal contamination of food and crops in Africa

Agricultural plant products in sub-Saharan Africa often decay fast due to infection of field crops and harvested products. Most of these plant pathogenic diseases are fungi. Despite the availability of chemicals for control of these pathogens, many farmers find it inaccessible for reasons of costs and lack of adequate knowledge on usage.

In Africa, the predominant food source for more than 70% of the population is grains such as maze and groundnuts. Even though there are new and improved methods for containing these diseases in food crops, there are still great losses due to fungal infections of the crops. A number of reports have shown that aflatoxins producing fungi are predominant with both field and stored maize and groundnuts. Aflatoxin (Aflatoxin B1) is produced by *Aspergillus flavus* or *Aspergillus parasiticus*, and effects of aflatoxin on crops like maize and groundnuts completely destroy the crop due to the toxicity of aflatoxin to humans.

4. Methodology

4.1 Isolation and identification of *Dermatophilus congolensis*

Traditional cultivation techniques were employed for isolation and identification. Swab samples were taken from the lesions on the animal and analyzed at the Phytobiotechnology Research laboratory for *Dermatophilus congolensis* culture. Initial cultures were done in thioglycolate broth and subculture after 48 hours on fortified chloramphenicol Sabouraud Dextrose agar and modified cycloheximide (Actidione)-chloramphenicol Sabouraud agar previously prepared and incubated for 48 hours at 35°C in an aerobic condition. For specific cultural distinct features on agar plate, blood and chocolate agar plates were prepared and distinct colonies from Sabouraud agar plates were transferred on to blood and chocolate agar plates aseptically incubated in air supplemented with 5% CO_2, and the blood agar was also incubated in an anaerobic atmosphere [4, 7].

At 24 hours, a pure culture with tiny, point-like, smooth, creamy, white-colored, beta-hemolytic colonies adherent to the media grew in aerobic blood agar and chocolate agar, with Gram staining showing hypha-like, branching filaments with "train track" forms and clusters of sporangia as well as Gram-positive coccoid forms, mostly in chains. After 48 h, crowded colonies became yellowish and mucoid, with a great variation in colonial morphology, for example, pulvinate, umbonate, or cake crumb-like forms were considered typical of *Dermatophilus congolensis*. This is shown in **Figures 1** and **2**.

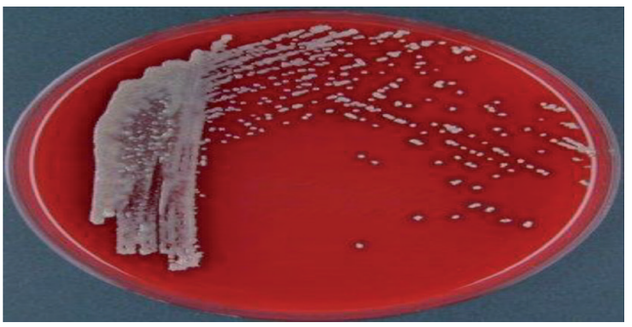

Figure 1.
Beta-hemolytic colonies after 2 days of incubation at 37°C on blood agar medium, with pleomorphic appearance in pulvinate, umbonate, or cake crumb-like form. Dermatophilus congolensis on blood agar plate.

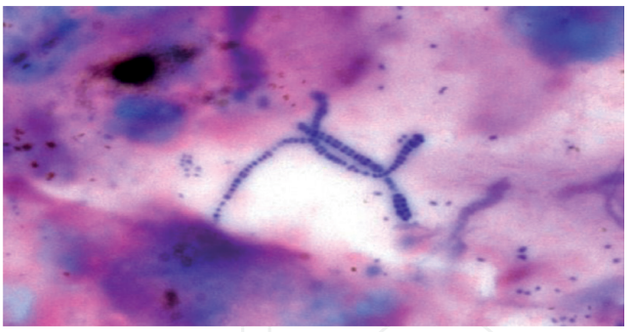

Figure 2.
Gram stain with characteristic branching filaments with "train track" forms or hypha-like chains that released sporangium Gram-positive cells (magnification, 1000×). Beta-hemolytic colonies after 2 days of incubation at 37°C on blood agar medium, with pleomorphic appearance in pulvinate, umbonate, or cake crumb-like form.

Figures 1 and **2** reveal the unique, distinct bacteriological features of *Dermatophilus congolensis.* The biochemical characteristics of *D congolensis* as basis for identification done according Monica Cheesbrough [8] revealed that beta hemolysis in 3–7 days, oxidase, gelatin, casein and starch all positive, while *D congolensis* fermented fructose, ribose and galactose.

5. Survey and extraction of bryophytes

A preliminary survey of liverworts in northwest and southwest regions of Cameroon was performed. Bryophytes (species of liverwort and moss) from Cameroon West/Central Africa were collected and complete sequences for the 18S-rRNA gene of bryophytes were used to construct a phylogenetic tree of bryophytes from Cameroon to fully identify the prevalent species in Cameroon.

5.1 Extraction procedures for the selected and identified bryophyte species

About 50 g of each of the bryophyte (*Marchantia debilis* and Plangiochila spp) plant material were added separately to 250 ml each of methanol and petroleum ether (1:5 w/v) in 250 beakers (Pyrex) for each plant mash and allowed to extract for 72 hours [6]. The extracts were filtered by gravity filtration using Whatman filter paper no 1 locally purchased in Bamenda, Cameroon, and the filtrate solvent was evaporated under vacuum using an incubator at 37°C and the resulting dried extracts were stored in sterile screw-capped bottles and kept at room temperature for further antibacterial testing using extracts of bryophytes. The morphology of the bryophytes and the nature of extracts is shown in **Figures 3–8**.

5.2 Antibacterial activity of the extracts of bryophytes

The agar diffusion method according to Yongabi et al. [9] was employed. Around 0.2 g of the *Marchantia debilis* and *Plangiochila* spp. extracts was reconstituted in 5 ml of distilled water. Antibiotic susceptibility will be determined by agar well diffusion method—commonly used and standardized in the US by National Committee for Clinical Laboratory Standards (NCCLS) [8, 10].

The zone of inhibition was measured and results interpreted as sensitive, intermediate resistant, or resistant. The zone sizes of inhibition were measured and interpreted using the NCCLS as recommended by WHO [8]. Each of the extracts was incorporated in a 6-mm well previously bored using a steel borer. A control set up was established by introducing the extracting solvent (methanol and petroleum) into the

different wells as well. The plates were incubated at 37°C for 36 hours. The development of inhibition by the extracts against the test organism was measured [11].

The differences between the inhibition rates of the extracts in the test setup and that of the control were recorded as actual diameter of zones of inhibition caused by the extract. The methanol and petroleum extracts did not exhibit any inhibition in this study, as shown in **Tables 1** and **2**.

5.3 Preparation of bryophyte extracts-based ointment using olive oil base

The organic extracts (200 mg each) of *Marchantia* spp. and *Plangiochila* were blended into 200 ml of olive oil and palm kernel oil. Standard organic chemistry protocols as described by Yongabi et al. [9] were applied.

	Fresh *Marchantia debilis*			**Dried *Marchantia debilis***		
Microbial test organism	**Hexane extracts**	**Petroleum extracts**	**Methanol extracts**	**Hexane extracts**	**Petroleum extracts**	**Methanol extracts**
Staphylococcus aureus	9 mm	12 mm	14 mm	9.5 mm	17.2 mm	12.5 mm
Pseudomonas aeruginosa	0 mm	0 mm	0 mm	10.5 mm	5.2 mm	12.5 mm
Bacillus spp	0 mm	0 mm	0 mm	11.5 mm	6.2 mm	13.5 mm
Dermatophilus congolensis	5 mm	5 mm	13 mm	6 mm	11 mm	12.5 mm
Aspergillus flavus	3 cm	2 cm	No Growth	2 cm	1 cm	No Growth

Dermatophilus congolensis is an isolate from cow. For Aspergillus flavus (an isolate from maize rot) the inhibition is given as growth of fungi in 7 days, where the control grew 10 cm.

Table 1.
Preliminary in vitro test showing zone of inhibition of organic extracts of Marchantia debilis on different microbes.

	Fresh *Plangiochila spp*			**Dried *Plangiochila spp***		
Microbial test organism	**Hexane extracts**	**Petroleum extracts**	**Methanol extracts**	**Hexane extracts**	**Petroleum extracts**	**Methanol extracts**
Staphylococcus aureus	9 mm	8 mm	14 mm	9.8 mm	8.8 mm	15.5 mm
Pseudomonas aeruginosa	0 mm	0 mm	0 mm	0 mm	0 mm	8.9 mm
Bacillus spp	0 mm	0 mm	0 mm	0 mm	0 mm	5 mm
Dermatophilus congolensis	6 mm	6 mm	7 mm	7 mm	8 mm	9.5 mm
Aspergillus flavus	1 cm	1 cm	No Growth	0.5 cm	0.5 cm	No Growth

Dermatophilus congolensis is an isolate from cow. For Aspergillus flavus (an isolate from maize rot) the inhibition is given as growth of fungi in 7 days, where the control grew 10 cm.

Table 2.
Preliminary in vitro test showing average zone of inhibition of organic extracts of Plangiochila spp. on different microbes.

Figure 3.
Marchantia debilis (Liverwort).

Figure 4.
Plangiochila spp (Moss).

Figure 5.
Two samples of fresh Marchantia debilis residues after extraction with hexane and petroleum ether.

Figure 6.
Two samples of partially dried Plangiochila spp residues after extraction with hexane and petroleum ether.

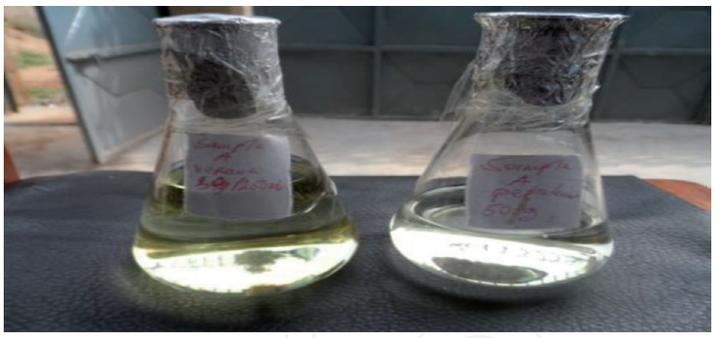

Figure 7.
Partially dried Marchantia debilis extract in hexane and in petroleum ether.

Figure 8.
Extract of Plangiochila spp in hexane and petroleum ether.

5.4 Ointment application and resultant outcome of application

An animal health officer applied the cream topically (by rubbing on affected parts of the animal, using hand gloves) once a day for 3 days in a week. Following this, a total drying off of the infected spot was noticed after 14 days. The dried, dead skin was then carefully peeled off.

6. The findings, discussion on the economic and environmental benefits of this study

The results show that extracts of *Marchantia debilis* and *Plangiochila* have antifungal activity against *Aspergillus flavus*, and antibacterial activities against *Pseudomonas* spp., *Bacillus spp*, and *Staphylococcus aureus* and *Dermatophilus congolensis* isolates (**Tables 1** and **2**). In **Figure 9**, a plate that demonstrates clear mycelia inhibition of *Aspergillus flavus* by extract of *Marchantia debilis* is shown. The product development focus has been on *Marchantia debilis* since Yongabi et al. [2] isolated a number of marchantins including a new marchantin Q from *Marchantia debilis* from Cameroon [2] (**Figures 10** and **11**).

Antimicrobial activity of liverworts is not new [1] but the testing of these liverworts and moss on isolates from plant pathogens in Africa is probably for the first time. The *Aspergillus flavus* isolate was provided for this study by a local laboratory in Cameroon. The effect of liverworts inhibiting the growth of *Dermatophilus congolensis* isolated from cattle is reported here for the first time. The synthetic agrochemicals used in daily farming in Africa are quite expensive and these synthetic products are normally out of reach of the rural farmer [5, 9, 12–14]. The result

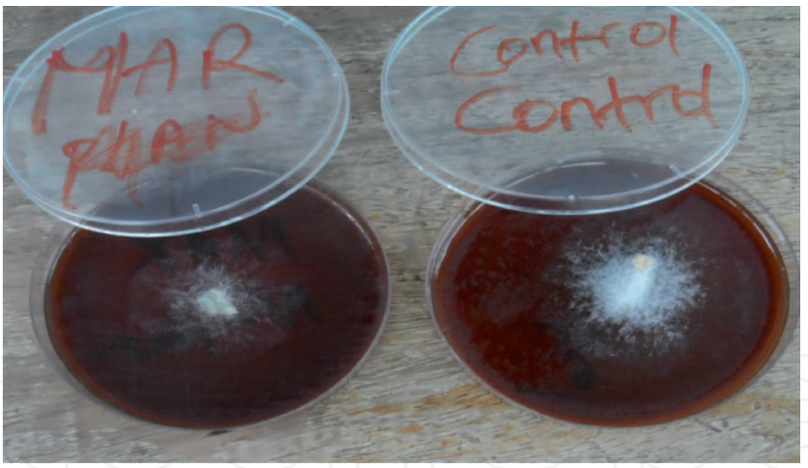

Figure 9.
Plate labeled marked (MAR) contains extract of Marchantia debilis Goebel with slow growth of Aspergillus flavus in 7 days as opposed to control plate (Marked: Control) with only n-hexane incorporated into the agar.

Figure 10.
A cured cow: management of Dermatophilosis in ruminants using Bryo-ointment.

Figure 11.
Maize drying sprayed with Bryo-extracts to inhibit moldy cotton wool Aspergillus flavus.

is that a lot of agricultural produce such as cattle and grains are lost to disease and the cost of these produce is prohibitive. More so because grains are not stored for a longer period to enable sales at off season [15–17].

The problem in cattle with Dermatophilosis is no different. In a lot of the developing countries, today, the problem of malnutrition is endemic and the related opportunistic infections lead to infectious disease, such as tuberculosis and malaria. Protein malnutrition in Africa is a serious problem, especially in rural Africa where approximately 70% of the population live [4, 7, 18, 19]. The chemical constituents of bryophytes are well studied [1, 2], but these rich chemical constituents have not yet been explored biotechnologically. Plants with bioactive ingredients abound in Africa [20], and bryophytes are even more abundant [3].

The production of plant-based products from the bryophytes in the treatment of Dermatophilosis shifts focus from the importation of orthodox drugs and conserves Africa's scarce foreign exchange reserve, and increases utilization of indigenous plant resources. Outside of the cheaper ointment product, a local industry for the production of this ointment is encouraged and the product would be available to a larger group of herders. This preliminary report details the first attempt. The multiplier effect is enormous; meat should be cheaper and malnutrition resulting from the lack of protein would drastically reduce, and possibly disappear. Moreover, the use of plant-based products could easily foreclose the emergent, resistant strains of *Dermatophilus congolensis* resulting from the frequent use and misuse of antibiotics.

Bryophytes are common and abundant, especially in the west and central African regions. The formulation process for the ointment is easy to follow and it is based on a technology that the rural populations could easily handle. The method of application is by glove-protected hands to animals, and the ointment is also effective against human skin infections [9, 14, 21, 22].

The ointment and bryosol (bryophyte solution suspended in glycerol) are observed to have astringent property when applied on sores. Thus, it not only heals but also smoothens the affected lesion to which it is applied. The oils from bryophytes when blended with *Vitellaria paradoxa* have improved cosmetic value and reduced treatment time, and this gives the formulated cream an added advantage. The Bryo-ointment is cheap and effective. For instance, 100 ml is sold at 1 US dollar, as against 5 US dollars for each of the antibiotics. Based on our findings, a container of 100 ml of the formulated cream can hardly be used completely in treating three animals once a day for 3 days a week, and for 2 weeks. However, this depends on the degree of infection.

7. Recommendations

The findings of the study suggest that one cannot begin addressing the problem of aflatoxin producing fungi on crops, grains contamination, and skin diseases in animals by simply relying on agrochemicals and introducing improved management practices. This requires a closer examination of the role of ecological technologies and approaches. Above all, studies on bryophytes are limited to taxonomy and molecular biological aspects with little effort toward actual biotransformation of bryophytes via appropriate biotechnology for direct applications in horticulture and animal husbandry.

From this study, it is therefore recommend that:

- A shift toward cost-effective technology will not take place unless a series of interventions via technology such as bryo biotechnology that can give necessary opportunities is provided to the farmers and other stakeholders.

- Bryophyte bioproducts offer an opportunity for sustainable animal husbandry and agriculture for Africans at potentially lower costs.

- Though farmers pay considerable attention to the selection of seed from their own produce, lack of awareness about identification of contamination in general prevents them from using aflatoxin-free seeds. Interventions such as treating grains with bryophyte-derived solution may ensure that farmers use grains free from contamination irrespective of the sources of supply.

Glossary

Dermatophilosis	bacterial skin disease of cows, sheep, goats, dogs and other animals, scab disease
Amblyomma variegatum	tick vector of Dermatophilosis (cutaneous streptothricosis)
Dermatophilus congolensis	the bacterial causative agent of Dermatophilosis
Kirchi	Hausa name for Dermatophilosis
Lamstreptocide	antibiotics used in treatment of Dermatophilosis
TLA	terramycin long-acting antibiotic used in the treatment of Dermatophilosis
Zoonotic	animal disease that could also be passed to infect man
Necrosis	the breaking down of cells/tissues resulting from an infection
Hausa	the language of Hausa people in Nigeria and Cameroon
Ecology	the study of the relations of living things to one another and to their surrounding

Author details

Kenneth Yongabi Anchang[1,2,3,4*] and Henrik Toft Simonsen[5]

1 Public Health Infectiology and Phytobiotechnology, Imo State University, Owerri, Nigeria

2 Ebonyi State University, Abakaliki, Nigeria

3 Phyto-biotechnology Research Foundation Clinic/Institute, Bamenda, Cameroon

4 Catholic University of Cameroon, Bamenda, Cameroon

5 DTU Bioengineering, Technical University of Denmark, Denmark

*Address all correspondence to: yongabika@yahoo.com

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