

POTENTIAL USE OF WILD *GANODERMA SP* BASED PETROLATUM PASTE AGAINST SOME MIXED DERMATOLOGICAL CONDITIONS IN GOATS IN MAIDUGURI, NIGERIA

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ABSTRACT

The effect of wild *Ganoderma sp* based petrolatum paste preparation against *Dermatophilosis* and other mixed fungal infecting organisms in goats was investigated. Twelve (12) adult goats, of different sexes and all with dermatological conditions were purchased from Maiduguri cattle market, and were then serially numbered 1-12 and divided into four (4) groups {A [nos.1-3], B [nos. 4-6], C [nos. 7-9] and group D [nos. 10-12]} of 3 goats/group. They were kept in separate pens and fed with mixtures of groundnut hay, wheat, corn and bean offal's, while clean drinking water was provided *ad libitum*. The goats were clinically evaluated and treated for other identified internal infections (Helminthes and Coccidiosis). Using standard methods, microbial analysis of scabs and skin scrapings from the lesion on the goat's skin

indicated the disease was mainly caused by *Dermatophilos congolensis* and in addition, other fungal infectious organisms such as *Rhizopus oryzae*, *Aspergillus niger* and *Penicilium sp* were all identified. Preparation of 10, 20 and 30 g of the powdered *Ganoderma sp* mixed in

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450 ml petroleum jelly was made, and it gave concentrations of 2.2 mg/ml, 4.4 mg/ml and 6.7 mg/ml respectively. These were used for topical application, in groups A, B, and C respectively, while goats in group D served as positive control and were treated with Charmil^R. The sick goats were treated by topical application of the wild *Ganoderma sp* based petrolatum paste for a period of two weeks. Results from this study showed that the petrolatum based wild *Ganoderma sp*. preparation treated the entire skin lesion in all the treated groups as against the Charmil^R treated group, and their skin integrity was restored to normal after 2 weeks of treatment compared to the Charmil^R treated group

KEYWORDS: Petroleum jelly, *Ganoderma sp*, Charmil^R, Mixed Dermatological disease, Goats.

INTRODUCTION

Wild *Ganoderma species* are medicinal mushroom found growing on logs of deciduous woods during the rainy season in tropical countries such as Nigeria. Many mushrooms have been shown to have antibacterial and mycotic effects against many pathogens of man and animals (Sonodiya *et al.*, 2009, Ge *et al.*, 2010, Ogbe *et al.*, 2011, Shamaki *et al.*, 2014a,b,c).

Dermatophilosis in animals is a global menace (Bida and Dennis, 1976). In Nigeria, dermatological diseases in ruminant animals are found in abundance especially during the rainy season, partly due to prevalence of predisposing vectors such as ticks (Shamaki *et al.*, 2007), The prevalence of predisposing factors, coupled with poor management practices, enable proliferation of skin diseases in ruminant animals, some of which are of zoonotic importance. These reduce the value of hides and skin in our leather industries, and generally, affect livestock production, with economic consequences.

Most of these vectors serve as predisposing factors to skin diseases such as *Dermatophilosis*, *Dermatophytosis*, *Scabies*, *streptothricosis* and *erysipelosis* that are caused by both fungal and bacterial organisms in ruminants, equidae and pigs (Radostits *et al.*, 1995).

Nigeria is currently ranked as the 187th out of 194th in terms of health in the world according to World health organization (2000). Most of human diseases do emanate from animal sources due partly to mishandling of diseases, poor management, negligence and carefree attitude. There are some of these diseases that are transmitted to man who work with or come in contact with these diseases (Lloyd and Sellers, 1976). Treatment of this disease include the

use of various antibiotics, antimycotics and other preparations from local farmers, while the use of insecticides and antiseptics is for prevention through tick control (Bida and Dennis, 1976). Treatments in most cases are prolonged and sometimes with little success, especially, when the infection is severe, however, spontaneous recoveries from mild to moderate infection have been reported (Bida and Dennis, 1976).

The uses of synthetic drugs require longer duration of administration, and can be associated with some unwanted side effects (Wagener *et al.*, 2012). Therefore, it is important to search for remedies to treat or manage these diseases using natural products, that can be accessible, affordable, effective and readily available anti-microbial agent, and which can easily be biotransformed and excreted from the body with minimum side effect. *Ganoderma sp.* is one of such mushroom (Yoon *et al.*, 1994, Obadai *et al.*, 2017)

Due to this drawbacks therefore, the search for remedies for these diseases in animals is therefore important, as this will reduce load on overstretched human hospitals, thus enabling Nigeria to achieve strategic goals and objectives of the national strategic health development plan of reducing diseases to its barest minimum, and to strengthen the slogan “one world, one health” by breaking the chain of zoonotic diseases transmission from animal to man (Chang and Wasser, 2012).

MATERIALS AND METHOD

Mushroom sample collection and preparation

Study area

The fruiting bodies of the wild *Ganoderma sp.* were collected in 2016 from Lafia, Nassarawa State, during the rainy season. It was dried under room temperature, grinded to coarse powder using Lister (China) grinding machine. This powder weighing 500g was carefully wrapped in a transparent polythene bag, inserted carefully in a plastic container, and transported to Maiduguri and kept at room temperature in the Pharmacology and Toxicology laboratory, Faculty of Veterinary Medicine, University of Maiduguri, until required for use.

Proximate Analysis of wild *Ganoderma* specie

Proximate chemical analysis of the powdered mushroom was conducted for dry matter, crude protein, crude fiber, fat (ether extract), ash, carbohydrates, and Nitrogen free extract (NFE) as described by American organization of Analytical Chemists (AOAC, 1990).

Phytochemical analysis of wild *Ganoderma* specie

Powdered *Ganoderma* sp. was measured (20 g) using Electronic weighing balance (METRA, China) and was extracted using distilled water, the extract was used to qualitatively analyze for phytochemicals such as soluble starch, alkaloids, tannins, flavonoids, carbohydrates, ketoses, cardiac glycoside, anthraquinone and combined anthraquinone, terpenoids and cardenolide, as described by Trease and Evans (2002).

Preparation of the wild *Ganoderma* specie based petrolatum paste

The grounded sample of Wild *Ganoderma* sp. was collected from the preservation container unto a clean paper using a clean spatula. Measurements of 10g, 20 g and 30 g were made using electronic compact scale (LabTech, China), and each of the measured weight of the powder was soaked in 10 ml of distilled water contained in a clean 250 ml beaker and allowed to stand for 24 hours. The content of the beaker was covered with a foil paper to avoid contamination. The whole contents of each beaker was mixed using a clean spatula, in 450 ml Vaseline jelly (blue seal, Unilever, South Africa, this gave concentrations of 2.2 mg/ml, 4.4 mg/ml and 6.7 mg/ml respectively. The Vaseline served as our petrolatum and the mixture was allowed to stand for another 24 hours before use.

Animals for the study

A total of twelve (12) mature Caprine (females) were purchased from the Maiduguri cattle market in Borno state. All the animals purchased were identified with a particular skin lesion whose cause was not identified. These animals were transported to Sahel goat research pen (Large Animal Clinic) in the Department of Veterinary Medicine, Faculty of Veterinary Medicine University of Maiduguri. The animals were fed with beans and wheat offal, groundnut hay and clean water was provided *ad libitum*. The floor of the pen was cleaned daily, feeding trough, and the drinkers were daily cleaned and fresh feed and water provided for the period of the research (12 weeks).

Microbial sample collection

Using a pair of sterile scalpel blade, a pair of disposable hand gloves, Petri dish and bijour bottles, samples from animals identified with skin diseased was collected. The Petri dish was placed just beneath the area of the identified scabs and crust lesions, the scabs or crust were gently removed unto the Petri dish, and this was transferred into a clean universal bottle. These samples were taken to the Medicine Research Laboratory, in the Department of

Veterinary Medicine, Faculty of Veterinary Medicine, University of Maiduguri for identification.

Isolation of the *Dermatophilos* organisms

Isolation of the *Dermatophilos* organisms was based on the principles of chemotactic release of zoospores described by Roberts (1963).

Principles: The collected scab samples were soaked in 10 ml of distilled water contained in a universal bottle. The universal bottle and its contents were placed in an anaerobic jar in which a candle light was placed, to produce carbon dioxide. It was incubated for 18 hours. Afterwards, 0.2 ml of each of the inoculums pipetted into a trypton broth, and was put in an anaerobic jar, incubated at 37°C for 24 hrs. The inoculum was observed for growth and sub-cultured in 9% bovine blood agar and these were also incubated at 37°C for 24 hrs. After incubation, plates were removed and examined for bacterial growth, the colonial appearance of the growth were also observed and those colonies giving the appearance of *Dermatophilos* strain found in all the samples collected from the Caprine, were further identified.

RESULTS

The microorganisms identified include *Dermatophilos*, *Rhyzopus oryzae*, *Aspergillus niger* and *Penicilium sp.* indicating mixed skin infection.

Extraction of the measured powder yielded 4.15g, and the pH was 4.8. Proximate analysis of the *Ganoderma* showed that it contains various amounts of organic compounds, dry matter 89.1 %, moisture contents 10.9 %, crude protein 6.3, ether extract/fats 1.0 %, crude fiber 29.0 %, ash 7.0 %, carbohydrates 46.8%, (Table 1).

The results of qualitative phytochemical analysis showed that the mushroom contain terpenoids, flavonoids, cardiac glycosides, tannin, cardenolide and carbohydrates. While soluble starch, alkaloids, ketoses and anthraquinone were absent (Table 2).

Table 1: Proximate analysis of wild *Ganoderma sp.* Powder.

S/No.	Dry matter (%)	Moisture contents (%)	Crude protein (%)	Ether extract or Fat (%)	Crude fiber (%)	Ash (%)	Carbohydrates (%)	pH	Yield (g)
1.	89.1	10.9	6.3	1.0	29.0	7.0	46.8	4.8	4.15

Table 2: Qualitative phytochemical analysis of wild *Ganoderma sp* extract.

S/No.	Phytochemical test	Results
1.	Soluble starch	-
2.	Test for alkaloids	
	Dragendorff's	-
	Meyers reagent	-
3.	Test tannins	
	Ferric chloride	+
	Lead acetate	-
4.	Test for flavonoids	
	Shinoda's test	-
	Ferric chloride	+
	Lead acetate	-
	Sodium hydroxide	-
5.	Test for carbohydrates	
	Molisch's test	+
	Barfoed's test (monosaccharide)	-
	Fehling's test	+
	Combine reducing sugar	+
6.	Test for ketoses	-
7.	Test for cardiac glycoside	
	Salkowski's test	+
	Lieberman	-
8.	Test for anthraquinone	
	Combined anthraquinone	-
9.	Test for terpenoids	+

Key: + = present, - = absent



Plate 1: A sick goat showing facial and earlobe skin infection.



Plate 2: Goats completely treated with the wild *Ganoderma sp.* based Petrolatum Paste at 6.7 mg/ml after 7 days of treatment.



Plate 3: Goats treated with 4.4 mg/ml after 7 days of daily topical application of the *Ganoderma sp.* based petrolatum paste.

DISCUSSION

Antimicrobial resistance threatens the effective prevention of an ever increasing range of infection caused by bacteria, viruses, parasites and fungi (WHO, 2017). This resistance mechanism that globally spreads limits our ability to treat common infectious diseases. This therefore, results to prolonged illnesses, disability and death in some cases (WHO, 2017), consequently losses to farmers. Although, not listed among antibiotic resistant “priority pathogen,” *Dermatophilosis*, cause by *Dermatophilos congolensis* is a bacterial pathogen that show resistance to antibiotic therapy (Scanlan *et al.*, 1984). This may be due to a combination of factors that may include poor nutrition, environmental conditions and the prevalence of ticks (Shamaki, *et al.*, 2007). The disease pathogen rarely penetrates the beyond the skin (Oduye, 1987, Radostits *et al.*, 1994), thus, making parenteral administration of antibiotic less effective due partly to poor blood supply to the skin, enzymatic deactivation by β -lactamase, in some cases, or its decrease accumulation at the target sites (Aminov and Mekie, 2007, Li and Nikaido, 2009). Contrary to reports by Hart and Tyszkiewicz (1968) that *Dermatophilosis* has no cure and have shown resistance to topical antibiotic and antifungal agents, using *pe wild Ganoderma sp* based petrolatum paste preparations and its topical application resulted in complete cure within seven days of application, this phenomenon was observed at all the prepared concentrations (2.2. 4.4 and 6.7 mg/kg). This curative ability can be attributable to presence of Pharmacologically active phytochemical constituent such as tannins, flavonoids, carbohydrates and terpenoids, found in this study, and were all reported to posses antibacterial activities (Shamaki, *et al.*, 2012, Santos *et al.*, 2014), However, worthy of mention here, is the absence of alkaloid which were reported in this mushroom by Shamaki *et al.*, (2012), this can probably be explained due to differences in analytical method used. Since variants of phytochemical constituents were equally observed by Obadai *et al.*, (2017). The presence of these pharmacologically active substances may provide an alternative to produce an antibiotic that may fight microorganisms that are resistant to antibiotics (Santos *et al.*, 2014, Xie *et al.*, 2014), this also corroborates findings of Rabi and Bushayee (2009) and Zhu *et al.*, (2015) who reported that terpenoids have useful effects in prevention and treatment of several diseases, While Wasser, (2002) and Lull *et al* (2005) reported anti-inflammatory and immune-modulatory properties of a metabolite isolated from *Ganoderma lucidum*.

The complete cure (100 %) of *Dermatophilosis* in goats also agrees with earlier findings of Ogbe *et al* (2011), who reported treatment of unspecified skin ailment, contaminated with

Staphylococcus aureus in an animal, with 97 % success rate using aqueous solution of *Ganoderma lucidum*, however, this preparation may be short acting compared to petrolatum preparation of the *Ganoderma* based products, that may have a longer lasting effect, thus allowing slow penetration of the skin with little requirements for blood supply.

Contrary to reports of appearance of skin lesions (*Dermatophilosis*) first in the muzzle and lips, before spreading, possibly by biting flies, to other parts of the body in goats, as against that of sheep (Radostits *et al.*, 1994), in this study, the lesions were first seen on the dorsum of the goats before spreading to other parts of the body, and not by biting flies, but probably by developing mycelia whose growth is facilitated by weakened skin tissues.

In addition to bacterial organism found in this study, other microbes such as *Rhizopus oryzae*, *Aspergillus niger* and *penicilium sp.* all fungal species, were also identified. This explains the multiple microbial implication in *Dermatophilosis* infection, and hence the reported resistance to treatment with antibiotic eg. Penicillin G, that only has anti bacterial activity against Gram positive bacteria, and information for combination of both antimicrobial and antifungal regimen in the treatment of *Dermatophilosis*. The resistance may be due to presence of *Aspergillus* and *Penicilium* species that are reported to have antifungal resistance (Srinivasan *et al.*, 2014). A combination therapy against *Dermatophilosis* may reduce multidrug resistant fungi (Costa *et al.*, 2014). However, in this study, the wild *Ganoderma sp.* based preparation has tremendous effects on all the identified microbe, including the resistant ones, and this agrees with findings of Sivaprakasam *et al* (2011) who reported that both aqueous and methanol preparation of *Ganoderma lucidum* at dose of 50-200 mg, has both antibacterial and antifungal activities respectively. The antifungal activity has been attributed to a protein- ganodermin, isolated from *Ganoderma lucidum* (Hexiang and Ng, 2005). This acidic protein may also enhance absorption of the extract into skin by softening skin tissues. Antifungal activities of extracts from *Ganoderma lucidum* has also been reported against *Candida albicans* (Aarati *et al.*, 2010, Alencar and Clemente, 2013), and also against plant pathogenic fungi (Shahid *et al.*, 2016), while Pushpa *et al* (2013) reported antimicrobial activity of *Ganoderma applanatum* to be more effective among selected *Ganoderma* species.

It is generally believed that medicinal mushrooms especially, higher basidiomycete, *Ganoderma sp.* inclusive, has high valuable biological resource of high exploitation

potentials (Gargano *et al.*, 2017), and that antibiotic treatment duration should be based on the infection and other health problems (NPS 2015, CDC, 2016).

In conclusion, wild *Ganoderma* sp. based petrolatum preparation applied topically, cured skin infection caused by a combination of *Dermatophilos congolensis* and other fungal organisms, thus contradicting earlier reports that it has no cure, the record treatment time (7 days) is highly reduced as against the usual prolonged treatment period, and also contradict the belief by some veterinarians that the disease is a product of malnutrition.

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