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Acaricidal Efficacy of *Cassia sieberiana* (*In-vitro* and *In-vivo*) against *Sarcoptes scabiei var cuniculli* on Experimentally Infested Rabbits (*Oryctolagus cuniculus*) in Maiduguri, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Cassia saberriana contains varying degree of glycoside, saponins, tannin, alkaloid and terpene as these were bases for selection in the study based on their current and past use in traditional medicine. Local and traditional knowledge has been the starting point for many successful drug development projects in the past and such approach is generally based on a detailed observation of how local populations uses the plants. Acaricidal Efficacy of *Cassia sieberiana* (*In-vitro* and *In-vivo*) against *Sarcoptes scabiei var cuniculli* on Experimentally Infested Rabbits (*Oryctolagus cuniculus*) in Maiduguri, Nigeria. Was evaluated using Eighteen (18) Rabbits both male (n=9) and female (n=9) at post-weaned ages (8-10 weeks), New Zealand white breed were used for the study. Table 1. Shows out of the 15 rabbits, 1 rabbit (6.7%) did not show any signs of infestation at all. Among the rabbits showing infestation,13.3% showed slight degree of infestation, moderate infestation was observed in 13.3% of rabbits. However heavy infestation was observed by high levels of pruritus, scaling and alopecia were observed in 66.7% of the rabbits (Table 1). All animals in the negative control (n=3) did not showed any level of infestation during the study period. only erythematous rash was observed on the infected area, no significant change in the behavior of the infected rabbits was observed. At day 14, the skin at the infected area becomes more erythematous and some raised papules were visible even with naked eye. Change in behavior of the infected rabbits was observed, they became restless and scratched the infected area. At day 21 microscopic examination of the skin scraping showed few adults mites and large number of larvae and nymphs. At day 28. In-vitro assay showed that at 12.5mg/ml 24hours post exposure the extract is capable of killing 2(20%), 28hours 7(70%) while 72hours 8(80%) but other concentrations have not shown effectiveness on mites even beyond 72hours. Moreover, the LC99 which is 12.5mg/ml when used In-vivo on experimentally infested rabbits did not show effectiveness even beyond 35days of treatment. Further studies on Methanol extract and other solvents and parts of the plant such as the leaves, flowers and the pods should be tried to demonstrate the effectiveness of the plants medicinal used or in other organisms such as gastrointestinal helminths.

Keywords: Experimental infestation; Sarcoptes scabiei var cunicull; acaricidal efficacy; rabbit; In-vitro; In-vivo.

1. INTRODUCTION

Rabbit has emerged as a key livestock that is increasingly being raised by farmers in many parts of our country and the globe at large; this is because of its small space utilization and use of fiber and domestic remnants from leafy vegetables [1-4]. However disease and inadequate technical knowledge amongst animal health providers on those diseases are the major challenge facing the sustainability of rabbit farming [5].

Sarcoptes scabiei commonly known as itch mite is an ectoparasite that burrows into the skin and causes a disease commonly known as mange in animals and scabies in humans. Such animals affected include wild and domesticated ruminants, dogs, cats, apes, as well as laboratory animals including rabbits among others [6].

The genus *Sarcoptes* is a part of larger family of mite collectively known as scab mites comprising of one specie *Sarcoptes scabiei* with further identification by variety of names indicating the host specie, e.g *S. scabiei var hominius* in humans and *S. scabiei var cuniculii* in rabbits. The complete stages of life cycle are found on the host, where the female oviposit into the tunnel made in the *stratum corneum* of the skin causing intense itchy skin rashes, hypersensitivity and inflammation [7]. The life cycle is 21 days but can be delayed during extreme weather and infestation is by contact [8,9] Hofing *et al*., 1994. Sarcoptic mange in rabbit has been described as an uncommon disease [10] however it has been reported from different countries [11]. Wild animals have been

reported to transmit sarcoptic mange in different animals including rabbits experimentally as well as naturally [12-14].

Due to resistance developed by many organisms against synthetic acaricide, its toxicity and environmental contamination [15], there is need for exploration of plant extract with medicinal properties as alternate in treatment. More plant has been shown to possess acaricidal properties against ectopararites;(Crude *Aloe vera* gel, [16] Methanolic extract of *Vitex negundo* dried stem and leaves, [17] *Cassia sieberiana*, [18] and Crude watery extract of *Onobrychis Ptolemaica,* Shahatha et al., [19] thus a trial of *Cassia sieberiana* using a dusting technique of powdered extract will be evaluated on mite.

2. MATERIALS AND METHODS

2.1 Study Area

The present study was conducted in Maiduguri Metropolis, formerly Yerwa was founded in 1902, the capital city of Borno state, Nigeria. It is the largest Metropolis in the northeastern region of Nigeria (Waziri, 2009). The study area lies within the semi-arid (Sahel savannah) zone of the north-eastern part of Nigeria, Maiduguri is located on latitude 11°48'N and 11052' N and longitude 13°02' E and 13912' E, at about 350m (1161 ft) above sea level with an ambient temperature range of 32°C to 45°C. The relative humidity is generally low throughout the state, ranging from as low as 13% in the driest months of February and March to the highest values of 70% to 80% in the rainy months of July and August, the rainy season averagely lasts for less than eighty days in the extreme north, the mean annual rainfall is about 600mm or less than 500mm in the extreme north around Chad republic(http://www.unimaid.edu.ng/about_Maid.aspx). The state shares borders with Cameroon to the east, Chad republic to the north-east and Niger republic to the north, (Abdulrahman et al.,2012)

2.2 Source of Experimental Rabbits

Eighteen (n=18) Rabbits both male and female at post-weaned ages (8-10 weeks), New Zealand white breed were used for the study, the animals were purchased from a commercial rabbit breeder in Maiduguri and on their arrival placed in a clean and well ventilated cages at large animal clinic Faculty of Veterinary Medicine, University of Maiduguri. They were routinely screened for ecto, endo and haemo-parasites using standard methods [8,15]. They were suitably housed in cages provided with sawdust as bedding, feeding with variety of vegetable and commercial grower feed, and water were provided *ad libitum*. They were allowed to acclimatize to laboratory conditions for two weeks prior to commencement of the experiment. Ethical approval was sought and approved by the ethical regulatory committee of the Faculty of Veterinary Medicine, University of Maiduguri before commencement of the experiment. At the end of the study period all infected rabbits will be immediately treated for sarcoptic mange.

2.3 Collection of Mites for the Study

Sarcoptes scabiei var cuniculi were collected using scab scraping from naturally infested rabbits in Maiduguri, Nigeria. The scabs containing mites were placed in a Petri dish and incubated at 35°C for 48 hours at Veterinary Parasitology and Entomology Research Laboratory. Under a stereomicroscope the motile larvae are distinguished with having six legs while the nymphs and adult have eight legs according to Soulsby [8] were all used for the experiments.

2.4 Collection and Identification of *Cassia sieberiana*

The plant *Cassia sieberiana* stem from mature plant were collected within University of Maiduguri campus, and identified based on its botanical features as described by Hassan *et al,* (2007) and further authenticated by a Botanist at the Department of Biological Sciences, Faculty of Science, University of Maiduguri and a voucher specimen number LCMC 228 was deposited in the herbarium.

2.5 Preparation of *Cassia sieberiana* Stem Extracts

Freshly harvested stem bark of the plant were cut into smaller pieces to enhance drying under shade at room temperature (±27°C). Dried plant was pulverized with pestle and mortar to obtained a fine powder as described by Tiwari et al., (2011), thus 100g of powdered plant material was extracted in 1000mls of distilled water to obtain the aqueous extract separately at 60°C for 8 hours in a Soxhiet extractor as described by Tiwari et al. (2011). The extract was concentrated on an aluminum tray using hot air

oven (40-50°C) to remove the solvent, leaving behind the dried extract which was weighed and stored at room temperature of 27°C in sealed plastic containers until required for use, as described by Biu et al. [18].

2.6 Phytochemical Screening of Aqueous Extract of *Cassia sieberiana*

Small portion of the extract was tested for the presence of secondary metabolites such as simple sugar, carbohydrates, soluble starch, tannins, phlebotannins, cardiac gylcosides, terpenoids, saponins, flavonoids and alkaloids using the methods described by Brain and Turner [20-23].

2.7 Chemical Acaricide

Permethrin is a synthetic pyrethroid used in treatment of lice infestation and scabies in humans and livestock. It is a yellow to light orange-brown, low melting solid or viscous liquid. Elimite^{R} was purchased which is a brand name of permethrin cream available in 50mg/ig for topical used marketed by Prestium Pharmacy Inc. indicated for treatment of infestation with *Sarcoptes scabiei*, and has a wider pharmacodynamics against Lice, ticks, fleas, mites and other arthropods. And the extract powders were also converted in to paste/gram according to their concentrations respectively for uniformity.

2.8 Acaricidal Bioassays

2.8.1 *In-vitro* acaricidal evaluation

The bioassay response of the extract acaricidal study was based on that of Khater *et al.,* 2007 and Politi *et al*., 2012. A dose dependent response was conducted using graded doses of the extract being tested, as described by Singh *et al*., 2014. Control group A: (normal control) was not treated but merely maintained at the same condition as the treated groups. Control group B: (positive control) was treated with 0.05% permethrin for 5 minutes, while treatment groups (C-I) were immersed in 10 mls of each extract concentration (2.5mg/ml, 5mg/ml, 7.5mg/ml,10mg/ml, 12.5mg/ml, 15mg/ml, and 17.5rng/ml) in a Petri dish followed by a gentle agitation for 5 minutes, before drying on a tissue paper. Each group was then transferred into a petri dish padded with Whatman's No.1 filter paper and incubated in a desiccator maintained at room temperature (27-28°C) and 85-90% RH (12/12h photoperiod) for 48hours. The effect of each extract concentration leading to mortality was monitored and death was confirmed by observing loss of motility and reflex after exposing to light for 48hours, dark discoloration of the cuticle and absence of movement (Politi et al.,2012; Singh et al., [24]. Numbers of dead and live mites were counted, post-exposure time was also considered and these were used to calculate the adult mortality rate (AMR) as corrected by Abbots formula and recommended by food and agriculture organization of United Nations (FAO 2004).

2.8.2 The *In-vivo* acaricidal evaluation

Experimental Rabbits with an average weight of 0.8kg were classified into six equal groups of three (3) each randomly, the first group 'A' were non-infected untreated (normal control), group 'B' were designed as infected untreated (negative control group), group 'C' were infected and treated with orthodox acaricide (positive control), group 'D' were uninfected and treated with orthodox acaricide, group 'E' uninfected and treated with Cassia extract while group 'F' were infected and treated with Cassia extract (Extract test control) using concentration that shows lethal concentration (LC99) value 24 hours' post-treatment in-vitro, according to Haussain (2002). The infestations were carried out on the dorsal back surface of the neck about 4x2cm after scratching the fur and until appearance of hyperemia. Mite suspensions were applied using an applicator, after induction of infection the rabbits were caged separately and monitored. The pathological changes in the infected area were observed and recorded on days 7, 14, 21, 28 and 35. Confirmation of infestation by collecting deep scrapings to re-harvest mites and their developmental stages, and the index scoring value used to describe lesions by Jensen et al., 2002 were used.

2.9 Data Analysis

All data obtained were subjected to accurate statistical package, Probit analysis were conducted on mortality data in bioassay tests, while One- way analysis of variance (ANOVA) with Duncan's multiple range test were carried out on biological data.

3. RESULTS

Varying degree of infestation was observed during the study period, the criteria for grading the intensity of infestation is presented in

Table 1. Out of the 15 rabbits, 1 rabbit (6.7%) did not show any signs of infestation at all. Among the rabbits showing infestation,13.3% showed slight degree of infestation, moderate infestation was observed in 13.3% of rabbits. However heavy infestation as observed by high levels of pruritus, scaling and alopecia was observed in 66.7% of the rabbits (Table 1). All animals in the negative control (n=3) did not showed any level of infestation during the study period.

At day 7, only erythematous rash was observed on the infected area, no significant change in the behavior of the infected rabbits was observed. At day 14, the skin at the infected area becomes more erythematous and some raised papules were visible even with naked eye. Change in behavior of the infected rabbits was observed, they became restless and scratched the infected area. At day 21, (Table 1) also shows infected area appears moist with serous exudates due to rapture of vesicles and papules from constant scratching, infected rabbits become more restless due to severe pruritus and show less interest in feeding. Microscopic examination of the skin scraping showed few adults mites and large number of larvae and nymphs. At day 28, the yellowish scab appeared on the infected area and in some rabbits with severe pruritus, lesion becomes hemorrhagic and signs of secondary infection with pyoderma were observed. Animals are more restless with skin scrapings showing large number of adult mites. At day 35, persistent excoriation and resultant sero-hemorrhagic exudation making the skin of the affected area thick and crusty. At this stage, histopathological changes were seen in the skin of the infected area, necrosis and degeneration of the epidermis was evident.

Table 1. Outcome of induction of infection

Days	Group A (n=3)	Group B (n=3)	Group C (n=3)	Group D (n=3)	Group E (n=3)	Group F (n=3)
7	N	-	-	-	-	-
14	N	+	++	++	++	+
21	N	+++	++	+++	++	+++
28	N	+++	+++	+++	++	++
35	N	++	+++	+++	+++	++

N = Normal skin and wool
+++ = heavy infestation
++ = moderate infestation
+ = slight infestation

Table 2. Phytochemical components of *Cassia sieberiana* Stem back aqueous extract

Components	Tests	Scoring
Alkaloids	Dragendorff's	++
Terpenes	Liebermann-Buchard	++
Saponins	Frothing	+++
Tannins	Ferric chloride	+
Glycosides	Salkowski's	++
Anthraquinones	Borntrager's	+
Combined anthraquinones	Sulphuric and Borntrager's	-
Flavonoids	Pew's	+++
Reducing sugar	Fehling's	++
Ketones	Standard	-
Pentoses	Standard	+
Monosaccharides	Barfoed's	-
General Carbohydrates	Molisch's	++

Keys:
- = Not detected
+ = Low concentration
++ = Moderate
+++ = High

Table 3. *In-vitro* mortality percentage of *S. scabiei* treated with *Cassia sabierriana*

Concentrations	Post treatment(hours)								
	24			48			72		
	D	L	M%	D	L	M%	D	L	M%
-ve control	0	10	0	0	10	0	3	7	30
+control	10	0	100	10	0	100	10	0	100
2.5mg	0	10	0	3	7	30	4	6	40
5.0mg	0	10	0	3	7	30	5	5	50
7.5mg	0	10	0	2	8	20	4	6	40
10.0mg	0	10	0	2	8	20	4	6	40
12.5mg	2	8	20	7	3	70	8	2	80
15.0mg	0	10	0	2	8	20	3	7	30
17.5mg	0	10	0	2	8	20	3	7	30

Keys
D = Dead
L = Live
M% = % of Mortality

Table 4. Index scoring of *S. scabiei var cuniculli* infested rabbits with extract LC_{99}

	Days post treatment				
	7	14	21	28	35
-Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
+Control	1.65±0.02	1.65±0.03	1.44±0.05	1.24±0.07	1.09±0.07
LC99	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Mean ± S.D

4. DISCUSSION AND CONCLUSION

Discussion; Almost all the mammalian host are known to be parasitized by itch mite *Sarcoptes scabiei*, they include a wide range of domestic mammals; pets, livestock and dairy animals, wild animals and humans [25,3] (Daszak *et al*.,2000).

In the current study, scabies was successfully transferred to laboratory rabbits.66.7% of the rabbits showed heavy infestation, 13.3% showed moderate infestation and 13.3% also showed slight infestation while 6.7% did not showed any degree of infestation. It was also observed in the current study that scabies mite is site specific, because they were introduced on the body of rabbits behind the neck back and could only produce disease at the extremities, this finding is in accordance with Licois et al., [26] whom reported occurrence of lesions at the extremities and hinders feed consumption in laboratory animals. Also Mendlowitz et al., [27] reported that lesions when present cause alopecia and dermatitis on the face, nose, lips, feet and external genitalia appear as light brown, thick crusty with foul smelling exudates that are erythematous and may lead to secondary bacterial infections.

Phytochemical screening indicates varying degree of organic compounds which accounts for the medicinal usage of the plant *Cassia saberriana*, [28,29] Ojo et al., 2006 and Buratai et al., 2011). These were the choice of selecting the plant *Cassia saberriana* for the study, coupled with their current and past use in traditional medicine. Local and traditional knowledge has been the starting point for many successful drug development projects in the past and such approach is generally based on a detailed observation of how local populations uses the plants. In this study, *In-vitro* assay showed that at 12.5mg/ml 24hours post exposure the extract is capable of killing 2(20%), 28hours 7(70%) while 72hours 8(80%) but other concentrations have not shown effectiveness on mites even beyond 72hours. Moreover, the LC_{99} which is 12.5mg/ml when used *In-vivo* on experimentally infested rabbits did not show effectiveness even beyond 35days of treatment. This may be due to varying concentration of the components as indicated in Table 2 or the solvent (aqueous) used in extraction, which does not agree with the findings of Biu et al., [18] that reported the effectiveness of *Cassia sieberiana* on *Hyalomma* ticks and Shahatha et al., [19] reported that Crude watery extract of *Onobrychis ptolemaica* at 10 mg/ml showed complete termination of scabies mite in sheep, whereas lemon oil at 50 and 100% have shown effectiveness to kill 99% of mites after (1) one

hour exposure and Methanolic extract of *Vitex negundo* dried stem and leaves *in vitro* showed 90% acaricidal activity, while *in vivo* showed 69, 73, 75, 77 and 78% acaricidal activity respectively.

In conclusion, Sarcoptic mites can be experimentally transmitted between different livestock host irrespective of the animal species [30,31]. However, this work shows that the organisms are site specific thus can only produce scab on the extremities of the ear, mouth, nose and paws. Also the plant *Cassia saberriana* despite the chemical component present and capable of killing *Hyalomma* ticks as reported by Biu et al., [18] cannot control scabies mite at varying concentration, further studies on Methanol extract and other solvents and parts of the plant such as the leaves, flowers and the pods should be tried to demonstrate the effectiveness of the plants medicinal used or in other organisms such as gastrointestinal helminths.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declared that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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