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Evaluation of Acaricidal Effect of Ethnoveteinary Medicinal Plant by *in vivo* and *in vitro* against *Sarcoptes scabiei* var. *caprae* of Infected Goats in North Shoa, Oromia Regional State, Ethiopia

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Abstract

This study was conducted to determine parasiticidal efficacy of seven ethnomedicinal plants against *Sarcoptes scabiei* var. *caprae* of goats using *in vivo* and *in vitro* techniques. *In vitro* techniques for evaluation of efficacy of medicinal plants essential oils and fixed oils of seven medicinal plant extracts diluted at different concentrations (essential oils from 2.5% to 0.15625% and fixed oils from 160 mg/ml to 5 mg/ml) were added to petridishes containing adult stage of *Sarcoptes scabiei* var. *caprae*. After 3 h of contact, all concentrations of essential oil of *Eucalyptus globulus* and *Cymbopogon citractus* showed a good *in vitro* acaricidal efficacy as compared with the non-treated controls ($p<0.05$). However, *Nicotiana tobacum* fixed oil had significant ($P<0.05$) effect at a concentration of 160 mg/ml and 80 mg/ml. *Pyrethrum cineraria folium* fixed oil showed lower acaricidal efficacy ($P<0.05$) in all the concentrations of the extract as compared to the reference drugs. *In vivo* techniques undertaken for evaluation of efficacy essential oil of *Eucalyptus globulus* and *Cymbopogon citractus* at a concentration of 0.625% in 2% Tween 80 on two groups of (six animals each) *Sarcoptes scabiei* var. *caprae* infested goats were topically treated two times at 14 days interval and its compared with non-treated and treated (diazinone and ivermectin) controls of six goats in each group. The infected goats treated with the essential oils were cured completely. Statistically insignificant ($p>0.05$) difference was never observed in mite count, Mean Recovery Response (MRR) and degree of Skin Lesion Quality (SLQ) between goats treated with plant extracts and those treated with diazinone and ivermectin. Therefore, *Eucalyptus globulus* and *Cymbopogon citractus* extracts should be licensed for the treatment of *Sarcoptes scabiei* var. *caprae*.

Keywords: *In vitro* test; *In vivo* test; Mange mites; Medicinal plants; Goat; *Sarcoptes scabiei* var. *caprae*

Introduction

Ethiopia's economy is based on agriculture that account for 85% of the total employment and 75% of exports [1]. Livestock is the second major source of foreign currency through export of live animals, skin and hides [2]. The leather industry is one of the fast growing economic sectors in the country. However, this sector of trade and the country as whole, lost revenue due to a decline in quality and fall in export prices [3]. The current utilization of hides and skins is estimated to be 77.3% for cattle hide, 58.4% for goats skin and 29.7% for sheep skin with expected off take rate of 33%, 35% and 7% for sheep, goats and cattle respectively [4]. Even though, small ruminants are important components of the farming system in Ethiopia, their contributions are far below the expected potential. This is because small ruminant production in Ethiopia is confronted by several factors like diseases, poor feeding and poor managements [5,6].

Ectoparasitic skin diseases of small ruminants caused by mange mites, lice, fleas, keds, ticks and fleas are among the major diseases causing enormous economic losses to smallholder farmers, the tanning industry and the country as a whole. Infestation with ectoparasites is responsible for blood loss, irritation which results in downgrading and rejection of skins, poor growth, decreased production and reproduction performances and mortality [5]. The major observed economic losses due to mites, lice and keds is associated with skin damage. In 1996/97 six tanneries in and around Addis Ababa have rejected 2,037,745 pieces of skins which caused loss of USD 6.3 million [7] and in 1998/99 three tanneries that are found in Amhara Regional State have reported 443,602 pieces of skin rejection per annum which worth USD 1.4 million loss. According to Kassa ectoparasitic skin diseases due to ticks, lice, sheep keds and mange mites cause 35% of sheep skin and 56% of goat skin rejections. The ectoparasitic mites of mammals and birds inhabit the skin, where they feed on blood lymph, skin debris or sebaceous secretions, which they ingest by puncturing the skin, scavenge from the skin surface or imbibe from epidermal lesions. Most ectoparasitic mites spend their entire lives in intimate contact with their host, so that transmission from host to host is primarily by physical contact. The generalized veterinary term for an infestation by mites in an animal is called acariasis and can result in severe dermatitis, known as mange or scabies, which may cause significant welfare problems, economic losses and outright deaths [8,9].

Mange is a widespread and most important ectoparasitic disease of animals. Mange infestation is spread mainly by direct contact between hosts and all the three stages: the larvae, the nymph and the adults are capable of migrating and inert materials such as bedding and grooming tools can act as a carrier. Adult mites do not usually survive more than two weeks away from the host, but in optimum conditions they may remain to three weeks [10,11]. Female mites produce relatively large eggs, from which a small, six-legged larva hatches. A few species are ovoviviparous, producing live offspring's. The larva moults to become an eight legged nymph. There may be between one and three nymphal stages, known respectively as the protonymph, deutonymph and tritonymph. At least one of these nymphal stages is usually inactive

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and development proceeds without feeding. The nymph then moults to become adult. The number of eggs produced per female is highly variable but lifetime reproductive outputs may be as low as 16 eggs per female. Nevertheless, the life cycle of many parasitic species may be completed in less than 4 weeks and in some species may be, as short as 8 days. Hence, the mites have the potential for explosive increases in their population size. High temperature, humidity and sunlight favor mange mite infestations [12]. The disease affects all age groups and runs a more chronic course in adults than younger animals. Animals in poor condition are most susceptible to manage [13]. Mange cases due to *Sarcoptes* and *Psoroptes* are often fatal. The mortality rate is higher in younger and poor condition animals [14]. Death may be due to dehydration, a direct result of the feeding of huge number of mites, inability to move and feed due to severe lesions on the face, muzzle and on the joints or to secondary causes such as pneumonia or bacterial septicemia introduced through self-inflicted bite and scratch wounds [13,15]. In infestations, which do not end fatally, a marked regression of lesions, with healing of the skin and re-growth of the wool or hair occurs during dry season. Exposure of lesion and mite to direct sunlight and desiccation may reduce the survival potential of mite populations.

The clinical signs of erythema, pruritus and scale or crust formation are due to the inflammatory response of the skin and resulting excoriation. This response is stimulated by feeding, burrowing or the production of antigenic material by the mite. Some observers suggest that infra-orbital, inguinal pouches, scrotum, under tail, ears, inter digital pouches, perineum, and skin folds are foci for mites and serve as potential dry season hiding places where the mites tend to migrate to the general body surface with the onset of cold season [16]. According to the study reports, mange was noticed throughout the year but the incidence was higher during the wet cold months where the moistness and temperature is optimum condition for mite development [17]. Mange in sheep and goats is caused by four genera of mites, namely Sarcoptes, Psoroptes, Chorioptes and Demodex [18].

Sarcoptic mange is burrowing mites. It occurs in all species of animals and is caused by mite *Sarcoptes scabiei* that has a number of subspecies that affect different hosts but this host specificity is not complete and transference from one host species to another can occur [9,13]. *Sarcoptes* may be transmitted to unusual host in which it might burrow in to the skin and set up a typical mange lesion [19]. *Sarcoptes mites* are economically the most important cause of mange in sheep and goats. Sarcoptic mange in sheep and goats is caused by *Sarcoptes scabiei* var. *ovis* and S*arcoptes scabiei* var. *caprae* respectively [20]. *Sarcoptic scabiei* var. *caprae* of goats and *Sarcoptic scabiei* var. *ovis* of sheep are widely distributed in many goat and sheep raising arid and semi-arid areas of Ethiopia. Sarcoptic mange seems more common in goats than in sheep. Sarcoptic mites are highly specialized for life with in the skin. Female mites burrow in to the skin and lay eggs in tunnels they made. Mating takes place on the surface of the skin [21]. The life cycle from egg to egg lying female may take 10-14 days [19]. The feeding activity of Sarcoptes causes intense itching and scratching due to a marked irritation, which causes self-inflicted lesions that aggravates the conditions [22]. Sarcoptic mange usually start on relatively hairless part of the skin and may latter generalize [19]. The course of Sarcoptic mange is rather more acute than the other forms of mange and may involve the entire body surface in a short time. It is highly contagious and the spread of *Sarcoptes scabiei* is usually by close physical contact. As a result single cases are rarely seen in groups of animals kept together. Infestation may also occur by indirect transfer, since the mites have been shown to be capable of surviving off the host for short periods. The length of time that *Sarcoptes scabiei* can survive off the host depends on environmental conditions but may be between 2 and 3 weeks [11]. The ideal approach for diagnosis of skin diseases in general is a logical progression from history to an overall clinical examination, to a detailed examination of the skin, and finally to confirmatory testing or diagnosis by response to treatment [22,23].

Ectoparasites of small ruminants can be controlled by using commercial acaricides, but their accessibility and affordability to the resource poor farmers, and safety towards the environment makes them less preferable compared to other alternatives such as medicinal plants. The majority of farmers and pastoralists in the developing countries rely on traditional health care practices to keep their livestock healthy. These indigenous practices include the use of medicinal plants or ethno-veterinary medicine. It provides a vital contribution to livestock health needs throughout Ethiopia and is especially important to the resource poor rural communities. Treatment of mange with various acaricides like diazinon, fenvalerate, deltamethrin and avermectin has been attempted with different grades of success. Rapid development of resistance [24,25], high cost and environmental contamination [26,27] and health hazards to humans during treatment of animals [28] are the major problems associated with the use of synthetic acaricides. Concern about toxicity of many acaricides limits their use and reduces the number of safe effective products available. These problems have lead to research efforts to discover new effective compounds. The identification of novel active plant derived natural compounds could increase the number of available chemotherapeutic agents, thereby reducing the frequency of development of resistance and providing alternative drugs with greater acceptance, especially in terms of environmental safety [29]. In view of all these problems, use of botanical acaricides against highly pathogenic and economically important ectoparasites like mange is extremely important. Different indigenous plants like *Cedrus deodara, Pongamia glabra, Diospyros scabra, Dobera glabra, Euphorbia abtyssinica,* and *Sterculia alexandri* have been tried against sheep mange mites [30]. Various parts of different plants are being used in Ethno Veterinary Medicine practices for the treatment of mange mites in animals in different part of the world. Therefore, the objective of this study is to evaluate the acaricidal ability of *Eucalyptus globulus, Cymbopogon citractus, Nicotiana tobacum, Jatropha curcas, Melia azadarachta*, *Ximenia caffra* and *Pyrethrum cinerariifolium* extracts by *in vitro* and *in vivo* techniques against *Sarcoptes scabiei* var. *caprae* on infected goats.

Materials and Methods

Study area

The study was conducted in Dera and Hidabu Abote districts of North Shoa, Oromia regional state from November 2011 to May 2012. These districts comprise different agro climates namely highlands (>2000 m), midlands (1500-2000) and lowlands (<1500 m). The production system of the area is mixed crop livestock.

Study animals

Indigenous goats managed under extensive management system in the different agro-climates were used for the present study. Mange mite infested goats from different parts of the study sites were brought to Ejere town for the experimental study. According to the guide line set by Vercruysse et al. [25], all animals were selected from the same parasitological background (exposure of parasite infestation) those acquired the disease naturally, having similar weight, age and breed. During the study, the animals were identified by uniquely numbered ear tags. Animals were managed similarly and with due regard to their

wellbeing. All study groups were confined separately, offered food under similar conditions according to local practice. Fresh water was available *ad libitum* throughout the study period. The health of the animals was also observed regularly. Other acaricides were never given to the study animals during the study period.

Study design

This study was designed using parameters of plant collection and extraction, *in vivo* and *in vitro* efficacy test and questionnaire survey.

Plant collection and extraction

The plants to be evaluated in this study were collected from their natural habitat and identified by taxonomists using standard flora, and voucher specimens was deposited in the national herbarium, Addis Ababa University. Air dried and powdered plant material (300-550 g) was extracted exhaustively with hydrochloric solvent by percolation at room temperature. The menstruum was filtered and concentrated in rotary evaporator or lyophilizer to give the crude extracts. The concentrated extracts were kept in tightly closed bottle in refrigerator until used for efficacy study. Small portion of the extract/fraction was used for the identification of the constituents (secondary metabolites) of the extract by examining the developed Thin Layer Chromatography (TLC) with appropriate chromogenic reagents.

***In vivo and in vitro* acaricidal efficacy test:** Goats with mange like lesion of natural infestation were selected for the *in vivo* study. Infestation was confirmed by skin scraping examination. Infested animals were randomly divided into 6 groups (n=6) and were assigned to different treatments randomly based on the result of *in vitro* test. Plant extracts with good *in vitro* efficacy were selected and tested *in vivo*. Animals in all the treatment groups were individually sprayed with approximately 1 liters of the extract (until sufficiently became wet by the fluid) at a concentration of 0.625% two times, at 14 days interval [31,32]. The positive controls were treated two times again with ivermectin (Chengdu Qiankum Pharmaceuticals Co. Ltd., China, Lot: 09YW0705, Mfg. Date 10/07/09, Exp. Date: 09/07/2012) at a dose rate of 0.2 mg/kg body weight subcutaneously and diazinon (Shandong Luxy Animal Medicine Share Co. Ltd. No.1, Zhquqiao Road, West Ofqihe County, 251100, Shandong PR China Bach No.:20080330, Mfg. Date: 03/30/ 2008, Exp. Date: 03/ 29/2011)topically in the form of spray 14 days apart. The negative control groups were left none treated. The animals in each group were housed in separate rooms (Table 1).

Clinical examination of animals was carried out on the day of the first treatment (D0), the day of the second treatment (14 days after D0) and subsequently 21, 28 and 56 days post treatment [31,33]. All animals were individually examined and response to the treatment was monitored on days 14, 21, and 28 and 56 days in terms of Mean Recovery Response (MRR) and % reduction in mite counts according to the method described by Vercruysse et al. After the start of the trial, degree of lesions on individual animals were ranked using grading codes from 1-4 indicating an increasing degree of skin reaction and mean Skin Lesion Quality (SLQ) of each group were determined. After treatment (from Day 0 till end of trial) recovery in individual animals were ranked with grade codes from 0 to 4 and MRR of each group were determined to compare the effect of treatments (Table 2).

For parasitological examination skin scrapings were taken from the part of the lesions bordering healthy tissue by scraping as the method described by Fthenakis et al. [26]. Samples were examined within 12 h of collection. Scrapings were collectively placed into a test tube with 5 ml of distilled water and 10% KOH and heated until hair and epidermal scales dissolved and centrifuged at 2000 rpm for 2 min. The sediment then suspended in distilled water and re-centrifuged. The sediment was examined under a microscope and mites were identified with the help of morphological characteristics described by Souls by, Das; Wall and Shearer [18,28,11].

The total numbers of mites present were counted and % efficacy of each treatment was calculated as given by Khan [30]:

$$\% \text{ efficacy} = \frac{\text{No. of mites before treatment - No. of mites after treatment}}{\text{No. of mites before treatment}} \times 100$$

Data Analysis

Collected raw data was carefully recorded and stored in Microsoft Excel database system used for data management. Statistical software package called SPSS for windows version 17.0 was used for data analysis. Statistical significance was set at $P<0.05$ and Analysis of variance (one-way ANOVA-Tukey test) was also used to compare the means of different treatments (concentrations) of the extracts and controls in different time used for *in vitro* and *in vivo* efficacy studies of medicinal plants.

Results

Questionnaire survey

The results of the questionnaire survey forwarded to small ruminant owners were summarized by agro-ecology.45/90 (50%) of the respondents indicated mange to be treated traditionally by local healers using herbal medicine 17/90 (18.9%), non-herbal treatment 14/90 (15.6%) and 17/90 (18.9%) both. Results of the questionnaire survey on the participation in the treatment campaign program launched by the government for sheep and goats against ectoparasites revealed that 88/90 (97.8%) of sheep and goat owners have participated and treated their animals. According to that program each animal should have been treated 4 times at 10-14 days interval annually for a maximum of three years, but only 65/90 (72%) of the respondents who have participated in the program have treated their animals more than 3 times. In addition the interval between treatments was not regularly performed.

Focus group discussion

They said that inhabitant of the districts use modern medical care for treatment of animal diseases in general and skin problems in particular. But due to inaccessibility and high costs of modern drugs, traditional medical care like drenching, branding, vein puncture and washing with crude plant extracts are highly practiced in the area. Most traditional healers use plant preparations to treat livestock skin diseases. The people usually seek assistance from knowledgeable community members. The knowledgeable community members (traditional healers) do not charge for their assistance. But the individual who need assistance offer materials in kind like coffee and sugar. They believe that the treatment

Animals	Control (non-treated)	Diazinone treated	*Eucalypus*	*Eucalyptus* 0.625%	*Cymbopogon* 0.625%	Total
	Group 1	Group 2	Group 3	Group 4	Group 5	
Goat	6	6	6	6	6	30

Table 1: Schematic design of the experimental study.

No.	Description of lesions	Grade codes
1	Reddening of skin	1
2	Bare, exposed, moist lesions with serious exudation	2
3	Dry lesions with scab formation and loss of hairs	3
4	Thick, wrinkled skin with hyper keratinization	4
	Description of recovery	
5	No response	0
6	Dryness of lesions and loss of itching	1
3	Start of shrinkage of lesions and hair growth	2
4	Marked hair growth with smooth skin surface	3
5	Complete recovery	4

Table 2: Degree of lesion and mean recovery response of experimental animals.

would be effective (curable) when they offer materials as a gift to the healers. But compared to the cost of modern drugs this contribution is too cheaper. The participants also indicated the presence of secrecy with the knowledge of traditional medicine to treat livestock diseases. The traditional healers believed that the treatment will remain curable if they keep it secret. They inform the name of the medicinal plants that are used for treatment of ectoparasites only to the respected family members especially their elder sons when they get mature. In addition to this there are some plants which are known by the public to treat mange mites. These plants include *Accacia tortilis* ('Dhadacha'), *Aloe scundiflora* ('Chakke'), 'Sensel' and Lemon. The interviewed persons forwarded suggestions for scientific investigation of medicinal plants so as to develop herbal based drugs. They also forwarded the idea that researchers should address traditional healers in the investigation and development process.

In vitro acaricidal efficacy evaluation

Mortalities for the mite treated with the different concentration of *Eucalyptus globulus* essential oil are shown in Figure 1. When compared to the reference drugs (diazinon and Ivermectin), the extract was found to have comparable effects ($p>0.05$) against *S. scabiei* var. *caprae* at all test concentrations of 0.15625-2.5%. The concentration of 2.5% of the extract had the highest acaricidal efficacy causing 100% mortality after 10 min of exposure. But the concentration of 1.25% and lower showed 100% mortality after 120' of exposure. 0.625% was the lowest concentration that caused 100% mortality of mites compared to others at this 120'. After 3 h of contact all the concentrations of *Eucalyptus globulus* essential oil showed statistical significant ($p<0.01$) difference in mortality of mites with respect to the solvents (negative controls) and untreated controls (distilled water).

Mortalities of the mite treated with the different concentration of *Cymbopogon citractus* are shown in Figure 2. When compared to the control, the extract was found to have significant effect against *Sarcoptes scabiei* var. *caprae* at all test times and concentrations of 1.25-2.5%. These concentrations of the extract had the highest acaricidal efficacy with 100% mortality after 10 min of exposure. There was no significant difference ($P>0.05$) at all concentration of 0.15625-2.5% after 3 h of exposure when compared to the positive control. This implies all concentrations had comparable effect with the reference drugs. But 0.625% was the lowest concentration that caused 100% mortality of mites compared the others after 2 h of exposure. After 3 h of contact all the concentrations of *Cymbopogon citractus* essential oil showed a high mortality ($p<0.01$) when compared to the solvent (negative control) and non-treated control (distilled water).

Mortalities for the mite treated with the different concentration of the extracts of *Nicotiana tobacum* fixed oil are shown in Figure 3. When compared to the positive control, the extract was found to have significant ($P<0.05$) effects against *Sarcoptes scabiei* var. *caprae* at all test times at a concentration of 160 mg/ml. 80 mg/ml and 160 mg/ml concentrations of the extract had the highest acaricidal efficacy with 100% mortality after 3 h of exposure. There was no significant effect ($P<0.05$) at a concentration of 40 mg/ml and below even after 3 h of exposure compared to the positive control. *Nicotiana tobacum* fixed oil 160-40 mg/ml showed a high mortality ($p<0.01$) When compared to the solvent (negative control). But only 20 mg/ml and above of the extracts with respect to untreated control (distilled water) showed statistically significance difference ($p<0.01$) in causing mortality of mites.

Mortalities for the mite treated with the different concentration of *Jatropha curcas* fixed oil are shown in Figure 4. When compared to the positive control, the extract was found to have comparable effects against *Sarcoptes scabiei* var. *caprae* at 120 min and concentrations of 160 mg/ml and 80 mg/ml. But after this time their effect seems to remain constant at about 80% when the positive controls continue to cause death of mites up to 100%. After 3 h of contact the extract showed no acaricidal efficacy ($P>0.05$) except the concentration of 160 mg/ml as compared to the reference drugs (diazinon and ivermectin). But when compared to the untreated control (distilled water) all the concentrations of the extract showed statistically significance difference ($p<0.05$) in causing mortality of mites. Only the concentration of

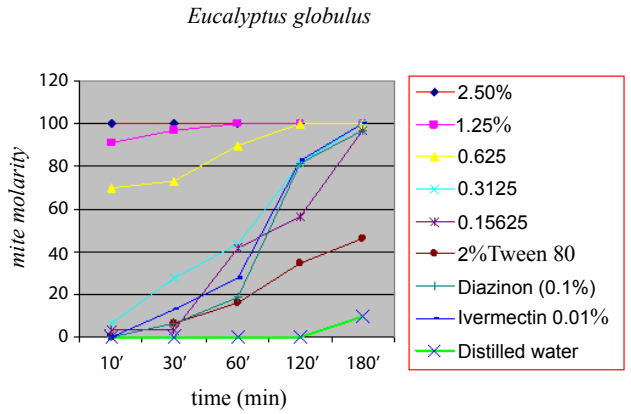

Figure 1: Mortalities of *Sarcoptes scabiei* var. *caprae* treated with the extracts of *Eucalyptus globules* essential oil *in vitro*.

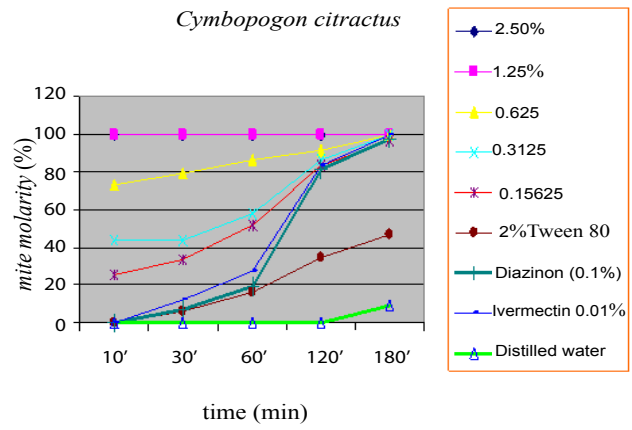

Figure 2: Mortalities of *Sarcoptes scabiei* var. *caprae* treated with the extracts of *Cymbopogon citractus* essential oil *in vitro*.

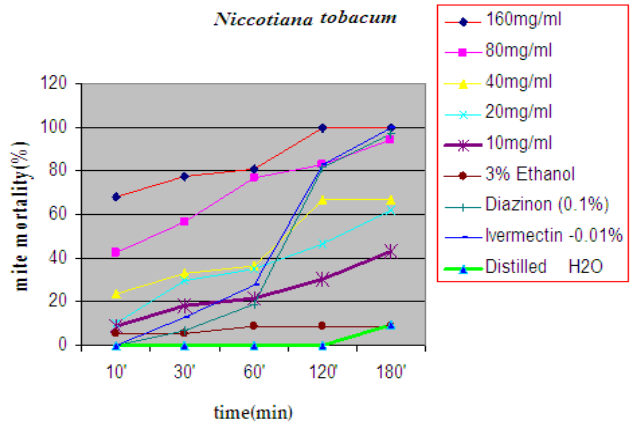

Figure 3: Mortalities of *Sarcoptes scabiei* var. *caprae* treated with the extracts of *Nicotiana tobacum* fixed oil *in vitro*.

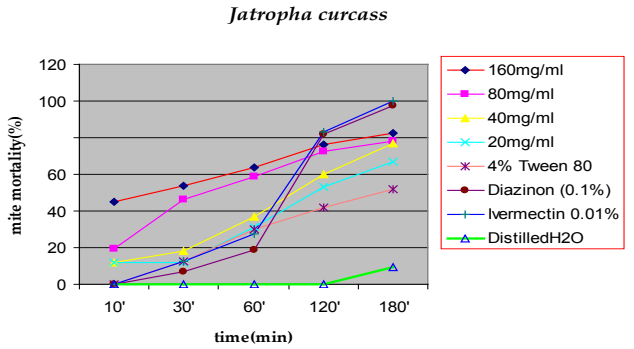

Figure 4: Mortalities of *Sarcoptes scabiei* var. *caprae* treated with the extracts of *Jatropha curcas* fixed oil *in vitro*.

80 mg/ml and 60 mg/ml showed statistically significance ($p<0.05$) difference on mite mortality to the solvent (negative control).

After 3 h of contact *Melia azadarachta* fixed oil showed comparable acaricidal efficacy ($P>0.05$) in causing mite mortality at concentrations of higher than 10 mg/ml of the extract when compared to the reference drugs (diazinon and ivermectin). But compared to non-treated control (distilled water) all the concentrations of the extract showed statistically significance difference ($p<0.05$) to cause mortality of mites (Figure 5).

After 3 h of contact the extract of *Ximenia caffra* (fixed oil) showed comparable acaricidal efficacy ($P>0.05$) in all the concentrations above 20 mg/ml of the extract as compared to the reference drugs (diazinon and ivermectin). But compared to untreated control (Distilled water) all the concentrations of the extract showed statistically significance ($p<0.05$) difference to cause mortality of mites (Figure 6).

After 3 h of contact *Pyrethrum cinerariifolium* fixed oil showed no acaricidal efficacy ($P<0.05$) in all the concentrations of the extract compared to the reference drugs (diazinon and ivermectin). The highest concentrations (160 mg/ml and 80 mg/ml) showed lower efficacy of mite mortality only 49% and 46.7% respectively (Figure 7). Even though there is statistically significant ($p<0.05$) difference compared to untreated control (distilled water) all the concentrations of the extract showed no significant ($p<0.05$) difference when compared with the solvent to cause mortality of mites.

In vivo acaricidal efficacy

For *in vivo* efficacy evaluation 0.625% concentration of both *Eucalyptus globulus and Cymbopogon citractus* was selected due to the promising efficacy of these plants after *in vitro* test. Table 3 shows the major results of the *in vivo* trial. At day 0 no difference was observed among all groups, both for the parasitological (presence of mites) and clinical score (presence of lesion). From day 21 after the beginning of the treatment, *Eucalyptus globulus* treated group, *Cymbopogon citractus* treated groups and treated control groups (ivermectin and diazinon treated groups) were negative for mites and/or eggs and significant differences ($p<0.05$) was recorded in their clinical lesion scores, Mean Recovery Responses (MRR) and percentage of mite count reduction

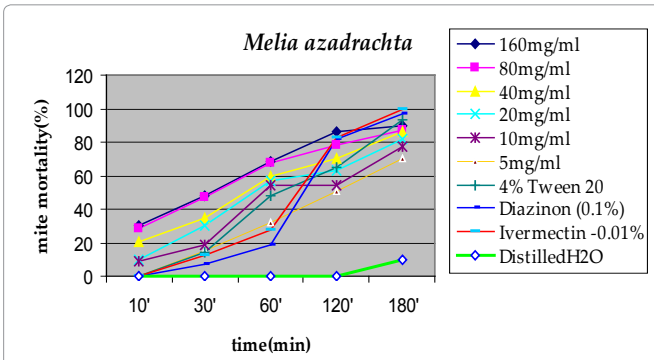

Figure 5: The mortalities of *Sarcoptes scabiei* var. *caprae* treated with the extracts of *Melia azadarachta* fixed oil *in vitro*.

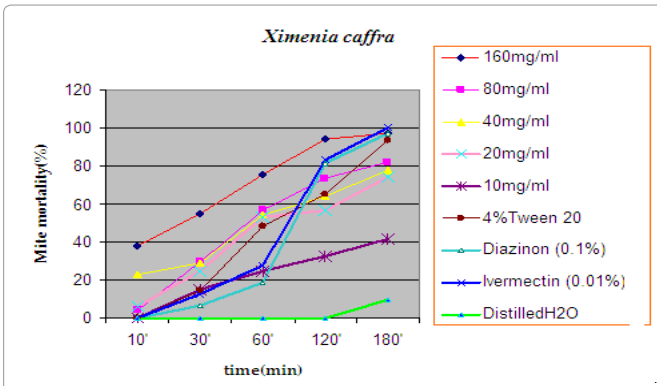

Figure 6: The mortalities of *Sarcoptes scabiei* var. *caprae* treated with the extracts of *Ximenia caffra* fixed oil *in vitro*.

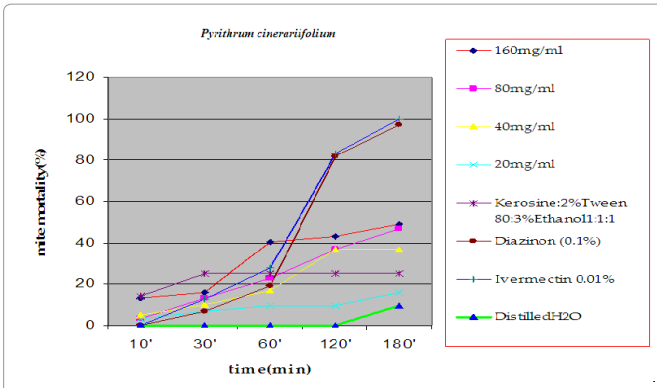

Figure 7: Mortalities of *Sarcoptes scabiei* var. *caprae* treated with the extracts of *Pyrethrum cinerariifolium* fixed oil *in vitro*.

	Eucalyptus globulus			*Cymbopogon citractus*			Ivermectin			Diazinon			Non-treated control		
Day	Mite count% efficacy	MRR	SLQ	Mite count %efficacy	MRR	SLQ	Mite count %efficacy	MRR	SLQ	Mite count %efficacy	MRR	SLQ	Mite count% efficacy	MRR	SLQ
0	0	0	3.2	0	0	3.2	0	0	2.7	0	0	2.8	0	0	3.3
14	45	2.0	1.8	83.3	3.1	1.17	80.7	2. 67	1.33	80.3	2. 67	1.5	-0.7	0	3.3
21	100	2.8	1.6	100	3.5	0.8	100	3.6	0.6	100	3.3	0.7	-0.7	0	3.3
28	100	3.0	1.0	100	3.8	0.33	100	4.0	0.00	100	4.0	0.00	-0.7	0	3.3
56	100	3.6	0.6	100	4.0	0.17	100	4.0	0.00	100	4.0	0.00	-0.7	0	3.3

Table 3: *In vivo* acaricidal efficacy of *Eucalyptus globulus* and *Cymbopogon citractus* against *Sarcoptes scabiei* var. *caprae*.

compared to non-treated group. Plant extracts of both of the essential oils and positive controls resulted in a clinical cure in all groups. The untreated control group remained positive for mite infestation until 14th day of the trial and the mite count increases progressively and the conditions of the lesions get worse and worse ending in death of the animals. On day 14, in this latter group the degree of infestation was significantly ($p<0.01$) higher when compared to *Eucalyptus globulus and Cymbopogon citractus* treated groups and treated control group in both the degree of skin lesion and mite count % efficacy. The Mean Recovery Responses in the treated groups and treated controls significantly increased without difference in these groups and the skin lesion reduced significantly ($p<0.05$) when compared to the non-treated groups and healing was observed after day 28 that was maintained to exist until day 56, the end of the *in vivo* experimental period.

Discussion

In the present study *Eucalyptus globulus* showed comparable acaricidal efficacy as compared to the reference drugs (diazinon and ivermectin) killing up to 100% of sarcoptic mites from goats. *Eucalyptus* essential oil has been previously reported to possess biocidal properties. *Eucalyptus globulus* essential oil has been tested for its acaricidal effect against other mites [34]. Oil from *Eucalyptus globulus* proved to be toxic to the mites in a separate study that tested the toxic effect of the essential oil from the same species of eucalyptus against *Dermanyssus gallinae* [35]. The present study confirmed *E. globulus* to be toxic to *Sarcoptes scabiei* var. *caprae*. In other studies, essential oils from other spp. of Eucalyptus proved to be effective in killing mites achieving more than 65% mortality. In contrary to the present study, essential oils from *Eucalyptus globulus* displayed a much reduced acaricidal effect, killing only 11% of mites after exposure [36,37]. This may be due to numerous factors which may affect the composition of essential oils. Geographic origin [38], seasonality [39], method of oil extraction [40], year of harvest [41] and even storage conditions are all factors that have been reported to influence essential oil chemistry and its efficacy against ectoparasites. The compound is commonly reported to have acaricidal [42,43] and pesticidal [44] properties. Essential oils with more complex chemical compositions may have an additional advantage over simpler oils if developed for use as acaricides. It has been observed the most important advantage of such products for pest management would be that the numerous active compounds in essential oils would make development of resistance to any essential oil based product extremely difficult [43].

All concentrations of *Cymbopogon citractus* essential oil had comparable efficacy with the reference drugs (diazinon and ivermectin). Even though there is very little study so far conducted on the acaricidal activity of this oil, several studies reported antimicrobial activities by *Cymbopogon citractus* essential oil [45-48]. Indeed, the oil exhibited a broad spectrum of fungi toxicity and its fungi toxic potency remained unaltered for 210 days of storage, with considerable interests in the application of the oil for the preservation of stored food crops [49]. *Cymbopogon citractus* essential oil inhibited microorganisms examined at ≤2% (v/v). The current study also showed mite mortality at ≤2.5% (v/v). Fungi colony growth and sporulation was completely retarded by this essential oil [50,51]. The initial idea of the current study was originated from these works on plant pest. Therefore the essential oil acts not only on crop pests but also animal pest particularly mange mites.

Nicotiana tobacum has a Jasmonic acid and its cyclic precursors and derivatives are members of a lipid-based signaling cascade originating from polyunsaturated fatty acids. It plays a role in development and defense including plant response to wounding and abiotic stress, and defenses against insects and pathogens [52]. This and other constituents of the plant may be responsible for the toxic effect of the mites that caused mortality of mites in the *in vitro* evaluation.

Jatropha curcas is used in the treatment of various disorders in man and animals, including goats and sheep, and are also ingested by grazing animals particularly at times of drought. The seed of this plant was used as purgatives, anthelmintic and molluscicides [53]. In the current study the extract was found to have goodacaricidal efficacy similar to conventional drugs *in vitro* against *Sarcoptes scabiei var. caprae* at concentrations of 160 mg/ml and 80 mg/ml even the effect seems to remain constant at about 80% when the positive controls continue to cause death of mites up to 100%. But in recent study by Abdel-Gadir et al. [52] the oral administration of *Jatropha curcas* seed to Nubian goat kids caused toxic manifestations and death of goats. But acaricides are applied topically; toxicity might not be as that of lethal as oral administration.

Some reports have confirmed that *Melia azadarachta* is an effective botanical acaricide for mange mites (*Sarcoptes scabies* var. *caniculi*) (*Sarcoptes scabies varovis*) [54,55] and ticks (*Boophilus microplus*) [56]. The pesticide activity of this fixed oil is generally thought to be due to azadirachtin, which is a well-known potent insecticide [57]. However, Walton et al., [58] reported that the product containing 0.3-0.5% azadirachtin had no effect on *Sarcoptes scabiei* var. *hominis* by *in vitro* test. Mortality of mites in the present study showed comparable efficacy with standard drugs even at lower concentrations.

Ximenia is a key part of native African medicine. Its principal use is as an emollient, hair oil, conditioner and skin softener, in soap manufacture, and as component of lipsticks and lubricants. The root, bark and leaves are used for medicinal purposes; the bark is used to treat toothache, mouth infections and stomach aches [59]. In the present study the extract of *Ximenia caffra* (fixed oil) showed comparable acaricidal efficacy with respect to the reference drugs (diazinon and ivermectin) *in vitro*.

The term "*Pyrethrum cinerariifolium*" refers to the plant, flower or flower extract, with the active insecticidal components known as "pyrethrins" [60]. *Pyrethrum cinerariifolium* is a plant widely used for insecticide production [61]. It also used as crop pest (grain weevil) [62].

It is a toxic agent for pest insects with a century-long history of safe use [63]. The great advantages of *Pyrethrum cinerariifolium* are its action against a wide variety of different insect species [64], a low mammalian toxicity and a rapid metabolism [63]. But in the current study *Pyrethrum cinerariifolium* fixed oil showed no acaricidal efficacy ($P<0.05$) in all the concentrations of the extract as compared to the reference drugs (diazinon and ivermectin). Even the higher concentrations (160 mg/ml and 80 mg/ml) showed lower acaricidal efficacy (only 49% and 46.7% respectively) against mites which is much lower to say effective. This may be due to physiological differences between acaris and insects.

Products prepared from both *Eucalyptus* and *Cymbopogon* extracts were found safe for goats in the *in vivo* application as they did not show the sign of irritation or restlessness at the time of application or afterwards. From the scanty previous information on the herbal acaricides *in vivo*, *Azadirachta indica* (50% oil) has been reported to cause 87.7% recovery [65,66], 100% recovery [32] in sheep with sarcoptic mange. Yand et al. [40], have also reported the efficacy of this ointment (neem) *in vivo* against ear canker of rabbits (caused by *Psoroptes cuniculi*). In the present study, 100% recovery was observed in goats treated with *Eucalyptus globulus* and *Cymbopogon citractus* at 0.625% concentration which were comparable with modern treatments. Other plant materials like linalool also showed acaricidal activity against Psoroptes mite *in vivo* on rabbits and goats [67]. In a recent study [45], linalool and cinnamyl acetate showed insecticidal activity against *Pediculus humanus capitis*.

In general along with the economic benefits, additional advantage of using plant pesticides is that, they have low environmental persistence [68] do not induce resistance readily in insects [69,70] and are relatively nontoxic to mammals. These results consolidate the belief that the use of herbal acaricides may provide a better way of combating a menace such as mange in domestic animals and they can be used more safely and effectively.

Conclusion and Recommendations

All medicinal plants showed acaricidal efficacy *in vitro* comparable to the reference drugs except *Pyrethrum cineraria folium* especially at higher concentrations. *In vivo* evaluation of both *Eucalyptus globulus* and *Cymbopogon citractus* resulted in complete elimination of mites. So results obtained in the *in vitro* and *in vivo* evaluation in the present study indicated that essential and fixed oils of the extracts tested could represent a possible alternative for the topical treatment of sarcoptic mange in goats.

Based on the above concluding remarks, the following recommendations are forwarded:

- The efficacy of the preparations, techniques and practices need to be further investigated in other areas to identify promising plants for use.
- Further studies should be conducted on the socio economic situation, drug formulation and licensing of the extracts so as to develop herbal based treatments.

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