

STUDY OF *TECOMELLA UNDULATA* G. DON. METHANOLIC EXTRACT AGAINST *SARCOPTES SCABIEI* L. IN VIVO AND IN VITRO

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ABSTRACT

Tecomella undulata G. Don. is long been used in ayurvedic system of medicine. The plant has been declared to exhibit diverse pharmacological activities. The antiacarid effect of methanolic extract of *Tecomella undulata* G. Don. with concentrations of 10 and 20% was evaluated. The extract was applied topically on scabies affected skin of camels, buffalos, goats, dogs and people and the effect was monitored through symptomatic relief and microscopic examination of the mange. The topical application of *Tecomella undulata* extract (10 and 20%), ivermectin (reference compound) and 100% methyl alcohol (control) on scabies affected buffalos gave 64, 78, 82 and 8%, on camels 64, 78, 82 and 4%, on dogs 62, 83, 85 and 7%, on goats 58, 73, 81 and 5% protection and on persons 61, 78, 80 and 5% protection, respectively after five weeks. In addition, the effect of *Tecomella undulata* extract with three different concentrations (i.e. 10, 20, and 30%) was studied to know the duration to be taken to kill *Sarcoptes scabiei* using *in vitro* laboratory test. The results revealed that 10, 20 and 30% concentrations of *Tecomella undulata* extract caused 45, 65, and 80% mortality of the *Sarcoptes scabiei* mites, respectively. Ivermectin caused 85% and in control treatment 10% mortality of the mite was recorded. It is concluded that *Tecomella undulate* has effective properties to be used as acaricidal.

Key words: *Tecomella undulata*, extract, efficacy, ivermectin and mite.

INTRODUCTION

Scabies is one of the notorious diseases affecting human and animal's skin. *Sarcoptes scabiei* L (a common itch mite) is the known cause of this disease. Scabies is a large scale disease affecting a wider section of a society throughout the world (Burgees, 1994). This disease has many manifestations such as classical, Norwegian and clean scabies. By and large, classical scabies is found common in people and animals round the globe and in countries like Pakistan. Skin diseases together with scabies, are reported to affect millions of people and animals (WHO, 2001; Hicks and Elston, 2009; Lusat *et al.*, 2009).

The gravid female lays two to three eggs a day in tunnel (several mm to several cm in length) created at the stratum corneum of epidermis. After 50-72 hours, the larvae emerge out of eggs and make new tunnels. After moulting from larvae to nymph form and subsequent two moults of the nymph reach to adult stage. This entire life cycle of *Sarcoptes scabiei* is completed in 10-17 days. Usually bathing can remove a significant number of *Sarcoptes scabiei* mites from the affected people and animal's body; but inappropriate bathing can affect their skins. This also cause skin dryness and scratches, and may prove harmful as dry skin is more susceptible to severe irritation and itching. In such circumstances people and animals affected with scabies may face other

health hazards i.e. dermatitis, pyoderma and sometimes eczema and urticaria. Treatment of scabies commonly includes the application of topical creams; alternatively, the oral administration of ivermectin is also considered effective to treat this disease (Mounsey *et al.*, 2008-a). Permethrin, as topical creams has been successfully used for the treatment of scabies in Australia (Carapetis *et al.*, 1997). However, a recent *in vitro* study on antiacarid activity has demonstrated an increased tolerance of *Sarcoptes scabiei* mite in people in northern Australia to permethrin (Mounsey *et al.*, 2008-b). Several constraints such as resistance of *Sarcoptes scabiei* to synthetic medicines that might come due to the repeated use of the same mode of action medicines in people and animals sick with scabies, side effects of synthetic medicines, their unaffordable cost and the long residual properties may justify the cause to find other approaches to manage scabies. One such approach could be the use of plants extracts that might be useful to cope with scabies on human and animals.

Tecomella undulata (Family-Bignoniaceae) is commonly known as rohira (Punjabi), lohira (Sindhi), and regions of India (Kritikar KR *et al.*, 1993). It can be found in Punjab, Sindh Khyber Pakhtunkhwa and Waziristan regions (Kritikar KR *et al.* 1993, Nadkarni, 2000). *Tecomella undulata* a shrubby small tree with drooping branches with small leaves 5-12.5 cm in length and 1-3.2cm in width and inodorous flowers. The screening of this plant for wide range of pharmacological

activities was conducted by various researchers. The methanolic extract showed anti inflammatory and analgesic effect through using rat paw oedema and tail immersion method respectively (Ahmad *et al.*, 1994). Flavones were reported from *Tecomella undulata* leaf (Verma *et al.*, 1986) and Iridoid glucoside undulatin assigned as 4'-O-P-coumaroyl-7, 8-dihydro-8-dehydroxymethylbartsioside structurally by chemical and spectroscopic analysis (Azam and Ghanim *et al.*, 2000). Quinonoid in heartwood and an iridoid glucoside, 6-O-veratryl catalposide has been isolated from the plant (Joshi *et al.*, 1974) and lapachol (Singh *et al.*, 1972). Several compounds isolated from this plant reported by different workers are Lapachol (Neamat *et al.*, 2010) Chromone glucoside (Gujral *et al.*, 1991), ferulic ester (Joshi *et al.*, 1986), quinines (Joshi *et al.*, 1977), iridoid glucoside (Joshi *et al.*, 1975), tecomin (Pandey and Dasgupta, 1971), rutin, quercetin, luteolin glucoside (Taneja *et al.*, 1975), tecoside (Verma *et al.*, 1979), undulatin (Verma *et al.*, 1986) and cirsimaritin and cirsilineol (Azam and Ghanim, 2000). Kritikar *et al.* (1993), Nadkarni (2000). Chromone glucoside by Gujral *et al.* (1991) and ester glucoside by Pandey *et al.* (1970) have been isolated from *Tecomella undulata* plant. Traditionally the plant has been reported to have insect repellent as well as pesticidal properties, however, the plant has also been suspected for its bio-medicinal values which may be useful for the treatment of various diseases. It is also important to recognize that its extract may be effective not only in the isolation but may have modulating effect when used in combination with other drug.

Keeping in views the suspected bio-medicinal properties of *Tecomella undulata* against scabies, the present study was designed to evaluate the effects of extracts of this plant for the treatment of scabies infested people and animals using *in vitro* and *in vivo* laboratory experimental trials.

MATERIALS AND METHODS

Processing of plant materials: The samples were collected from four sites each in district Bannu and district Karak, Khyber Pakhtunkhwa during summer 2008-2009. Branches of *Tecomella undulata* were chopped into small pieces by a manual cutter, collected and placed in brown paper bags. All samples were placed in the Laboratory of Organic Chemistry Gomal University Dera Ismail Khan and air dried for seven days at room temperature. The dried samples were then grinded to powder form in a grinding machine. The powder (500 grams) was mixed with 2 L of 100% methyl alcohol (CH₃OH) in a beaker. This mixture was stirred twice a day regularly for 20 days to mix the powder with CH₃OH to obtain the standard extraction of *Tecomella undulata*. The methanolic extract was filtered into a

beaker using muslin cloth and stored in a refrigerator. The filtered mixture stored in refrigerator was subjected to Rotavapour at 55-63 °C for 9-10 hours and achieved 5.2% crude extract out of the total extracted volume of *Tecomella undulata*. Thus we achieved the *Tecomella undulata* crude extract (free of CH₃OH). The crude extract was stored in a refrigerator at 4 °C for use in the trial. For extraction of *Tecomella undulata* crude extract the experimental protocol was adapted as reported by Daniel (1991).

Preparation of the stock solutions: The methanolic extract obtained from *Tecomella undulata* (50 g) was weighed by using an electronic balance and poured into 100 mL flask. To each flask 50 mL CH₃OH was added to get the final volume of 100 mL of methanolic concentration of *Tecomella undulata*. This constituted 50% CH₃OH diluted extract was designated as 50% stock solution. Three concentrations 10, 20 and 30% were prepared for *in vitro* and *in vivo* experiments by diluting the standard stock solution serially with CH₃OH. The ivermectin at the dose rate of 0.2 mg kg⁻¹ body weight was used for human and animals as a reference compound recommended for scabies treatment (Sparsa *et al.*, 2006). The CH₃OH was used as a control treatment.

Sarcoptes scabiei mites rearing: A mass culture of *Sarcoptes scabiei* was established inside the laboratory on rabbits at the Faculty of Pharmacy Gomal University D. I. Khan from August 2008 to September 2009 according to Briemer *et al.* 1993 with some modifications. The rabbits were offered suitable environment with the prerequisites (adequate oxygen supply, 21-25 ± 2°C temperature and 65-71 ± 5% relative humidity (RH)) for rearing of mites. The rabbits were provided fodder and water twice a day to fulfil their food requirements.

Mites collection: Ten rabbits infested with *Sarcoptes scabiei* were kept for 30 days to get maximum typical skin lesions. After noting maximum lesion (approximately 65%) on the rabbit's skin, were scrapped with the help of scalpel and placed in Petri dishes and were enriched in 5 mL of 10% solution of Potassium Hydroxide (KOH) for microscopic examination. Three times greater quantity of Sodium Chloride (NaCl) was added to the actual quantity of skin lesions mass (10 grams) and mixed together. The mixture was centrifuged at 1500 rpm. To identify the infested population of *Sarcoptes scabiei* mites, the centrifuged supernatant solution was discarded and a drop of the sediments was put on counting chamber slide to count the number of mites. The counting chamber slide was checked under Stereo Binocular Microscope (Model Sz2-ILST Sz61, Olympus, Tokyo Japan). The sediment of the skin lesions having 50 mites per drop was used as standard infestation good for live mites collection.

To collect *Sarcoptes scabiei* mites, five infested female rabbits were confined separately in a 5 linen bags (2 x 2 feet) covering its all parts except mouth, eye and nose. The vaginal and anal openings were connected to the plastic tube outside the linen bag. The mites were collected every 24th hour from the linen bag and transferred to cells (3 x 4 inches) containing nutrients (i.e. fish flakes, multivitamins, amino acids, glucose, skin shaving and horse serum semisolid globules). The house dust mite (*Dermatophagoides farinae* Hug.) collected from the common home used carpets and beds were also added to the plastic cells in order to provide suitable environment to the *Sarcoptes scabiei* mites. The cells containing mites were tightly closed with bulldog clips and placed in a glass container on a steel gauze (covering the filled water in the 2 L glass container) in order to keep cells away from direct water contact. The container was closed with a lid having only a small opening for Oxygen (O₂) supply and kept in a room with a tightly closed door (18-24 ± 2 °C temperatures) with 65-71 ± 5% RH for periodic use.

Cell structure for enclosing mites: All cells used to house the mites were constructed from concave black plastic discs with a varnish attached black filter paper at the opening of the discs to provide sufficient RH to the mites. The mites along with food were enclosed in the hollow section of the discs covered by glass square and tightly closed using Vaseline and bulldog clips. To feed the mites, aquarium gold fish flakes and tetra-mine tropical fish flakes were provided once a week and shaken on weekly basis to activate the mites. Optimum climatic conditions (25 ± 2 °C temperature and 65-70 ± 5% RH (Hallas, 1991; Arlian and dippold, 1996) for the growth of mites were maintained inside the Postgraduate Microbiology Research Laboratory in the Department of Biological Sciences, Gomal University D.I.Khan. All cells were placed in sealed desiccators and RH was provided by mixing KOH and water placed at bottom of desiccators (70 pellets of KOH + 85 mL tap water to provide 65% RH at 25 ± 2 °C) and was checked on weekly basis. The desiccators were placed in growth cabinet (10 x 8 feet) having 65% RH and 25 ± 2 °C temperature to assure suitable environment for the mites.

Media preparation: To provide the required nutrients to the mites during the trial, a media was prepared adapting methodology of Brimer *et al.*, (1993). This media was warmed using a spirit lamp and normal horse serum added into it. The media was again warmed and autoclaved at 121 ± 4 °C for 10 min to eradicate undesired micro-organisms, then added human skin shavings (mites feed) and yeast (to discourage the growth of other microorganism) and incubated at 37 °C for 24-48 hours to make it stable for use in *in vitro* technique.

Screening of *Tecomella undulata* extract through *in vitro* technique: The experiments were performed using 96 wells prepared tissue culture plates made of polyethylene. To each well 3.5 mg of media was added. An adequate dilution (30µl) of 10, 20 and 30% *Tecomella undulata* extract was added into the bottom of the wells and allowed to dry for 24 hours at room temperature (37 ± 4 °C) prior to launching the experiment. In the opposite wells 30µl of ivermectin (10 mg mL⁻¹), was put and used as reference compound. The control wells were only added 30µl CH₃OH.

Into each well, 20 mites were released using a teasing needle while observed under microscope. To avoid escape of mites, each well was covered with 16 mm diameter paper disk (porous) and incubated at 21 ± 2 °C and 70 ± 4% RH for 72 hours and sufficient O₂ was maintained in the dark room having 21 ± 2 °C temperature and 70% ± 4% RH. The mites mortality was recorded with a Stereo Binocular Microscope after 24, 48 and 72 hours incubation period. The criteria for conclusion of result was on the basis of movable (live) and non-movable (dead) when seen under the microscope checking through teasing needle.

Screening of *Tecomella undulata* extract through *in vivo* technique: For topical use on scabies affected animals, 10 and 20% concentrations of methanolic extract of *Tecomella undulata* in saturated CH₃OH was prepared by diluting 50% stock solution. The two concentrations (10 and 20%) of *Tecomella undulata* extract as well as ivermectin and CH₃OH (control) each were applied separately on 10 scabies positive buffalos, camels, dogs, goats and persons at one and two weeks interval (i.e. on 1st, 7th, 14th and 28th day of experiment). The data was recorded on 7th, 14th, 28th and 35th day intervals.

Statistical design and analysis: Both the experiments were established using completely randomized design with three replications (10, 20 and 30% *Tecomella undulata* extracts) for the *in vitro* experiment and both the treatments (10 and 20% *Vitex negundo* extracts) for the *in vivo* experiment. Ivermectin treatment and that of a control was kept for comparison with the other treatments. A t-test was performed on the means of the data (at *P* < 0.05) to see if there were significant differences among the treatments.

RESULTS

Mitocidal effect of *Tecomella undulata* plant extracts: On Day7, Day14, Day28, and Day35 topical use of 10%, 20% methanolic extract of *Tecomella undulata*, subcutaneous use of ivermectin @ 0.2mg/kg b.wt and topical use of concentrated methyl alcohol as control on scabies affected buffalos-calves gave 7%, 26%, 34% and 27% protection (10% *Tecomella undulata*), 13%, 34%, 44% and 34% protection (20% *Tecomella undulata*),

23%,59%, 69% and 68% protection (Ivermectin) and 0, 0, 8 and 10% protection (concentrated methyl alcohol) respectively; protection observed in Camels on Day7, Day14 Day28, and Day35 was 15%, 31%, 37% and 30% (10% *Tecomella undulata*), 25%, 45%, 54% and 45% (20% *Tecomella undulata*), 27%, 57%, 56% and 69% (ivermectin s/c) and 0, 0, 4,6 % (concentrated methyl alcohol) respectively; In Dogs on Day7, Day14 Day28, and Day35 protection observed was 9%, 25%, 32% and 25% (10% *Tecomella undulata*),15%, 49% 58% and 49% (20% *Tecomella undulata*), 17%, 69%, 71% and 72% (Ivermectin) and 0, 0, 7 and 10% (concentrated

methyl alcohol); protection seen in Goats on Day7, Day14 Day28, and Day35 was 10%, 20%,28% and 22% (10% *Tecomella undulata*),12%, 22%, 33% and 28% (20% *Tecomella undulata*), 15%, 39%, 49% and 68% (Ivermectin) and 0, 0, 5 and 8% (concentrated methyl alcohol); Whereas the protection observed in Human on Day7, Day14 Day28, and Day35 was 15%, 29%, 35% and 28% (10% *Tecomella undulata*), 25%, 35%, 45% and 40% (20% *Tecomella undulata*), 22%, 54%, 68% and 66% (Ivermectin) and 0, 0, 5 and 7% (concentrated methyl alcohol), (Table/Figure-II-a).

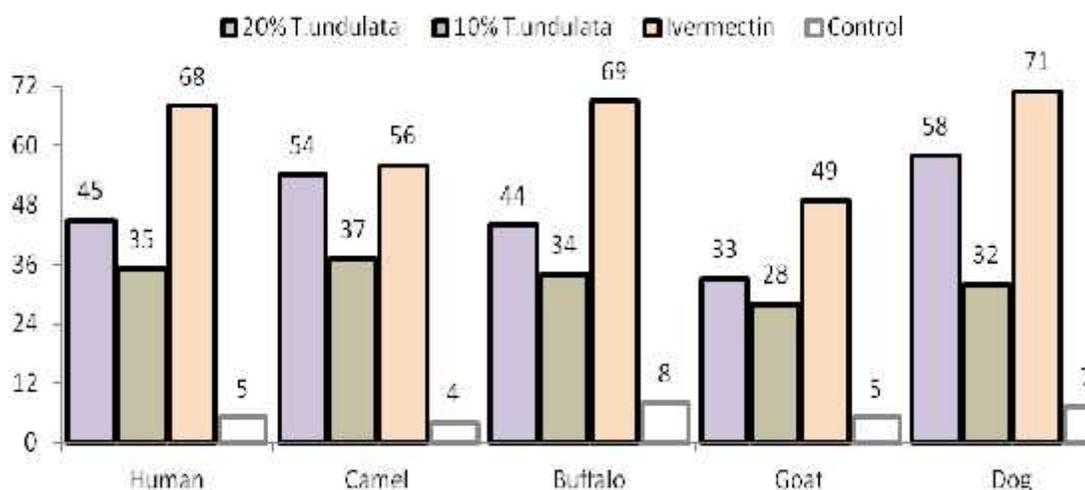


Figure-II-a: Miticidal activity of *Tecomella undulata* (10 and 20%) extract, ivermectin and control treatments against the *Sarcoptes scabiei* mite using *in vivo* laboratory test after 4th week of application.

The different concentrations of *Tecomella undulata* extract showed significantly different effect on *Sarcoptes scabiei* mite mortality ($P < 0.05$) (Table II-b). The results revealed that the 10, 20 and 30% concentrations caused 45, 65, and 80 % mortality of the mites, respectively whereas Ivermectin gave 85% mortality and only 10% mortality of the mite was observed in the control treatment after 48 hours (Figure/Table-II-b).

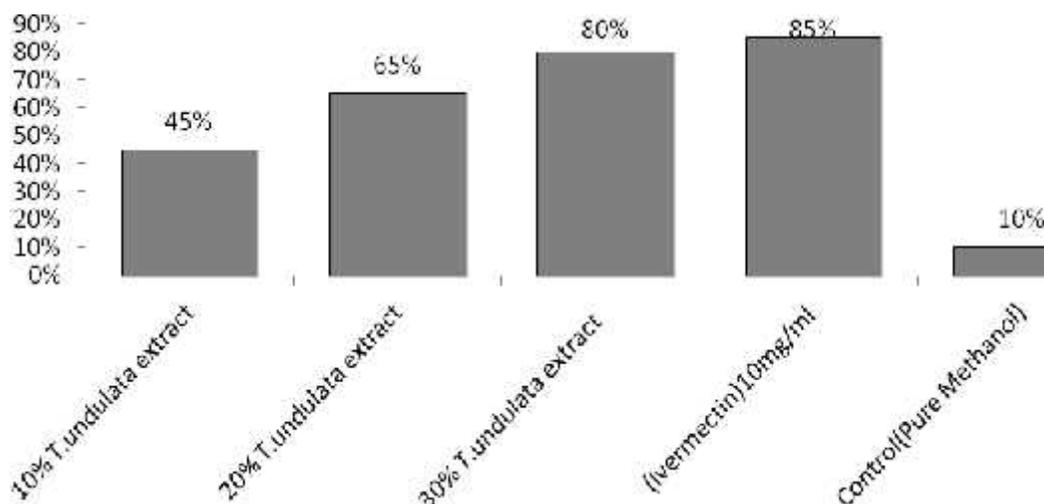


Fig-(II-b). Miticidal activity of *Tecomella undulata* (10, 20 and 30%) extract, ivermectin and control treatments after 48 hours of application against the *Sarcoptes scabiei* *in vitro* test.

Table-II-a: Miticidal effect of *Techomella undulata* methanolic extract *in vivo*

READING TIME	PERCENT SUSPENSION	BUFFALO	CAMEL	DOG	GOAT	HUMAN
1ST WEEK	10% <i>T. undulata</i> methanolic extract	7	15	9	10	15
	20% <i>T. undulata</i> methanolic extract	13	25	15	12	25
	Ivermectin	23	27	17	15	22
	Control	0	0	0	0	0
2ND WEEK	10% <i>T. undulata</i> methanolic extract	26	31	25	20	29
	20% <i>T. undulata</i> methanolic extract	34	45	49	22	35
	Ivermectin	59	57	69	39	54
	Control	0	0	0	0	0
4TH WEEK	10% <i>T. undulata</i> methanolic extract	34	37	32	28	35
	20% <i>T. undulata</i> methanolic extract	44	54	58	33	45
	Ivermectin	69	56	71	49	68
	Control	8	4	7	5	5
5TH WEEK	10% <i>T. undulata</i> methanolic extract	27	30	25	22	28
	20% <i>T. undulata</i> methanolic extract	34	45	49	28	40
	Ivermectin	68	69	72	68	66
	Control	10	6	10	8	7

Table-(II-b). Miticidal effect of *Tecomella undulata* plant extracts versus Reference and Control compound against *Sarcoptes scabiei* mite *in Vitro* (every time 20 mites were used in 96 wells Micro titration plate for this bioactivity test)

Test compound	Scabies mites tested	Dead			Average	Mortality Percentage
		1 st reading	2 nd reading	3 rd reading		
10% <i>T.undulata</i> extract	20	8	9	10	9	45%
20% <i>T.undulata</i> extract	20	12	14	13	13	65%
30% <i>T.undulata</i> extract	20	15	17	15	16	80%
Reference compound (Ivermectin) 10mg/ml	20	17	18	16	17	85%
Control(Pure Methanol)	20	01	02	03	02	10%

DISCUSSION

Due to resistant strains of *Sarcoptes scabiei* mite and adverse effects of some medicines upon the curing persons or animals, have made scabies a challenging disease to be diagnosed and properly cured (Anderson, 1982). Though the modern miticides are effective upon the scabies, but most of them possess adverse effects on people and animals receiving treatment as has been found for ivermectin used for the treatment of Norwegian scabies, showed resistance to the disease caused by the mite (Currie *et al.*, 2004) and toxicity (O, Brien, 1999) environmental contamination and environmental persistence (Halley *et al.*, 1993; O, Brien, 1999). Treatment generally entails the application of topical creams for classical scabies, while oral Ivermectin is recommended for crusted scabies (Mounsey *et al.*, 2008-a).

The topical use of *Tecomella undulata* extract showed a moderate miticidal activity against the *Sarcoptes scabiei* mites on people and animals while *in*

vitro study against *Sarcoptes scabiei* showed high miticidal response. This indicates that *Tecomella undulata* possess effective bio-miticidal compounds against the *Sarcoptes scabiei* mite and may be used to cure humans and animals suffering from scabies infestation. In the present study, ivermectin showed 85% and 30% *Tecomella undulata* showed 80% miticidal activity *in vitro* and 71% protection by Ivermectin injection and 58% by 20% *Tecomella undulata* extract through topical use against *Sarcoptes scabiei* mite. Our results are in accordance with the findings of other ectoparasiticidal medicinal plants (Maqbool *et al.*, 1992). The miticidal effect of *Tecomella undulata* extract against the *Sarcoptes scabiei* mite may be due to the presence of compounds such as Lapachol, flavonoids and other compounds present in this particular plant as has previously been reported by Neamat *et al.*, (2010), Sacaua *et al.*, (2003). This efficacy of *Tecomella undulata* containing Lapachol (naphthaquinine) may be due to interference of naphthaquinine with the oxygen metabolism of the mite cells, blocking of cell respiration generation of free radicals and nitric oxide production

resulting in mite hypoxic and toxic death. The 10% mortality of the *Sarcoptes scabiei* mites in the control treatment (without the application of *Tecomella undulata* extract or ivermectin) might be occurred due to the experiment handling as the mite is very sensitive to manipulation process. Although the present study has shown that *Tecomella undulata* methanolic extract could be effective against the *Sarcoptes scabiei* mites induced disease (Scabies) and could be used to control this disease, however, further studies is needed to find out the active ingredient and its biochemical composition for standardization of its proper therapeutic use both in animal and human population.

Conclusions: It is concluded from the therapeutic and laboratory trials that methanolic extract of *Tecomella undulata* is as useful as other synthetic therapeutic agents like lindane, permethrin and ivermectin available in the market. It is an effective bio-medicine for the treatment of scabies in humans and animals, however further research studies may be needed to further explore the medicinal efficacy of the plant against scabies in more detail.

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