Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(6): 1618-1622

Received: 15-08-2020
Accepted: 07-10-2020

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Antifungal activity of selected medicinal herbs against canine dermatophytosis

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DOI: https://doi.org/10.22271/j.ento.2020.v8.i6v.8051

Abstract
The current study was conducted to evaluate the clinical efficacy of the polyherbal combination containing *Acalypha indica*, *Curcuma longa* and common salt for the treatment canine dermatophytosis. Qualitative phytochemical screening was carried out to assess the bioactive components present in extracts of *Acalypha indica* and *Curcuma longa.* Three different polyherbal combinations were prepared in different ratios and evaluated for its efficacy. The clinical evaluation was carried out in dogs with dermatophytosis brought to Veterinary Clinical Complex, Veterinary College and Research Institute, Orathanadu. The phytochemical screening of the selected herbs revealed the presence of phytoconstituents viz. alkaloids, flavonoids, glycosides, phenols, tannins, terpenoids, and carbohydrate compounds. The clinical recovery in fungal dermatophytosis in pet animals with an application of polyherbal combination in the ratio of 7:2:1 comprising *Acalypha indica*, *Curcuma longa* and saturated salt solution for four times a day for 10 days indicated that topical therapy not only eliminates fungal infection but also control the skin problem without any side effects. Also, the polyherbal antifungal combination will be an efficient and economically affordable formulation in the field to treat dermatophyte infection in pet animals.

Keywords: Dermatophytosis, Herbal drugs, *Acalypha indica*, *Curcuma longa*, common salt

Introduction
Dermatophytosis in companion animals is a skin disease caused by a superficial fungal infection of keratinized skin structures by zoophilic, geophilic or anthropophilic fungal organisms, most commonly *Microsporum canis, M. gypseum and Trichophyton mentagrophytes* [17, 22]. These fungi digest the keratin protein complex in the skin by keratinases and others enzymes that allows the dermatophyte to burrow deeper into the stratum corneum in the host and elicit an inflammatory reaction [14, 15]. Because of the pleomorphic presentation of clinical signs, its infectious and contagious nature, and zoonotic potential, dermatophytosis is an important disease in small animal medicine. Treatment is recommended with the goal of shortening the course of the disease to prevent spread to other animals and human being [22].

The incidence of dermatophytes in animals especially in dogs has been high and serious problem today. This is because the antifungal drug resistance of the pathogen and the side effect exhibited by the drugs used to cure fungal diseases. Hence there is great demand for an efficient, alternative and safer treatment. Recently, there has been increasing interest in the use of various herbal materials as an alternative medicine to treat some of the fungal infections and many compounds of natural products have been specifically targeted against pathogenic fungi. Medicinal herbs could be an alternate source for the treatment of canine dermatophytosis. Herbal medicines are safe and generally free from side effects. Another important reason for this revival is that the effectiveness of many traditional medicines is now an accepted fact.

With the above background, the current study has been taken to evaluate the antifungal action of certain herbal plants against canine dermatophytosis. Based on the literature survey and traditional use,the following herbs having the antifungal action had been selected, viz. *Acalypha indica* [1, 7, 26], *Curcuma longa* [4, 24, 36], sodium chloride (common salt) [10] and evaluated for the antidermatophytic activity.

Materials and Methods
Selection of Materials
Medicinal plants with antifungal effects like *Acalypha indica, Curcuma longa* and common salt were selected for this study.

Collection of plant materials

Fresh leaves of *Acalypha indica* and rhizome of *Curcuma longa* were collected from Herbal garden at Veterinary College and Research Institute, Orathanadu, Tamilnadu, India. The taxonomic identities of plants were confirmed by Botanical Survey of India, Coimbatore, Tamilnadu, India. The collected plants were washed with running tap water, air dried, homogenized to a fine powder and stored in air-tight container.

Preparation of crude extracts for phytochemical analysis

About 30 g of dried powder material of *Acalypha indica* and *Curcuma longa* were extracted with 100 ml of distilled water and methanol respectively using soxhlet apparatus. The extract was then concentrated using Rotary Evaporator (Buchi Rotavapor R-300) and stored at 4°C until use.

Estimation of yield percentage

The extraction percentage of extracts of *Acalypha indica* and *Curcuma longa* was calculated by using the following formula:

$$\text{Percentage of extraction} = \frac{\text{Weight of the extract (g)}}{\text{Weight of the plant material (g)}} \times 100$$

Phytochemical screening assay

Qualitative phytochemical screening was carried out to assess the bioactive components present in extracts of *Acalypha indica* and *Curcuma longa* as described by Trease and Evans (1989) [35].

Preparation of polyherbal combination

The plant leaves were washed twice thoroughly with sterile distilled water in order to remove traces of dust and soil particles and the fresh leaves of about 75 g of *Acalypha indica* were taken and ground thoroughly using mixer grinder by adding 100 ml of water (75%) and the ground leaves mixture was filtered using Whatman filter paper No 1 and the filtrate was collected. The turmeric powder was prepared by grinding the dry turmeric rhizome using mixer grinder. Fifty percent turmeric extract was prepared by adding 50 gram of turmeric powder to 100 ml distilled water and keeping it in a magnetic stirrer rotating at 400 rpm at 50 degree celsius. Saturated salt solution (SSS) was prepared by adding enough quantity of salt to distilled water until it gets precipitated.

The different combinations of crude extracts of selected plants and saturated salt solution (SSS) were tried to find out the most effective combination against canine dermatophytes. The crude extracts of plants were taken in different ratio randomly and the clinical trials were carried out for all the combinations and it is presented in the Table 1. The most efficient combination was then determined by comparing the results of clinical trials. The combination was mixed thoroughly and ready for use in animals.

Table 1: Different ratio of polyherbal combination

Combination	*Acalypha indica*	*Curcuma longa*	SSS
I	5	4	1
II	6	3	1
III	7	2	1

Experimental design

The study was carried out in dogs with dermatophytosis brought to Veterinary Clinical Complex, VCRI, Orathanadu. Dogs with clinical signs of pruritus with regular and circular alopecia, scales and crust with erythematous margin, hyperpigmentation and a thin desquamation were selected. The Wood's lamp test was performed on dogs with the clinical signs of dermatophytes to establish the tentative diagnosis of dermatophytosis in dogs. The animals with the clinical signs of dermatophytosis that were brought to Veterinary Clinical Complex, VCRI, Orathanadu were divided into four treatment groups with six animals in each group and it is presented in the Table 2. Group I was treated with topical Ketaconazole which serves as the positive control. Group II, III and IV were treated with polyherbal combination I, II and III respectively.

Table 2: Experimental design

Group	Number of animals	Treatment	Route of administration
Group I	6	Treated with topical Ketaconazole	Topical application, Four times daily on affected area for 10 days
Group II	6	Combination I	Topical application, Four times daily on affected area for 10 days
Group III	6	Combination II	Topical application, Four times daily on affected area for 10 days
Group IV	6	Combination III	Topical application, Four times daily on affected area for 10 days

The clinical efficacy was assessed by topically applying the polyherbal combination on the dogs with clinical signs of dermatophytosis. A score card was prepared with selected clinical signs viz. alopecia, scales and crust, erythema, hyperpigmentation, thin desquamation for assessing the clinical efficacy of polyherbal combination.

Results

Plant extraction yield percentage

The ethnobotanical data of the selected plants and their extract percentage yield are given in Table 3. The percentage yield of plant extract obtained from *Acalypha indica* and *Curcuma longa* were (28.09%±0.19) and (14.51%±0.23) respectively.

Table 3: Ethnobotanical data of selected plants and their extract yield percentage

Plant	Family	Local name	Plant part used	Extract yield (%) (±SD)
Acalypha indica	Euphorbiaceae	Kuppaimeni	Leaves	28.09% ±0.19
Curcuma longa	Zingiberaceae	Turmeric, Manjal	Rhizome	14.51%±0.23

Qualitative phytochemical screening

The result of phytochemical screening of extracts of *Acalypha indica* and *Curcuma longa* are presented in Table 4. The preliminary screening for the phytochemicals showed the presence of phytoconstituents viz. alkaloids, flavonoids, glycosides, phenols, tannins, terpenoids, and carbohydrate compounds.

Table 4: Qualitative phytochemical screening of extracts

S. No	Phytochemicals	Extract of *Curcuma longa*	Extract of *Acalypha indica*	Polyherbal combination
1	Alkaloids	Positive	Positive	Positive
2	Glycosides	Positive	Positive	Positive
4	Phenols	Positive	Negative	Positive
5	Tannins	Negative	Positive	Positive
6	Phlobatannins	Negative	Negative	Negative
8	Flavonoids	Positive	Positive	Positive
9	Terpenoids	Positive	Positive	Positive
10	Saponins	Negative	Negative	Negative
11	Carbohydrates	Positive	Positive	Positive
12	Steroids	Positive	Positive	Positive

Assessment of efficacy of polyherbal formulation

Clinical evaluation was done in the dogs having dermatophytosis that are brought to the Veterinary Clinical Complex, Orathanadu. The combination was used four times daily for 10 days. The dogs recovered from the clinical lesions in 10-15 days. The efficacy of the polyherbal combination was assessed with the score card given in Table 5.

Table 5: Score card analysis for clinical evaluation (after 15 days)

Clinical signs	Before treatment (0 day)	Positive control	Combination I	Combination II	Combination III
Alopecia	Present	Absent	Present	Absent	Absent
Scales and crust	Present	Absent	Present	Absent	Absent
Erythema	Present	Absent	Absent	Absent	Absent
Hyperpigmentation	Present	Absent	Present	Present	Absent
Thin desquamation	Present	Absent	Present	Absent	Absent
Woods lamp examination	Positive	Negative	Positive	Negative	Negative

Out of the three combinations prepared combination III comprising *Acalypha indica*, *Curcuma longa* and SSS with the ratio of 7:2:1 was found to be effective against canine dermatophytosis when compared to the other two combinations. In alopecic areas growths of new hairs with visible improvement in the clinical signs were noticed and Woods lamp examination also found to be negative. The results were comparable with that of the positive control (Group I).

Discussion

Dermatophytosis is a specific fungal disease of the epidermal tissues in the skin, caused by keratinophilic and keratinolytic genera such as *Microsporum*, *Trichophyton* and *Epidermophyton*. It is an endemic infection in many countries throughout the world affecting companion animals (dogs, cats), domestic animals (calves), and laboratory animals (rabbits) as well as humans [23, 30]. The most frequently found dermatophytes infestation in dogs are *Microsporum spp*. and *Trichophyton spp*. Some dermatophytes are zoonotically important which infect primarily the animals and are transmitted from infected animals to human beings on many occasions [25]. In dogs, nearly 70% of cases are caused by *Microsporum canis*, 20% by *M. gypseum*, and 10% by *Trichophyton mentagrophytes* [11]. Dermatophytes are significant due to their zoonotic potential and the concern of owner of pets with some time severe inflammatory diseases. The antifungals commonly used in systemic treatment of dermatophytosis in dogs and cats include itraconazole, terbinafine and griseofulvin [19]. The quick development of resistance and rapid global spread of resistant fungi demands to find new antifungal agents. Currently emergence of antifungal resistant clinical isolates leads to failure in the treatment of mycosis [2]. Keeping this in view, the current study had been carried out to identify the therapeutic potential of certain herbal plants for their antifungal activity particularly against dermatophytes.

Acalypha indica commonly known as kuppaimeni in Tamil and *Curcuma longa* (turmeric*)* were identified to have antifungal property which is in accordance several researchers [1, 7, 26].

Acalypha indica belongs to the family Euphorbiaceae, is used to treat superficial fungal infections [1]. The plants belonging to Euphorbiaceae family contains high concentration of flavonoids, phenols and alkaloids. The presence of these phytochemicals may be responsible for the potent antifungal activity [26]. Oksana *et al*. [27] reported that flavonoids (quercetin, kaempferol, isorhamnetin, isoquercitrin), phenolic derivatives (gallicin, gallic, syringic, and caffeic acids), and coumarin (scopoletin) containing plants have potent anti-fungal activity against *Microsporum* spp. and *Trichophyton* spp. Dinesh kumar *et al.* [16] reported that leaves and flowers of *Acalypha indica* contain flavonoids kaempherol, glycosides, clitorin, mauritianin and nictiflorin.

Acalypha indica leaf juice is added to oil or lime to treat a variety of skin disorders and other ailments [33]. Azmahani *et al.* [7] reported the leaves and roots of the aqueous extract of *Acalypha indica* inhibit the growth of *Microsporum canis*.

Curcuma longa belongs to Zingiberaceae family, possesses powerful antifungal activity, as demonstrated in many studies [4, 24, 36].

Curcumin, demethoxy curcumin and bis-demetho-hydroxycurcumin, are three pharmacologically important Curcuminoids that have been isolated from *Curcuma longa* [20]. They have been shown to possess anti-oxidant, anti-inflammatory, anti-carcinogenic, anti-mutagenic, anti-fungal, anti-viral and anti-cancer properties [12]. The therapeutic properties of *C. longa* include insecticidal [3], antimicrobial [18], antifungal [21], antimalarial, antiviral and antioxidant properties. Hu *et al*. [21] reported that the inhibition behaviour of *C. longa* on fungal growth (*Aspergillus flavus*) is involved in its ability to disrupt the integrity of plasma membrane and mitochondrial dysfunction, inducing metabolic stagnation. Chen *et al*. [13] reported that the ethanol extract of *C. longa* can disrupt the synthesis of critical proteins and enzymes, which may ultimately inhibit the growth of fungi (*Fusarium*

graminareum). The antifungal effects were found to be related to the disruption of fungal cell membrane systems, specifically the inhibition of ergosterol synthesis and the respiratory chain.

Sodium chloride salt is a known antifungal agent that acts by altering osmotic gradients, forcing organisms to expend energy in osmoregulation, diverting it away from growth at concentrations beyond tolerance limits, the osmoregulatory processes are overloaded and death occurs [10].

In the present study, the polyherbal combination containing *Acalypha indica*, *Curcuma longa* and sodium chloride is found to be clinically effective against dermatophytes in pet animals. The results of the current study are in accordance with the reports of the previous studies of antidermatophytic activity of the herbal plants selected. Leaves and roots of the aqueous extract of *Acalypha indica* inhibit the growth of *Microsporum canis* [7]. Somchit *et al.* [32] reported that the non-polar extract of *Acalypha indica* showed antifungal action and at 30 mg/mL chloroform extract, the activity was statistically similar to the antifungal drug ketoconazole. Sudhakar Chekuri *et al.* [33] reported that, methanol, ethanol and acetone shade dried extract of *Acalypha indica* leaf extract showed wide range of antibacterial and antifungal activity and can be used and administered in medical practice. Herbal drug preparation containing rhizome powder cured ringworm infection caused by *Trychophyton verrucosum* in 12 cattles and *Microsporum canis* in 21 dogs within12-15 days of treatment [31]. The crude methanol extract of *C. longa* has inhibitory effect against some clinical isolates of dermatophytes. It was demonstrated that 18-month-old and freshly distilled oil isolated from rhizome of *C. longa* showed the most potent antifungal effect against 29 clinical isolates of dermatophytes with MIC values of 7.2 and 7.8mg/mL, respectively. *Trichophyton rubrum*, *T. mentagrophytes*, *Epidermophyton floccosum*, and *Microsporum gypseum* were suppressed by 1: 40–1: 320 dilutions of turmeric oil. An *in vivo* study on infected guinea pigs with *T. rubrum* demonstrated that dermal application of turmeric oil (dilution 1: 80) induced an improvement in healing of the lesions after 2–5 days and it caused the lesions after 6-7 days of consumption to vanish [6]. Oil from *C. Longa* proved to be more effective against dermatophytes than curcumin. Vaijayanthimala *et al*. [36] demonstrated that *C.longa* rhizome ethanolic extract showed more activity against *T. rubrum* (0.57 mg/ml) than *T. mentagrophytes* (1.15 mg/ml).

In the current study, the phytochemical analysis of the aqueous extract of *Acalypha indica* leaves showed the presence of alkaloids, glycosides, tannins, terpenoids, flavonoids and carbohydrates. The results are in accordance with the phytochemical analysis done earlier in several studies in which the qualitative phytochemical screening indicated the presence of alkaloids, phenols, saponins, steroids, flavonoids and catechol [9, 29]. Takle *et al*. [34] also stated that qualitative chemical examinations of various extracts and fractions of *Acalypha indica* revealed the presence of alkaloids, steroids, flavonoids, glycosides, tannins and carbohydrates. The preliminary phytochemical analysis of the methanolic extract of turmeric also showed the presence of tannins, alkaloids, saponins, flavonoids, terpenoids and cardiac glycosides which is in accordance with the study of Rajesh *et al*. [28]

Somchit *et al.* [32] reported that the non-polar extract of *Acalypha indica* leaves especially at 30 mg/mL chloroform extract, has showed antifungal activity which was as potent as to the conventional antifungal drug ketaconazole and fluconazole. Hence, they can be used in treatment of fungal diseases caused by *Microsporum canis* and some *Candida sp* which is in correlation with the current study. Bhadauria and Kumar [8] studied that the bound flavonoid extracts as well as free flavonoid extracts present in certain herbal plants showed prominent antidermatophytic activity against all the dermatophytes. The phytochemical studies carried out in the present study indicate the presence of flavonoids in both *Acalypha indica* and *Curcuma longa* as well as in the polyherbal formulation. The presence of flavonoids in the selected herbs and in polyherbal antifungal combination could be attributed for the antidermatophytic action.

The clinical recovery in fungal dermatophytosis in pet animals with an application of polyherbal combination for four times a day for 10 days indicated that topical therapy not only eliminates fungal infection but also control the skin problem without any side effects. Also, the polyherbal antifungal combination will be an efficient and economically affordable formulation in the field to treat dermatophyte infection in pet animals.

Conclusion

In conclusion, it is suggested that the application of polyherbal combination containing *Acalypha indica*, *Curcuma longa*, and sodium chloride in the ratio of 7:2:1 for 10 days has the potential to reduce the fungal infection in pet animals. Further studies are warranted to explore the possible mechanisms of antifungal action behind the selected herbs.

Acknowledgments

The authors are thankful to Tamil Nadu Veterinary and Animal Sciences University (TANUVAS), Chennai and TANII Scheme, Tamil Nadu Government.

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