Ringworm in calves: Risk factors, improved molecular diagnosis, and efficacy of Aloe vera gel extract for treatment

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Abstract

Background: Calves dermatophytosis is a major public and veterinary health problem worldwide due to its zoonotic potential and economic losses in cattle farms. However, it has lacked adequate attention; thereby for effective control measures it is worth determining ringworm prevalence, risk factors and direct sample nested-PCR diagnostic indices as compared to conventional methods for dermatophytes identification. Moreover, Aloe vera gel extract (AGE) phenolic composition and its in-vitro and in-vivo anti-dermatophytic activity in comparison to antifungal drugs were evaluated.

Results: Of 760 examined calves, 55.79 % showed ringworm lesions. 84.91% were positive for fungal elements in direct microscopy, and 79.72% were culture positive. Trichophyton verrucosum was the most frequently identified dermatophytes (90.24%). Risk of dermatophytosis is high in 4-6 month than 1-month aged calves (60% versus 41%), in summer and winter compared to spring and autumn seasons (66% and 54% versus 48%). Poor hygienic conditions, intensive breeding system, animals raised for meat production, parasitic infestation, crossbreed, and newly purchased animals were statistically significant risk factors correlated with dermatophytosis. One-step PCR targeting conserved regions in the 18S and 28S genes achieved unequivocal identification of T. verrucosum and T. mentagrophytes in hair samples. Nested-PCR achieved an excellent performance in all tested diagnostic indices and increased the species-specific detection of dermatophytes by 20 % as compared to culture. Terbinaine and miconazole were the most active antifungal agents for dermatophytes. Gallic acid, caffeic acid, chlorogenic acid, cinnamic acid, aloe-Emodin, quercetin, and rutin are the major phenolic compounds of AGE identified by High-performance liquid chromatography (HPLC). These compounds increased and synergized the anti-dermatophytic activity of AGE. The treated groups showed significantly lower clinical scores than the control group ( P < 0.05). The calves were successfully treated with topical AGE (500 ppm) resulting in clinical and mycological cure within 14-28 day of the experiment.

Conclusions: Implementation of nested-PCR assay providing a rapid diagnostic tool for dermatophytosis augments and complement the conventional methods for initiating targeted treatments of calves ringworm. The recognized anti-dermatophytic potential of AGE is advantageous countenance to commercial drugs to go used in therapeutics.

Background

Fungal infections associated with zoonotic transmission are an important public health problem worldwide [1]. Cattle dermatophytosis is a major public and veterinary health concern not only for its highly zoonotic impact but also economic losses in cattle farms due to hides damage, loss of weight, decimated meat and milk, contagiousness among animals, treatment cost and difficulty of control measures[2, 3]. Ringworm is usually enzootic in cattle herds and is more prevalent in calves [2]. This may be due to the stressors of rapid growth, weaning, or parasite burdens that weaken their immunity, healthiness of the skin, close confinement, dietary factors deficiencies and production system [4]. Importantly, *Trichophyton verrucosum* is the predominant zoophilic dermatophyte species causing
dermatophytosis in cattle and can occasionally spread to humans through direct contact with cattle or infected fomites causing highly inflammatory skin and hair dermatophytoses [4–6]. As a consequence, a precise laboratory diagnosis for identification of dermatophytes species is pivotal for prevention and effective control of dermatophytoses [2]. Considering the previously mentioned contemplations, scant researches have been found so far studying the prevalence, risk factors and treatment of calves’ ringworm in Egypt.

Furthermore, there are scarcity publications on the direct molecular diagnostic assays for detection and identification of dermatophytes from animals clinical samples[7, 8] to surpass the time consuming conventional microscopy and fungal culture that require weeks for a positive identification[8]. The nested polymerase chain reaction (PCR) technique proved to be an effective practical approach for dermatophytosis diagnostic, helping clinicians in initiating rapid and targeted as opposed to empirical treatments of animal ringworm [7].

Dermatophytosis in animals remains difficult to eradicate because of the antifungal resistance, the scarcity of accessible and authorized antifungal agents for use in veterinary practice, the restricted systemic treatment in livestock due to the hepatotoxicity, drug residues in products consumed by human [2, 9]. Thus, the discovery of natural, less toxic and more specific therapeutic alternatives is gaining ground. Although the antidermatophytic potentials of natural products promising, are plagued by a lack of in-vivo studies to affirm the antifungal activity of bioactive compounds announced in-vitro [9]. Aloe vera is a plant of Liliaceae family has multiple applications including antifungal, antibacterial, antioxidant, antiseptic and in cosmetics industries [10]. Investigations for in-vitro and in-vivo anti-dermatophytic potential of Aloe Vera and determination of its bioactive compounds are still meager.

Hence, this work was designed for investigation of a) the prevalence and risk factors of calves ringworm in Egypt, (b) diagnostic indices of the direct nested PCR for detection and identification of dermatophyte species from hair and scale samples in comparison with the conventional microscopic and culture methods, (c) Aloe Vera gel extract (AGE) biological activity and phenolic composition, (d) the antifungal activity of AGE in comparison to the antifungal drugs and (e) its application for treatment of calves ringworm.

Results

Prevalence of dermatophytosis among clinically examined calves

On clinical examination, 55.79 % of calves (424/760) showed grayish-white, crusty, circular circumscribed discrete lesions (Figure 1A) whereas; alopecic, erythematous areas left after removal of raised greasy crusts were occasionally observed (Figure 1B). The skin lesions were mostly found on the head and neck (46.69%) and all over the body (44.81%). Some cases (8.49%) had lesions on the head, neck and trunk as well. The degree of infection diversified from moderate (55.18 %) to severe (44.81%).
Direct microscopy of skin scraping and hair samples revealed ectothrix arthroconidia in 84.91 % (360/424) of samples whereas, 79.72% (338/424) were positive by mycological culture. Fungal culture resulted in *T. verrucosum* (90.24%) and *T. mentagrophytes* (9.76%) from the infected animals.

### Potential risk factors for calves’ ringworm

There is highly significant association between ringworm infection and the age of calves ($\chi^2 = 19.7$, $P < 0.001^{**}$). Risk of infection in 4–6 month aged animals is higher than younger calves (60% versus 41%). Risk ratio and odds of disease in 1-month aged animals were 68% and 45% lower than 4–6 month animals, respectively.

Seasonal variation was significantly highly associated with skin lesion in the examined animals ($\chi^2 = 19.58$, $P < 0.01^{**}$). The high risk of calves’ dermatophytosis was in summer and winter as compared to spring and autumn seasons (66% and 54% versus 48%). Also, there is highly significant association between calves ringworm and each of breed type, production system, breeding system, origin of the animals at the farm, ventilation, and pattern of disinfectant used ($\chi^2 = 63.18$, $P < 0.001^{**}$). Risk of infection in cross breed, animals for meat production, parasitic infestation, intensive breeding system, newly purchased animals introduced to the farm, bad ventilation, and irregular use of disinfectant are higher as compared to others, respectively (85 % versus 49%).

The area available per calf highly affects the infection potential. The narrower the area the more skin lesion spread among animals ($\chi^2 = 7.79$, $P < 0.01^{**}$). The relationship is weak (Phi & Cramer’s V = 0.16, $P < 0.001^{**}$). Risk of infection in animals reared in 1.5mr/animal is higher than animals reared in 6mr/animal (62% versus 52%). Ringworm lesion in animals reared in 1.5mr/animal is 1.19 times more likely higher than animals reared in 6mr/animal (Table 1). The random forest classification model confirmed the same observation, where age of calf was the most important risk factor, followed by production system, parasitic infestation, and irregular use of disinfectant was the fourth most risk factor (Figure 2).

### Nested PCR for detection and identification of dermatophytes in clinical samples

Pan-dermatophytes, one-step and nested PCR were evaluated for dermatophytes identification in 75 direct microscopy and culture-positive samples, 36 microscopy +/culture -, 9 microscopy -/culture + and 30 negative samples. The Pan-dermatophytes PCR had the ability to specifically detect dermatophytes DNA in 58%, one-step PCR in 62% and nested PCR in 72 % of 150 samples (Table 1) with 440 bp pchs-1 amplicons, ~ 900 bp ITS+ and 400 bp ITS−1 amplicons, respectively (Figure 3).

Nested PCR increases the dermatophytes species-specific detection by 20 and 10 % as compared to culture alone or the combination of culture and direct microscopy, respectively.
Fungal culture identified dermatophytes in 56% (84/150), whereas direct microscopy identified 74% (111/150) of samples. Out of 66 samples that were negative for dermatophytes by culture, non dermatophyte-moulds were cultured from 21 samples that were test-positive only by one-step PCR. In addition, non dermatophyte-moulds were co-cultured with dermatophytes from six samples that were negative by the pan-dermatophytes, one-step and nested PCRs (Additional table 1).

As depicted in table 2 the performance of nested PCR assay was excellent in all tested diagnostic indices. Based on fungal culture as a reference standard, a sensitivity of 82.14 %, 71.43 % and a specificity of 72.73 %, 50 % were recorded for pan-dermatophytes and one-step PCRs, respectively. Meanwhile, the respective values were 92.86 and 54.55 % for nested PCR. Whereas using the combination of culture and nested PCR as a gold standard, nested PCR was superior achieving a sensitivity value of 94.74 %, whilst culture and direct microscopy demonstrated 73.68% and 78.95%, respectively. Specificity and PPVs were 100% for nested PCR and dermatophytes culture so they were considered as the gold standard, whilst the competent values were 41.67 and 81.08 % for direct microscopy. Nested PCR was very accurate (AUC = 96%), whereas pan-dermatophytes PCR (82%) and culture (80%) were moderately accurate. Less diagnostic accuracy was recorded for direct microscopy and one-step PCR (50 % AUC ≤ 70 %). DOR of nested PCR is much higher than any other test this means that the diagnostic performance of nested PCR is the best and showed strong agreement with culture and nested PCR results (Kappa value = 0. 91and P < 0.001).

Confirmatory ITS sequencing was performed and the BLAST search results for the sequence (GenBank accession no. MK918485) corresponded to various T. verrucosum isolates, with the highest-ranked genetic identity to T. verrucosum isolated from inflammatory tinea capitis (KY623478 (100% identity) and tinea corporis in a 3-years old child in China (KY623476 (99.89% identity). Identity percent for the sequence (MK918486) was 100% with T. mentagrophytes (MK447604 and MK447606) isolated from tinea capitis and tinea faciei in 8 and 44-years old Indian boy and man, respectively.

### Susceptibility of dermatophytes to antifungal drugs

The mean MICs and MFCs values of the five antifungal drugs for T. verrucosum and T. mentagrophytes are presented in additional table 2. When the values of the five antifungals for the two species tested were compared, those for terbinaine were the lowest followed by miconazole (MIC range 0.03–0.5, 0.03–1 µg/mL; MFC range, 0.06–1, 0.06–2µg/mL, respectively). Mean MIC values ± SD of the tested antifungal agents did not differ for T. verrucosum and T. mentagrophytes (P< 0.05). Fluconazole was the least effective drug with overall MIC range 8–64 µg/ml.

### Yield, TPC, TF, Phenolic compounds, antioxidant and antifungal activity of AGE
As depicted in Table (3) the extract yield was 1.02 g extract 100 g⁻¹ Gel. The amount of total phenolics as mg GAE g⁻¹ AGE was (111.78 mg GAE g⁻¹). The flavonoid contents of extract as mg quercetin equivalent/g AGE was (45.6 mg QE g⁻¹). Flavonoids have an expansive range of chemical and biological activities, including radical-scavenging properties. For this reason, extract was analyzed for total phenolic and flavonoid contents. The major phenolic compounds of AGE were identified by HPLC as presented in Table 3. These components were gallic acid, caffeic acid, chlorogenic acid, cinnamic acid, aloe-Emodin, quercetin, and rutin. All these compounds increased and synergized the antidermatophyte activity of AGE.

The results of DPPH radical-antioxidant activities of AGE were demonstrated in (Figure 4a) with antioxidant activity 85.3 % for AGE and 92.2% for gallic acid and TBHQ (88.6%) after 2 h. of reaction. The obtained results clearly demonstrate that this extract displayed antioxidant activity. As revealed in Figure 4a AGE inhibited the bleaching of β-carotene by scavenging linoleate-derived free radicals. The decreasing efficacy was ordered as TBHQ > AGE > gallic acid. The results showed a comparable scavenging ability 72.3 % for AGE to the synthetic antioxidants gallic acid 65.7 % and TBHQ 81.22%. AGE displayed a ferric reducing power compared with TBHQ and gallic acid (Figure 4b). The ferric reducing power of AGE was 1.96 compared with gallic acid (2.23) and TBHQ (2.57). AGE showed an inhibitory effect for T. verrucosum and T. mentagrophytes at MIC values ranged from 300–400 ppm and 400–500 ppm, respectively.

**Effectiveness of AGE in the eradication of T. verrucosum from calves**

In the treated calves, gradual improvement of the lesions was seen within 7 to 12 days post-treatment. Complete clinical recovery (full hair growth) was observed within 14 –19 days of the study for calves in G2 & G4 and within 21–28 day for animals in G1 and G3 (Additional figure 1). Meanwhile, the lesions on the control animals (G5) progressed and did not heal till 42 day of the study.

Direct microscopic examination and fungal cultures were negative within the 4th week of starting treatment. In contrast, samples from the untreated control calves repeatedly demonstrated positive mycological results during the investigation period.

As revealed in Figure (5), clinical scores of the five animal groups did not differ significantly on days 0 and 7, while the treated groups showed significantly lower clinical scores than control untreated group (G5) on days 14, 21, 28, and 42 ($P < 0.05$).

At day 14, significant improvement in the clinical scores ($P < 0.05$) was detected between miconazole treatment (G2) and untreated control group (G5), G2 and terbinafine treatment (G1) and G2 and AGE (G3). Moreover, significant changes were detected between terbinafine treatment (G1) and the untreated group (G5) and AGE (G3) and G5 (Figure 5).
Neither recurrence, nor grossly side effects were observed throughout the study period and during the clinical follow-up after treatment.

**Discussion**

An enzootic circumstance of animals' dermatophytosis is the outcome of the confinement of animals in breeding and the viability of the arthrospores in the environment for many months[2]. Prevention is difficult, but periodic surveys on the prevalence and risk factors of cattle ringworm may permit the adoption of increasingly effective prophylaxis and control measures to prevent the infection both to other animals and humans [2, 11, 12]. In the current study, the prevalence rate of ringworm in calves of 1 to 6 months was 55.79 % that is nearly similar to the 57.5% found in Iran [5]. Furthermore, the infection rate is higher than the 1.6% found in Chitral district of Pakistan[12] but lower than the 87.7% in Tuscany region [4] and 71.7% in nearby Umbria, Central Italy[11]. This discrepancy among different countries perhaps due to cattle breed, production, breeding system, origin of the cattle in the farm and climatic conditions[11]. In accordance with other studies[4, 11], random forest classification and box plot model indicated that age is the most important risk factors whereas, the risk of infection in 4–6 month aged calves is higher than the younger suckling calves (60% versus 41%) and this could be attributed to the stressors of weaning and rapid growth. Furthermore, there is a highly significant correlation of several risk factors found in the examined calves population and ringworm infection, mainly season, bad ventilation, overcrowding and irregular use of disinfectants. This participate in reinforcing the broadly accepted concept that high humidity, close contacts between calves, poor hygienic conditions of the stable play a significant role in the ringworm prevalence[4, 5, 11, 12]. Hence, repeated topical treatment of all infected animals, together with good ventilation, thorough disinfection of the stable, halters, fences, cleaning tools and all the materials in contact with the animals is the basis for cattle ringworm effective control[11, 13]. Of interest, there is highly significant ($P < 0.001$) association between the risk of dermatophytosis and the newly introduced animals to the farm. In support of this finding,[4] debate that the newly introduced calves in the herd spread the infection to both calves and humans as they are carriers of dermatophytes before the development of clinical signs.

As described in the literature [2, 5, 11, 14], the detected clinical signs of cattle dermatophytosis were crusty lesions on the head, neck regions and other parts of the body. Though, other study conducted in Tanzania[15] occasionally reported the widespread lesion of alopecia and erythema that were observed herein as well. The detection rates were 84.91 % by direct microscopy and 79.72% by fungal culture. In such a context, inadequate scraping of the lesion and the slow and poor growth of *T. verrucosum* that hampered its detection probably the reasons for direct microscopy and culture false-negative results, respectively[4].

According to Nweze; Agnetti et al. [11, 14] *T. verrucosum* is the main dermatophytes causing cattle ringworm, although *T. mentagrophytes* that is usually associated with the presence of small rodents in the farm has been isolated. The present findings showed that calves ringworm caused by *T. verrucosum*
(90.24%) and *T. mentagrophytes* (9.76%). Nevertheless, Aghamirian and Ghiasian [5] exclusively isolated *T. verrucosum* from 352 infected cows in Iran.

To date, direct molecular assays are used for the detection of *T. verrucosum* in clinical samples and also as culture confirmation tests [16]. Wollina and coauthors have failed to cultivate *T. verrucosum* from clinical sample and paraffin-embedded skin tissue from a patient with severe tinea barbae. But, real-time PCR and subsequent sequencing of the ITS2 region of rRNA could successfully identify *T. verrucosum*. No previous studies attempted to identify dermatophyte species from hair samples of calves using direct nested PCR assay. The obtained findings revealed that one-step PCR could correctly identify *T. verrucosum* and *T. mentagrophytes* in samples, that were culture positive (n = 72/150), with an amplicon size of 900 bp and 872 bp, respectively. Meanwhile, nested-PCR amplified ITS+ of both species in 108 samples producing 400 bp ITS−1 amplicons. This coincides with another study [7] that the one step-PCR accurately identified *Microsporum canis* in hair samples from canine and felines at 922 bp, but ~ 851 to ~ 872 bp ITS+ amplicons were obtained for *T. mentagrophytes, T. terrestre* or *M. gypseum*, whereas nested PCR achieved unequivocal identification. The highly sensitive nested PCR also had high specificity and positive predictive value and detecting additional dermatophyte-positive samples that were missed by culture (n = 30/150) or both microscopy and culture (n = 15). Other studies highlighted the importance of direct sample PCR incorporation in the laboratory diagnosis of onychomycosis for increasing the detection of dermatophyte-positive samples that were negative by culture [17, 18]. Despite direct microscopy and /or culture positive (n = 27/150), or negative samples in both (n = 15), negative PCR results, except 21 samples that were positive by one-step PCR and with non-dermatophytes mould grew in culture, could be explained by PCR inhibition by components in samples from non-dermatophytes contaminant/colonizer or the existence of smaller amounts of dermatophytes DNA in samples [7, 17]. A possible reason for the low specificity and accuracy of one-step PCR than pan-dermatophytes and nested PCRs is employing the universal fungal regions of rDNA. Additionally, the low sensitivity of culture could be attributed to overgrowth of non-dermatophytes contaminating moulds in the culture or dermatophytes cultures were not yet positive after 4 weeks of incubation [4, 7]. Other reasons are the non-viable fungal material in specimens from treated calves or that the DNA extraction step ease overcoming the impediment of trapping fungus in the keratin [18].

As previously reported [19, 20], terbinafine and miconazole were effective antifungal drugs for dermatophytes followed by itraconazole and griseofulvin whereas; fluconazole was the least active antifungal agent. Recently, Pal [21] recommended the conduction of further research for the development of cheap, safe and potent chemotherapeutic agents for cattle dermatophytosis management. AGE is a cheap, easily obtainable and safe natural product. Besides an endless source of bioactive compounds that clearly recognized for having antifungal activities that correlated with the antioxidant activities [22]. So, in this study, antioxidant activities were measured by three methods (DPPH• free radical, β-carotene/linoleic emulsion bleaching, and FRAP). DPPH• is a model of a stable nonpolar radical. A chain reaction of radicals is initiated by lipid autoxidation. Antioxidants react with DPPH•, reducing the number of DPPH• free radicals to the number of their available hydroxyl groups [23]. The antioxidant activity of
AGE against DPPH was concentration-dependent. In β-carotene/linoleic emulsion bleaching assay, oxidation of linoleic acid results in free radicals derived from hydroxide that assault the chromophore of β-carotene, leading to bleaching the emulsion of the reaction. An extract can inhibit/retard the oxidation of β-carotene might be portrayed as a free radical scavenger and initial antioxidant[24]. Antioxidant compounds cause the reduction of ferric (Fe$^{3+}$) form to the ferrous (Fe$^{2+}$) form because of their reductive capabilities[25]. According to our results, ferric reducing power and TPC content are related to one another. Fe (III) reduction is commonly utilized as a marker of electron-donating activity, which is a significant mechanism of the phenolic antioxidant action[26]. Considering the results of all three assays, phenolic compounds can explain high antioxidant capacity[27]. The antioxidant activity of phenolic compounds is chiefly because of their redox properties, which can assume a significant role in the absorption and neutralization of free radicals, decomposing peroxides or triplet oxygen, quenching singlet and reductive heavy metals with two or more valence states [28]. The phenolic compounds are the active antimicrobial constituents of various plants extract. However, the whole extract has more noteworthy antifungal activity. Accordingly, AGE might be more advantageous than the isolated component, since a bioactive individual constituent can change its properties in the existence of other compounds[29]. The additive and synergistic impacts of phenolic compounds in fruits and vegetables are accountable of their efficient bioactive properties, which clarifies the purpose behind which no single antimicrobial can supplant the combination of these natural components to achieve the antifungal activity [30]. The recognized anti-dermatophytic activity of AGE was in consistent with previous report[31] that the water extract of Aloe vera was effective on T. mentagrophytes. However, no reports were found about AGE activity on T. verrucosum. Nonetheless, most investigations are performed on fungal isolates, thereby it hard to extrapolate the findings to real conditions. Therefore, more in-vivo studies are needed to ensure reliable results[9]. The efficacy of 2 weeks’ twice-daily topical application of AGE was compared with topical miconazole 2%, oral terbinafine with topical AGE application and once-daily oral terbinafine in proven T. verrucosum infected calves. There were significantly lower clinical scores in all treated groups after 14 day than untreated group ($P < 0.05$) but complete clinical recovery was achieved earlier in miconazole group and AGE with oral terbinafine than both oral terbinafine group and AGE alone. This confirms the high efficacy of AGE for the treatment of calves dermatophytosis and suggests that the combination of AGE with oral terbinafine was highly effective. The achieved results were comparable with the treatment findings of calves dermatophytosis with topical applications of propolis and whitfield’s ointment[32] and polyherbal lotion, along with levamisole and griseofulvin [33].

**Conclusion**

This study highlights the need for good hygienic conditions, regular disinfection of holdings, rapid treatment of infected calves and examination of the incoming calves so as to prevent dermatophytic epizoonoses in calves and human as well. Implementation of nested PCR assay providing a rapid diagnostic tool for dermatophytosis augments and complement the conventional methods for dermatophytes species-specific detection for initiating targeted treatment which would reduce the burden of economic losses due to the ringworm infection. The recognized anti-dermatophytic potential of AGE is
advantageous countenance to commercial drugs to go used in therapeutics. Further studies are recommended to use the AGE for large scale treatment of calves’ dermatophytosis.

Methods

Population and collection of clinical samples

From May 2015 to December 2018, a total of 760 Holstein cow calves (597 weaning and 163 suckling calves) raised in different farms in Egypt, were clinically examined for evidence of ringworm infection. Data about age, breed, farm production, breeding system, production management system, origin of calves of the farm were obtained for each calf. For examination of parasitic infestation, fecal samples were examined for enteric parasites and thin blood films were prepared, fixed in absolute methyl alcohol and stained with freshly filtered and diluted 10% Giemsa stain. After cleaning the skin lesion of suspected ringworm-affected calf with 70% ethanol, scales and dull hair samples from the margins were collected using a sterilized plastic hair brush and tweezers, respectively [4].

 Portions of hair and scales were examined microscopically after clearing with 20% potassium hydroxide (KOH), cultured on Mycobiotic agar (Remel™, Thermo Fisher Scientific) slants with 10% thiamine and inositol, incubated at 30 °C for 4–6 weeks and observed for growth at 3 days intervals. Dermatophyte isolates were identified according to their macro-and micromorphological characteristics [34].

Extraction of DNA from hair and scales samples and PCR amplification

The direct molecular identification of dermatophytes was executed in 150 representative clinical samples that were selected on the basis of direct microscopy and culture results. For high-throughput disruption of samples, 50 mg of hair and scales were placed in a 2 ml safe-lock tube and incubated overnight at 55°C with 360 µl of ATL buffer and 20 µl QIAGEN protease (QIAamp DNA Mini kit, Qiagen, Germany, GmbH). Subsequently, tungsten carbide beads were added and tubes were placed into the TissueLyser adapter set for disruption using the TissueLyser for 2 min at 20–30 Hz two times. Then, DNA extraction was performed utilizing QIAamp DNeasy Plant Mini kit (Qiagen, Germany, GmbH) following the manufacturer’s instructions. DNA was eluted with 50 µl of elution buffer and the concentration was assessed using NanoDrop ™2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

One-step PCR

According to Cafarchia et al. [7] the chitin synthase (chs-1) gene was amplified using DMTFchsF1 (5′-CGAGTACATGTGCTCGGCAC-3′) and DMTFchsR1 (5′-CGAGGTCAAARGCACGCCAGG-3′) primers to assess the presence of dermatophytes amplifiable DNA in the clinical samples. Then, further one-step
PCR was performed using the primers DMTF18SF1 (5'-CCAGGGAGGTTGGAAACGACCG-3') and DMTF28SR1 (5'-CTACAAATTACAACCTCGGACCC-3') amplifying a 900 bp of the conserved regions in the 18S and 28S genes that includes internal transcribed spacers regions of ribosomal DNA (ITS–1, 5.8S and ITS–2).

**Nested PCR**

A nested PCR was applied to amplify a 400 bp of a conserved region in the dermatophytes 5.8S gene from the ITS+ amplicons of the primary PCR using DMTF18SF1 and DMTFITS1R (5'-CCGGAACCAAGAGATCCGTGTTG-3') primers [7].

PCR was performed in an amplification reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer (20 pmol), 6 µl of DNA template in case of primary PCR or 1 µl of diluted product from the primary PCR (dilution 1:1 with molecular grade water) for nested PCR and nuclease-free water up to 25 µl. *T. verrucosum ATCC® 28203™* and an amplification reaction without DNA template were utilized as positive and negative control, respectively. The thermo-cycling conditions previously described[7] were employed in an applied biosystems 2720 thermal cycler (Thermo Fisher Scientific, USA).

The amplified products were electrophoresed on ethidium bromide-stained 1.5% agarose gel (Applichem, Germany, GmbH). A gelpilot 100 bp DNA Ladder (Qiagen, Gmbh, Germany) and 100 bp DNA ladder H3 RTU (Genedirex, Taiwan) were used to determine the amplicon sizes. The gel documentation system (Alpha Innotech, Biometra) was used to photograph the gel and the data analysis was done through the computer software.

**DNA sequencing and sequence analysis**

Two representative ITS+ PCR products were purified using QIAquick PCR Product extraction kit (Qiagen, Valencia) and then sequenced using Bigdye Terminator V3.1 cycle sequencing kit (Perkin-Elmer) in Applied Biosystems 3130 genetic analyzer (HITACHI, Japan). DNA sequences were compared with those available in NCBI databases (National Center for Biotechnology Information, [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) using Basic Local Alignment Search Tool (BLAST). MEGA5 program, product version 5.1 ([www.megasoftware.net](http://www.megasoftware.net)) was used for sequence analysis. The ITS sequences were available under GenBank accession no. MK918485 and MK918486.

**Antifungal susceptibility testing of dermatophytes isolates**
Broth micro-dilution method in accordance with the CLSI M38-A2 [35] was employed for testing the sensitivity of dermatophytes isolates to the most commonly used antifungal drugs. Fluconazole were obtained from Pfizer International, New York, NY, USA, itraconazole, and miconazole from Janssen Research Foundation, Beerse, Belgium. Griseofulvin was bought from Sigma Chemical Company, St. Louis, MO, USA, and terbinafine from Novartis, Basel, Switzerland. All drugs were dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) except fluconazole in RPMI1640 medium (Sigma Co. St. Louis, USA) buffered at pH 7.0 with 165 mM of 3-(N-morpholino) propanesulfonic acid (MOPS; Sigma) and two-fold serially diluted to final concentrations of 0.125 to 64 μg/mL for fluconazole and 0.03 to 16 μg/mL for the other antifungal agents. Minimum inhibitory and minimum fungicidal concentrations (MIC and MFC) were determined.

Preparation of *Aloe vera* gel extracts (AGE)

*Aloe vera* leaves were obtained from Agriculture Faculty, Zagazig University, Zagazig, Egypt. *Aloe vera* gel was obtained from leaves by scratching. The aqueous extract of gel was prepared utilizing magnetic stirrer (Fisher Scientific) and filtered using Whatman No.1 filter paper. The extraction ratio was 1 W: 5 V (gel: solvent). The filtrate was freeze-dried (Thermo-Electron Corporation-Heto power dry LL300 Freeze Dryer), the extract was then weighed to decide the yield and stored at −20°C.

Chemical characterization of AGE

Determination of phenolic compounds

The concentration of total phenols in extract was measured by a UV spectrophotometer (Jenway-UV–VIS Spectrophotometer 6705), based on a colorimetric reduction of the reagent by phenolic compounds, as described by Škerget et al.[36]. Total phenolic content expressed as gallic acid equivalent (GAE) was calculated: \( y = 0.0228x + 0.0086 \) and \( R^2 = 0.9969 \), where \( x \) is the concentration (µg GAE) and \( y \) is the absorbance.

Determination of total flavonoids

Total flavonoids content expressed as quercetin equivalent (QE) in AGE at a final concentration of 1 mg mL\(^{-1}\) was calculated: \( y = 0.0142x - 0.007 \) and \( R^2 = 0.9994 \), where \( x \) is the concentration (µg QE) and \( y \) is the absorbance [37].

Determination of phenolic compounds by HPLC
HPLC analysis was executed as previously described [38] with slight modifications using an Agilent Technologies 1100 series liquid chromatograph equipped with an autosampler. The analytical column was Agilent Eclipse XDB C18 (100 x 4.6 µm; 3.5 µm particle size). Diode array detector (DAD) was set to a scanning range of 180–420 nm. The mobile phase consisted of methanol (solvent A) and 0.1% formic acid (v/v) (solvent B). The flow rate was kept at 0.4 mL min$^{-1}$ and the gradient program was as follows: 10% A - 90% B (0–5 min); 20% A - 80% B (5–10 min); 30% A - 70% B (10–15 min); 50% A - 50% B (15–20 min); 70% A - 30% B (20–25 min); 90% A - 10% B (25–30 min); 50% A - 50% B (30–35 min) and 10% A - 90% B (35–36 min). There was 5 min of post-run for reconditioning. The injection volume was 10 µL and peaks were monitored simultaneously at 280, 320 and 360 nm for the benzoic acid and cinnamic acid (Sigma, St. Louis, MO, USA) derivatives and flavonoids compound, respectively. All samples were filtered through a 0.45 µm Acrodisc syringe filter (Gelman Laboratory, MI) before injection. Peaks were identified by congruent retention times and UV spectrum and compared with those of the standards (Sigma, St. Louis, MO, USA).

**Antioxidant and biological activity of AGE**

1, 1-Diphenyl–2picrylhydrazyl (DPPH˙) radical-scavenging activity

The electron donation ability of AGE was measured by bleaching of the DPPH˙ (Sigma, St. Louis, MO, USA) purple colored solution using a UV spectrophotometer (Jenway-UV–VIS Spectrophotometer 6705) [39]. The absorbance was determined against control at 515 nm[40]. Percentage of scavenging activity of free radical DPPH˙ was calculated as follow:

\[
\text{Scavenging activity (Inhibition) %} = \left[ \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \right] \times 100
\]

Where $A_{\text{control}}$ is the absorbance of the control reaction and $A_{\text{sample}}$ is the absorbance in the presence of plant extract. Gallic acid and Tert-butyl hydroquinone (TBHQ) (Sigma, St. Louis, MO, USA) (1 mg / 1mL methanol) were utilized as a positive control. Samples were tested in triplicate.

β-Carotene/linoleic acid bleaching

The ability of AGE and synthetic antioxidants (gallic aid and TBHQ) to hinder the bleaching of β-carotene (Sigma, St. Louis, MO, USA) was examined according to Dastmalchi et al. [41] A control sample with no added extract was also analyzed. Antioxidant activity was calculated as follows: Antioxidant activity (%) = \[
\left[1 - \frac{(A^0_{\text{sample}} - A^{120}_{\text{sample}})}{(A^0_{\text{control}} - A^{120}_{\text{control}})}\right] \times 100
\]
Where \( A_{\text{sample}}^0 \) is the absorbance of the AGE or synthetic antioxidant at 0-time, \( A_{\text{sample}}^{120} \) is the absorbance after 120 min, \( A_{\text{control}}^0 \) and \( A_{\text{control}}^{120} \) are the absorbance of control at 0-time and after 120 min, respectively.

**Ferric reducing antioxidant power (FRAP)**

The extract reducing power was assessed [40]. Distilled water was employed as a negative control and gallic acid and TBHQ as positive control. Absorbance of this mixture was measured at 700 nm using a UV spectrophotometer (Jenway-UV–VIS Spectrophotometer 6705). Decreased absorbance demonstrates ferric reducing power capability of sample.

**Testing AGE antidermatophyte activity**

The procedure of Silva et al.[42] was used to test the antidermatophyte activity of AGE. The freeze-dried AGE (3.5 gm) was dissolved and serially two-fold diluted in RPMI–1640 broth to obtain a concentration range of 1000–20000 \( \mu \text{g/ml} \) as TPC. A final concentration of 50–1000 \( \mu \text{g/ml} \) was obtained by mixing 2 ml of this solution with 18 ml of liquefied Mycobiotic agar medium (Remel™, Thermo Fisher Scientific) at 45 °C in sterile Petri dish. Next, wells of 3 mm diameter were made in the centre of this agar plate and filled with 10µl of fungal spore suspension (10\(^6\) cfu/ml) that was prepared from freshly cultured isolates. The plates were incubated for 5 days at 25 °C. The assay was carried out in triplicate and growth and drug control were incorporated into the test. The concentration that inhibits the fungal growth was considered as MIC.

**Investigation of AGE effectiveness for treatment of calf ringworm**

Seventy-five calves showing evident clinical signs of ringworm infection were used for investigation of AGE effectiveness in comparison with antifungal drugs for the treatment of calf ringworm after obtaining informed consent from the farm owners. The enrolled calves proved positive on mycological examination and \( T. verrucosum \) was isolated from clinical samples. Calves were randomly allocated into five groups, 15 animals in each group (G). Animals in G1 were treated orally with 250 mg /day terbinane (Lamisil®; Novartis, Basel, Switzerland). The crust on the skin lesions was removed with a brush, subsequently, topical miconazole (Janssen Research Foundation, Beerse, Belgium) (G2), AGE solution (500 ppm) (G3) or oral terbinafine in combination with AGE (G 4) were applied twice a day for two weeks. Animals in G5 were left untreated as controls. Calves were observed daily for six weeks. At the beginning, during and after treatment, the clinical efficacy was assessed by scoring alopecia, scaling, crusting, the numbers and spreads of lesions on a 0–3 scale. The sum of the scores assigned to each lesion on the evaluated area was divided by the numbers of affected areas yielding the total score for each calf, and the same lesion
was assessed on every examination. The mycological examination was performed every week until two consecutive fungal cultures give negative results [32, 43]. The control animals were treated after the observation period.

Antifungal disinfection for the entire stable and all materials with which animals come in contact was performed using 0.2% enilconazole (Clinafarm® EC; Merck Animal Health USA).

**Data analysis**

Data analysis was performed using IBM SPSS Statistics for Windows, version 24.0 (released 2016), and MedCalc 2014 (MedCalc Software) were used to analyze data. Chi-square test and Odds ratio analyses were performed to determine the association of ringworm infection with different risk factors. Prevalence and risk ratio was used to compare between groups at risk to those not at risk. To confirm the results, random forest non-parametric classification method was done using MetaboAnalystR web server [44]. Briefly, the occurrence of each variable was firstly used to build up random forest classification model (an ensemble of 500 tree trial; out of bag error (OOB) = 0.6) in the respective outcome. The importance of the risk factor was determined by measuring the increase of the OOB error when the respective factor is permuted. The sensitivity, specificity, negative, positive predictive values (NPV, PPV), positive, negative likelihood ratio (LR+, LR-), and diagnostic odds ratio (DOR), that express strength of association between test result and disease, with 95 % confidence intervals (CIs) for direct sample PCR assays were estimated. All diagnostic indices were predestined based on (a) culture and (b) culture and nested PCR as the gold standard for detection/identification of dermatophytes causing calves ringworm. Kappa value was used to test the agreement between test results. Independent samples t-test was run to compare between the mean MIC values ± SD of each antifungal drug for the tested species. Kruskal-Wallis test was used to analyze the differences in clinical score changes within and among the treated and untreated groups over time. Differences in clinical scores between groups were assessed by Mann-Whitney U test after significant Kruskal-Wallis test. \( P < 0.05 \) was considered significant.

**Abbreviations**

*ITS*: internal transcribed spacers regions of ribosomal DNA, *MIC*: Minimum Inhibitory Concentration, *AGE*: Aloe vera gel extract, *HPLC*: High-performance liquid chromatography, *TPC*: Total phenolic compounds, *TF*: total flavonoids, *QE*: quercetin equivalent, *GAE*: gallic acid equivalent.

**Declarations**

*Ethics approval and consent to participate*: The protocol of this study was approved by the Ethical Committee on animal care and use Experimentation in Zagazig University (ZU-IACUC/2/F/11/2018). Written informed consent was obtained from the farm owners for the treatment of calves showing clinical signs of ringworm infection.
Consent for publication: Not applicable

Availability of data and material: The datasets of this study are included within the article and its additional files.

Competing interest: Authors have no competing interests to declare.

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Authors’ contributions: YT, WE conceived and designed the work; YT, WE, AM, MA contributed to execution of the experiments, data acquisition, analysis and interpretation; YT performed the molecular analyses and wrote the manuscript. All authors read and approved the submitted version.

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Tables

Table (1): Calves’ dermatophytosis prevalence and risk ratio of different risk factors

† Risk ratio and ‡ odds of summer to winter season. **, *** significant P-values.

Table (2): Diagnostic indices of direct sample PCRs for detection and identification of dermatophytes from calves ringworm based on (a) culture, (b) culture and/or nested PCR as the gold standard
| Variables          | Categories          | Ringworm lesion Positive cases | Prevalence & Relative risk | $X^2$ & $P$ value | Odds ratio |
|--------------------|---------------------|-------------------------------|---------------------------|------------------|------------|
|                    |                     | Total animals                 |                           |                  |            |
| **Age**            | 1 month             | 66                            | 41%                       | $X^2 = 19.7, P < 0.001^{**}$ | 0.68        |
|                    | 4-6 month           | 358                           | 60%                       | $X^2 = 63.18, P < 0.001^{**}$ | 1.50        |
|                    | **Breed**           | **Pure breed**                | 297                       | 49%               | 0.68/1.5=0.45 | 0.95       |
|                    |                     | 610                           |                           | $X^2 = 63.18, P < 0.001^{**}$ | 0.95        |
|                    | **Cross breed**     | 127                           | 85%                       | $X^2 = 0.001^{**}$ | 5.52       |
|                    |                     | 150                           |                           | $Phi = 0.16^{**}$ |            |
|                    |                     |                               |                           | $Phi = 0.29^{**}$ |            |
|                    | **Production system** | **Milk**                 | 297                       | 49%               | 0.49/0.85=0.58 | 0.95       |
|                    |                     | 610                           |                           | $X^2 = 63.18, P < 0.001^{**}$ | 0.95        |
|                    | **Meat**            | 127                           | 85%                       | $X^2 = 0.001^{**}$ | 5.52       |
|                    |                     | 150                           |                           | $Phi = 0.29^{**}$ |            |
|                    |                     |                               |                           | $Phi = 0.29^{**}$ |            |
|                    | **Breeding system** | **Semi-intensive**           | 297                       | 49%               | 0.49/0.85=0.58 | 0.95       |
|                    |                     | 610                           |                           | $X^2 = 63.18, P < 0.001^{**}$ | 0.95        |
|                    |                     |                               |                           | $Phi = 0.29^{**}$ |            |
|                    | **Intensive**       | 127                           | 85%                       | $X^2 = 0.001^{**}$ | 5.52       |
|                    |                     | 150                           |                           | $Phi = 0.29^{**}$ |            |
|                    | **Origin of the animals** | **Born at the farm** | 297                       | 49%               | 0.49/0.85=0.58 | 0.95       |
|                    |                     | 610                           |                           | $X^2 = 63.18, P < 0.001^{**}$ | 0.95        |
|                    |                     |                               |                           | $Phi = 0.29^{**}$ |            |
|                    | **Newly purchased** | 127                           | 85%                       | $X^2 = 0.001^{**}$ | 5.52       |
|                    |                     | 150                           |                           | $Phi = 0.29^{**}$ |            |
|                    | **Parasitic infestation** | **Absent**             | 297                       | 49%               | 0.49/0.85=0.58 | 0.95       |
|                    |                     | 610                           |                           | $X^2 = 63.18, P < 0.001^{**}$ | 0.95        |
|                    |                     |                               |                           | $Phi = 0.29^{**}$ |            |
|                    | **Present**         | 127                           | 85%                       | $X^2 = 0.001^{**}$ | 5.52       |
|                    |                     | 150                           |                           | $Phi = 0.29^{**}$ |            |
|                    | **Summer**          | 190                           | 66%                       | $X^2 = 1.9$       |            |
| Season   | Winter | Spring | Autumn |
|----------|--------|--------|--------|
|          | 81     | 83     | 70     |
|          | 150    | 173    | 147    |
|          | 54%    | 48%    | 48%    |
|          | 19.58, | 0.92   | 0.91   |
|          | $P < 0.01^{**}$ |            |
|          | $\Phi = 0.16^{**}$ |       |

| Area per calf | overcrowding (1.5 mr/calf) | 194 | 314 |
|              | No overcrowding (6 mr/calf) | 230 | 446 |
|              |                          | 62% | 52% |
|              | $x^2 = 7.79$, $P < 0.01^{**}$ | 1.62 |
|              | $\Phi = 0.01^{**}$ | 1.06 |
|              | $\Phi = 0.16^{**}$ |       |

Area per calf: 1.62 / 1.06 = 1.53

| Ventilation | Good | Bad |
|-------------|------|-----|
|             | 297  | 127 |
|             | 610  | 150 |
|             | 49%  | 85% |
|             | $x^2 = 63.18$, $P < 0.001^{**}$ | 0.95 |
|             | $\Phi = 0.29^{**}$ | 5.52 |

Use of disinfectant: 0.49 / 0.85 = 0.58 / 5.52 = 0.17

Use of disinfectant: 0.95 / 5.52 = 0.17

| Use of disinfectant | Regular | Irregular |
|---------------------|---------|-----------|
|                     | 297     | 127       |
|                     | 610     | 150       |
|                     | 49%     | 85%       |
|                     | $x^2 = 63.18$, $P < 0.001^{**}$ | 0.95 |
|                     | $\Phi = 0.29^{**}$ | 5.52 |

Use of disinfectant: 0.49 / 0.85 = 0.58 / 5.52 = 0.17

Use of disinfectant: 0.95 / 5.52 = 0.17
| Diagnostic index† | Pan-dermatophyte | One step-PCR | Nested-PCR |
|-------------------|------------------|--------------|------------|
| **Culture as a gold standard** | | | |
| Se (95 % CI) | 82.14% (72.26% to 89.65%) | 71.43% (60.53% to 80.76%) | 92.86% (85.10% to 97.33%) |
| SP (95 % CI) | 72.73% (60.36% to 82.97%) | 50% (37.43% to 62.57%) | 54.55% (41.81% to 66.86%) |
| PPV (95 % CI) | 79.31% (71.86% to 85.20%) | 64.52% (57.96% to 70.57%) | 72.22% (66.48% to 77.32%) |
| NPV (95 % CI) | 76.19% (66.40% to 83.82%) | 57.89% (47.58% to 67.56%) | 85.71% (72.91% to 93.05%) |
| LR+ (95 % CI) | 3.01 (2.01 to 4.52) | 1.43 (1.08 to 1.88) | 2.04 (1.56 to 2.68) |
| LR- (95 % CI) | 0.25 (0.15 to 0.40) | 0.57 (0.38 to 0.87) | 0.13 (0.06 to 0.29) |
| DOR (95 % CI) | 12.36 (5.64 to 26.7) | 2.5 (1.72 to 4.19) | 15.6 (5.965 to 40.799) |
| Accuracy | 78.00% (70.51% to 84.35%) | 62.00% (53.72% to 69.79%) | 76.00% (68.35% to 82.59%) |
| Kappa value | 0.55*** | 0.217 ** | 0.493*** |

| Nested PCR and Culture as gold standard | | | |
|-------------------|------------------|--------------|------------|
| **Direct microscopy** | | | |
| Se (95 % CI) | 78.95% (70.31% - 86.02%) | 73.68% (64.61% - 81.49%) | 63.16% (53.61% - 72.00%) | 94.74% (88.90% - 98.04%) |
| SP (95 % CI) | 41.67% (25.51% - 59.24%) | 100.00% (90.26% - 100.00%) | 41.67% (25.51% - 52.94%) | 100.00% (90.26% - 100.00%) |
| PPV (95 % CI) | 81.08% (76.19% - 85.16%) | 100.00% | 77.42% (71.56% - 82.37%) | 100.00% |
| NPV (95 % CI) | 38.46% (26.99% - 51.38%) | 54.55% (46.88% - 62.00%) | 26.32% (18.47% - 36.02%) | 85.71% (73.36% - 92.89%) |
| LR+ (95 % CI) | 1.35 (1.01 - 1.81) | ‡ | 1.08 (0.79 - 1.48) | ‡ |
| LR- (95 % CI) | 0.51 (0.30 - 0.85) | 0.26 (0.19 - 0.36) | 0.88 (0.56 - 1.39) | 0.05 (0.02 - 0.11) |
| DOR (95 % CI) | 2.68 (1.2 - 5.9) | 0.46 (0.35 - 0.59) | 1.22 (0.57 - 2.62) | 19.6 (8.7 - 41.4) |
| Accuracy | 70.00% (61.99% - 77.20%) | 80.00% (72.70% - 86.08%) | 58.00% (49.68% - 66.00%) | 96.00% (91.50% - 98.52%) |
| Kappa value | 0.2 ** | 0.57** | 0.04 | 0.91*** |

†Se: Sensitivity, Sp: specificity, PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-negative likelihood ratio, DOR: diagnostic odds ratio and AUC: accuracy
‡Cannot be estimated/infinity. **, *** significant P-values.

Table 3: Yield, TPC, TF and phenolic compounds of AGE
| Unit             | $\text{Unit}$ | $\text{\$AGE}$ |
|------------------|---------------|----------------|
| **Extract Yield** | g/100 g *Aloe vera* gel | 1.02 ± 0.052   |
| $^\dagger$TPC    | mg GAE/g extract      | 111.78 ±10.62  |
| $^\ddagger$TF    | mg QE/g extract       | 45.6 ±8.45     |

**Phenolic compounds**

| Phenolic compound | Unit    | $\text{AGE}$  |
|-------------------|---------|---------------|
| Gallic acid       | mg/ g AGE | 0.12 ±0.06    |
| Caffeic acid      |         | 0.23 ±0.11    |
| Chlorogenic acid  |         | 0.54 ±0.07    |
| Cinnamic acid     |         | 0.98 ±0.25    |
| Aloe-Emodin       |         | 28.02 ±4.67   |
| Quercetin         |         | 1.54 ±0.22    |
| Rutin             |         | 1.14 ±0.86    |

$^\dagger$TPC: Total phenolic compounds, GAE: gallic acid equivalent, $^\ddagger$TF: total flavonoids, QE: quercetin equivalent, and $^\ddagger$AGE: *Aloe vera* gel extract.

**Figures**
Figure 1

Calves ringworm due to T. verrucosum with typical grey-white raised crusty lesions on head and neck regions (A) and extensive alopecia, erythema, and scales after removal of thick greasy crusts firmly attached to erect and matted hair over the back (B).
Figure 2

(A) Random forest classification showing the predictive accuracy of the studied risk factor (X-axis). The mini heat map shows the frequency distribution of each factor across the two outcomes (ringworm lesion and without lesion). Each dot refers to value of mean decrease accuracy of one risk factor, (B) box plot for a normal distribution of age (as a continuous variable) across all examined animals (n=760), each dot represent one case and horizontal line refers to the median of age distribution.
Figure 3

Agarose gel electrophoresis for amplicons of direct PCR assays for testing dermatophytes DNA from calves’ hair samples. (A) Pan-dermatophytes PCR amplicons of pchs-1gene at 440 bp, (B) One-step PCR ITS+ amplified products for T. verrucosum at 900bp (lanes 2-8) and T. mentagrophytes at 872 bp (lanes 11, 12) and (C) ITS-1 amplicons of Nested-PCR at 400bp. Lanes M: 100bp molecular size marker, lane Pos.: positive control and lane Neg.: negative control.
Figure 4

(A) Antioxidant activity of Aloe vera gel extract (AGE) against 1,1-Diphenyl-2-picrylhydrazyl (DPPH·) radical and β-Carotene/linoleic emulsion compared with gallic acid and Tert-butyl hydroquinone (TBHQ),
(B) Absorbance of ferric reducing power of AGE against gallic acid and TBHQ.
Figure 5

Score lesions (mean and standard deviations) for treated groups and control untreated group from 0 day to 42 day of experiment. There is a non-significant difference between the clinical scores of groups on days 0 and 7, while the treated groups displayed significantly (P < 0.05*) lower clinical scores than the control group on days 14, 21, 28, and 42. Clinical scores carrying asterisks within the same day was statistically different. ** indicating significant difference between control untreated group and all other groups within the same days.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1S.docx
- Table2S.docx
- AdditionalFigure1.tif
• supplementarymaterials.docx