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LITERATURE REVIEW

Plant-based therapies for dermatophyte infections

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Background: Resistance and side effects encountered with use of common topical and systemic antifungal drugs for dermatophyte infections highlights the need for novel therapies. Medicinal plants, which have been traditionally utilised for their antimicrobial properties to treat superficial skin infections, serve as an abundant source for the identification of new antifungal compounds.
Aim: To summarise the current evidence for plant-based natural therapies for dermatophytic infections.
Methods: A comprehensive literature search was performed across databases PubMed, Embase and ScienceDirect using keywords 'dermatophyte' or 'anti-dermatophytic' or 'antifungal', combined with 'natural, 'ethnomedical', 'plant', 'botanical', 'treatment' or 'remedy'. Additional studies specific to the plant extract were searched using genus and species.
Results/ Discussion: Seventy plant extracts demonstrating *in vitro* anti-dermatophytic properties are summarised in this review. Among these, common antifungal phytochemicals found include phenolic compounds, terpenoids, terpenes, alkaloids, xanthones and glycosides including saponins. Only 21 plant extracts or their active components have been evaluated in *in vivo* bioassays in clinical trials and animal studies. Multiple mechanisms of action have been elucidated, including disruption to cell wall and cell membranes, inhibition of cell wall synthesis, hyphal growth, and spore germination, as well as possible *in vivo* anti-inflammatory and immunomodulatory effects.
Conclusion: Based on *in vitro* studies, numerous plant extracts show significant therapeutic potential for the treatment of dermatophyte infections. However, more *in vivo* studies are required to assess the clinical effectiveness of plant extracts.
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Introduction

Superficial fungal infections are estimated to affect one billion people globally each year.[1] Dermatophytes are a group of keratinophilic filamentous fungi responsible for a significant proportion of fungal skin, hair and nail infections.[2] These include nine genera, of which *Trichophyton*, *Microsporum* and *Epidermophyton* most commonly infect humans.[3] The anthropophilic species *T. rubrum* is responsible for most infections world-wide. However, significant geographic and demographic variation exists, with a recent emerging predominance of zoophilic species *M. canis* and *T. mentagrophytes,*[4] in some areas likely due to increased contact between animals and humans. Additionally, there has been unprecedented growth of dermatophytoses in developing countries, including

increases in chronic and relapsing cases associated with significant morbidity, financial burden and impaired quality of life.[5]

Concern has been raised regarding the development of dermatophyte resistance to existing antifungal agents, including azoles and allylamines, contributing to therapeutic failure. Although already a major public health threat in endemic areas such as India, reports of dermatophyte resistance to terbinafine have increased globally in recent years including in Japan, European countries, Iran, Mexico and the US.[6-11] Whilst previously limited to *T. rubrum,* resistance is now being reported in *T. mentagrophytes* and *T. interdigitale.*[12] Mechanisms for terbinafine and azole resistance include alteration of drug targets, drug efflux and biofilm formation.[6,13] Selective pressure for resistant dermatophyte strains is multi-factorial, and may be due to poor compliance, sub-inhibitory doses, widespread self-treatment and steroid misuse.[14] Moreover, cellular drug targets are limited and overlapping, for instance, allylamines, azoles, morpholines and thiocarbamates all inhibit ergosterol biosynthesis, albeit through different enzyme targets.[14] This has stimulated the search for new formulations to enhance current drug effectiveness, or the development of novel antifungal compounds.[15]

Plants possess natural defence systems against external microbial pathogens, and serve as a limitless source of antimicrobial compounds.[16] Several plants have been utilised historically by native populations to treat fungal infections, particularly in Latin America, Asia and Africa. For example, a survey conducted among 34 Ayurvedic practitioners in Sri Lanka identified 165 plants used for skin diseases, including neem (*Azadirachta indica* A.Juss.*)* and turmeric (*Curcuma longa* L.*)*.[17] Many of these species have been scientifically investigated *in vitro*, and are often chosen based on their uses in traditional medicine.

The aim of this review is to summarise the current scientific evidence *in vitro* and *in vivo* for plant based natural therapies for dermatophyte infections.

Methods

A comprehensive search of the literature was conducted across databases PubMed, Embase, and ScienceDirect from their inception to January 2021. Keywords included 'dermatophyte' or 'anti-dermatophytic' or 'antifungal', combined with 'natural', 'ethnomedical', 'plant', 'botanical', 'treatment' or 'remedy'. A comprehensive list of plants was extracted from the initial search, and databases searched again using each individual plant name and 'dermatophyte', to identify all relevant studies. *In vitro* studies included described methods of plant extraction and anti-microbial susceptibility testing methods. Minimum inhibitory concentration (MIC) and phytochemical composition was reported if investigated within the *in vitro* studies selected. *In vivo* studies included both animal models and clinical trials. One hundred and ten studies of 70 plant extracts were selected after the exclusion of duplicates and irrelevant articles by AM. Concentrations of compounds are reported here using the same units as in the cited study.

Results 1: Plants with *in vitro* anti-dermatophytic activity

Seventy plants demonstrated *in vitro* anti-dermatophytic activity (Appendix 1). Interpretation of MIC values is difficult due to variations in methodology and no standardised minimum breakpoint concentration deemed to indicate effectiveness against dermatophytes. Additionally, comparison between studies of the same plant extract frequently reveals discrepancies of reported MIC or diameter inhibition zone values. Of the 70 plant extracts included in this review, 25 had more than two dedicated *in vitro* studies, but very few revealed consistent data. Variations in methodology include inoculum size and preparation, incubation duration and time, criterion used for MIC determination, differences in solubility of phytochemicals in solvents used, environmental growth conditions affecting compound composition, and fungal sensitivity, all of which all significantly influence outcomes. The use of negative and positive controls would be helpful in determining usefulness in clinical application, but these are not always applied. Of thirty-six plant families, *Lamiaceae* (mints) and *Myrtaceae* (Myrtle) comprised the most, (10 and 8 plants respectively). Notable plants with the highest antifungal activity as demonstrated by an MIC of < 50 μg ml^{-1} were *Azadirachta indica* A. Juss, *Piper betle* L.*, Vitis vinifera* L.*, Terminalia chebula* Retz.*, Lawsonia inermis* L.*, Ocimum sanctum* L.*, Libidibia ferrea* (Mart. Ex Tul.) L.P. Queiroz*, Mimosa tenuiflora* (Willd.) Poir*, Foeniculum vulgare* Mill*, Anagallis arvensis* L. and *Piper regnellii* (Mia.) C. DC. Across studies of plants with the lowest MIC values, many common constituents are identified. For instance, essential oil of *P. betle* comprising primarily eugenol reported an MIC of 0.2 – 0.4 μgml^{-1} against *Trichophyton* and *Microsporum* species.[18] Eugenol was also the main component of *O. sanctum* (MIC 0.4 – 0.8 μgml^{-1})[18] and *Syzygium aromaticum* L. (MIC 160 μg ml^{-1})[19]. Several other studies showed a weak to moderate inhibitory effect, with MICs ranging up to 160 mg ml^{-1} reported as having antifungal activity. The impact of methodological variations is highlighted by a

2004 study of *T. chebula* aqueous extracts, which yielded an MIC against *T. mentagrophytes* of 600 µg ml^{-1}, whereas a 2013 study using lyophilised ethanolic extract yielded an MIC of 3.125 µg ml^{-1}.[20]

In general, plant extracts were less effective *in vitro* than commercial antifungal drugs. However, isolated phytochemicals showed greater inhibitory growth effects than that of the plant extract alone, and sometimes had efficacy comparable or greater than their positive controls. For example, macrocarpal C isolated from *Eucalyptus globulus* Labill. had an MIC of 1.95 µg ml^{-1}, similar to terbinafine (0.625 µg ml^{-1}) and nystatin (1.25 µg ml^{-1}).[21] Isolated thymoquinone from black cumin *(Nigella sativa* L.*)* had an MIC of 0.125 – 0.25mg ml^{-1} compared to the plain ether extract (40 mg ml^{-1}).[22] Other examples include terpinen-4-ol from tea tree oil (*Melaleuca alternifolia* (Maiden & Betche) Cheel), limonene and methyleugenol from *Thapsia villosa* L., and thymol from *Thymus pulegioides* L.[23,24,25]

Synergistic activity is seen when plant extracts are used in combination with existing antifungal drugs. In a study by Khan and Ahmad (2011), the addition of cinnamaldehyde to fluconazole reduced the MIC 8-fold against *T. rubrum* from 200 µg ml^{-1} to 40 µgml^{-1}.[19] Similarly, the addition of eugenol or *Cinnamomum verum* J. Presl reduced the MIC of fluconazole to 80 µgml^{-1}.[19] Synergy was also observed against *Aspergillus* species suggesting broad spectrum antifungal activity.[19] Strong synergism is also noted in studies of *Mentha piperita* L. and its components menthone and menthol with itraconazole against *T. mentagrophytes,*[26] as well as citronellol and geraniol from *Pelargonium graveolens* L'Hér combined with ketoconazole against *Trichophyton* spp.[27]

Some plant extracts are also reported to be effective against resistant strains of dermatophytes. The extract of *Pothomorphe umbellata* (L.) yielded an MIC of 78 µg ml^{-1} against *T. rubrum* containing genes associated with multi-drug resistance.[28] Cinnamaldehyde isolated from *C. verum* was also effective against fluconazole and itraconazole resistant strains of dermatophytes with an MIC of 40-80 µgml^{-1}.[19] Essential oil of *Cymbopogon citratus* (DC.) Stapf and citral also generated inhibited zones of diameter 24.7 – 32.6 mm against azole resistant strains of *T. rubrum*.[29] The proposed mechanisms and utility of such plant extracts in overcoming drug-resistant fungi are discussed below.

Results 2: Plants with *in vivo* anti-dermatophytic activity

Twenty-one plant extracts were evaluated using *in vivo* bioassays. Of these, only 6 plant preparations have been tested in clinical trials, summarised in Table 1. Tea tree oil was evaluated in four randomised controlled trials, of which two demonstrated significantly better outcomes compared to placebo, and were comparable to commercial antifungal preparations topical butenafine and topical clotrimazole.[30, 31] However, one randomized double-blind trial randomizing 104 patients with tinea pedis to 1% tolnaftate, placebo or 10% tea tree oil cream showed significantly lower mycological cure rates with tea tree oil (30%) compared to tolnaftate (85%).[32] Nonetheless, tea tree oil was significantly better than placebo.[32] Isolated encecalin from snake root (*Ageratina pichinchensis* (Kunth) R.M.King & H.Rob.) was evaluated in five small randomised, double-blind controlled trials in patients with tinea pedis and onychomycosis and showed comparability to topical ketoconazole and ciclopirox respectively. In patients with onychomycosis, topical lacquer made from norway spruce (*Picea abies* (L.) H.Karst.) was as effective as 5% amorolfine (13% vs 5% cure, respectively), but significantly less effective than oral terbinafine in achieving mycological cure (56% at 10 months).[33] Garlic (*Allium sativum* L.), snow gum (*Eucalyptus pauciflora* Sieber ex Spreng.) and Devil's fig (*Solanum chrysotrichum* Schltdl.*)* have also been tested in clinically but are preliminary and of small size.[34-36]

The remaining plant extracts tested *in vivo* were animal studies (Appendix 2), most commonly in guinea pigs, but also in mice, sheep and cattle. Five studies used anthropophilic dermatophyte species. Studies consistently demonstrated efficacy through both clinical cure and mycological cure, comparable to pharmacological preparations. Effects were dose-dependent with negligible dermal irritation. Synergistic activity was also observed in a study of twenty-four cattle with *T. verrucosum* infections exposed to enilconazole, *N. sativa* extract, or combined treatment of enilconazole and plant extract. Combined treatment resulted in healing of all 8 cattle, whereas single agent enilconazole or *N. sativa* treatment resulted in healing in 5 and 6 animals respectively.[22]

Table 1: Summary of clinical trials of anti-dermatophytic plants

Ref	Scientific name	Study design	Study population	Experimental intervention	Control(s)	Main results
37	*Allium sativum* L.	Double-blind, randomized comparative trial	47 patients with tinea pedis	0.6% ajoene, 1% ajoene	1% topical terbinafine	At 60 days, mycologic cure rates were 72% for 0.6% ajoene, 100% for 1% ajoene, and 94% for 1% terbinafine.
34		Single-arm trial	34 patients with tinea pedis	0.4% ajoene cream	None	Clinical and mycological cure were 79% at 7 days and 100% at 14 days of ajoene treatment, without recurrence at 90 days.
30	*Melaleuca alternifolia* (Maiden & Betche) Cheel	Double-blind, randomized controlled trial	60 patients with toenail onychomycosis	5% TTO cream	2% butenafine hydrochloride, Placebo	At 36 weeks, overall cure rates were 80% for butenafine and TTO and 0% for placebo.
32		Double-blind, randomized controlled trial	158 patients with tinea pedis	50% TTO, 25% TTO	Placebo	At 4 weeks, clinical response was 39% in the placebo group, 72% in the 25% TTO group and 68% in 50% TTO group. Mycological cure rates were 31% in the placebo group, 55% in the 25% TTO group and 64% in the 50% TTO group.
38		Double-blind, randomized, trial	104 pts with tinea pedis	10% TTO cream	1% tolnaftate, Placebo	At 4 weeks, mycologic cure was 85% of tolnaftate group, 30% in TTO and 21% in placebo. No significant difference between groups. Clinical response was significantly better in tea tree oil group compared to placebo group.
31		Double blind, randomized, controlled, multi-centre trial	117 patients with distal subungual onychomycosis	100% TTO	1% Clotrimazole	At 6 months, mycologic cure was seen in 11% of clotrimazole patients and 18% of TTO patients. No significant differences in culture or clinical outcomes
33	*Picea abies* (L.) H.Karst.	Observational single-arm trial	37 patients with onychomycosis	30% Resin lacquer	None	At 9 months, by 14 compliant patients, resin lacquer achieved complete healing in 9% and partial healing in 85%.
39		Prospective, randomized, controlled, investigator-blinded study	73 patients with onychomycosis	30% Resin lacquer	5% amorolfine lacquer, 250mg oral terbinafine daily	At 10 months, topical amorolfine and resin lacquer were comparable but both significantly less effective than oral terbinafine in mycologic cure and clinical response.
35	*Solanum chrysotrichum* Schltdl.	Randomized, controlled, double-blind clinical trial or	101 patients with tinea pedis	Standardised phytodrug from *S. chrysotrichum*	2% topical ketoconazole	At 4 weeks, clinical effectiveness achieved for 96% plant extract group and 91% treated with ketoconazole. Mycological effectiveness was 78% and 77% respectively of plant and ketoconazole groups.
40	*Ageratina pichinchensis* (Kunth) R.M.King & H.Rob.	Double-blind, randomized controlled pilot trial	97 patients with tinea pedis	10% plant extract	2% topical ketoconazole	After 4 weeks, clinical effectiveness was achieved in 80.3% of patients treated with the plant extract and 76% treated with ketoconazole with no significant difference.
41		Double-blind, randomized controlled trial	160 patients with tinea pedis	0.76% encecalin, 1.52% encecalin	2% topical ketoconazole	After 4 weeks, therapeutic cure was achieved in 34.1% and 41.8% of patients receiving the plant extract at the lower and higher dose respectively, and 39.53% of patients receiving ketoconazole with no statistical difference between groups.
42		Double-blind, randomized controlled trial	96 patients with onychomycosis	Encecalin lacquer	8% ciclopirox lacquer	At 6 months, therapeutic effectiveness was achieved in 71.1% of encecalin group, and 80.9% of ciclopirox group with no significant difference.
43		Double-blind, randomized controlled trial	122 patients with mild to moderate onychomycosis	Topical lacquer at 12.6% and 16.8% plant extract	None	At 6 months, the higher concentration extract achieved therapeutic effectiveness in 79.1% of patients, which was significantly higher than the lower concentration of 67.2%.
44		Double-blind, randomized controlled trial	71 patients with diabetes mellitus and onychomycosis	Encecalin lacquer	8% ciclopirox	At 6 months, clinical efficacy was achieved in 78.5% of the experimental group and 77.2% of the control group with no significant difference.
36	*Eucalyptus pauciflora* Sieber ex Spreng.	Single-arm clinical study of, cruris or corporis.	50 patients with tinea pedis, cruris or corporis	1 % essential oil in ointment	None	At three weeks, 60% of patients recovered completely and 40% showed significant improvement. After the second week of treatment, all patients were KOH-negative with no recurrence at 2 months analysis.

Mechanism of action of natural anti-dermatophytic compounds

The anti-dermatophytic mechanism of action in plants is multi-modal and includes disruption to cell membranes and cell wall synthesis as well as inhibition of hyphal growth and spore germination. These actions are mediated by abundant active plant compounds, of which phytochemical screening indicate the presence of phenolic compounds, terpenoids, terpenes, alkaloids, xanthones and glycosides including saponins, and have been well-characterised.[45]

Phenolic compounds are found abundantly in plants and include phenols, flavonoids, tannins, quinones, coumarins and phenolic acids. They are extractable by water, methanol or acetate.[46] Gallic acid has been shown to inhibit ergosterol biosynthesis by reducing activity of sterol-14α-demethylase P450 and squalene epoxidase in *T. rubrum*.[47] Ergosterol content in the fungal membrane was significantly decreased at all tested concentrations of gallic acid (between 43.75 and 83.33 µg/ml).[47] Correlation between content of phenolic compounds and antifungal activity is

observed in a study of *Vitis vinifera* L., where grape varieties Alphonse-Lavallee containing the highest flavon-3-ol content also exhibited the highest antifungal activity.[48] Tannins, found in many plants including *Mimosa tenuiflora* Benth., *Hypericum perforatum* L., *Moringa oleifera* Lam. and *Urtica dioica* L., inhibit cell wall synthesis through formation of irreversible complexes with proteins.[49]

Saponins have been previously reported to cause leakage of protein and enzymes from the cell by complexing with sterols in the cell membrane.[50] They were the most effective anti-dermatophytic compound in *Polyscias fulva* (Hiern) Harms.[51] Saponins have also been studied in the context of *Candida* which have also concluded alteration to cell membranes as the main mechanism.[52] Increased permeability to propidium iodide for *Candida* in a study by Pinto (2009) suggests that there is a primary lesion of the cell membrane leading to cell death.[53]

Terpenoid phenols also exhibit strong broad-spectrum antifungal activity. They include eugenol, which was reported as a main constituent of thirteen antifungal plants, as well as thymol and cavacrol found in *Thymus vulgaris* L., *T. pulegioides* and *Origanum vulgare* L. Essential oil of *O. gratissimum* containing 57.8% eugenol, viewed under transmission electron microscopy, caused morphological changes to dermatophyte hyphae, with damages to cell wall and membranes and expansion of endoplasmic reticulum.[54] Flow cytometric studies with lemon thyme and active compounds thymol and cavacrol, showed lesion formation in the cytoplasmic membrane and a reduction on ergosterol content.[25] Suggested mechanisms of cell membrane disruption and cell death occur include calcium stress (increased cytosolic calcium leading to loss of cell viability) and inhibition of the Target of Rapamycen (TOR) pathway.[55]

Interference of hyphal growth and spore germination contributes to the anti-dermatophytic action of plant extracts. Extract of *Tetradenia riparia* (Hochst.) showed strong inhibition and irregular growth pattern of hyphae through scanning electron microscopy.[56] Additionally, essential oil volatiles of *Agathosma betulina* (P.J.Bergius) Pillans inhibited spore production of *T. rubrum*.[57] Further subcultures of mycelia did not result in growth suggesting strong fungicidal action due to irreversible damage.[57] This is significant, as spores are asexual reproductive structures which can initiate skin infections and are responsible for transmission in the community. The hydroalcoholic extract of *Piper regnellii* was further able to inhibit spore germination in a dose-dependent manner, and 7.8 μg ml^{-1} inhibited *T. rubrum* spore generation by 100%.[57] Nail fragments that were saturated and immersed in *P. regnellii* extract at a concentration of 1.2 $mgml^{-1}$ and inoculated with spore suspension did not experience mycelial growth, whereas the nail fragments not exposed to the plant extract had vigorous growth.[58]

Other compounds investigated for their mechanism of action include monoterpenes, terpenoids generally and sesquiterpenoids. Macrocarpal C, a sesquiterpenoid isolated from *E. globulus,* was effective against *T. mentagrophytes* though several mechanisms including increased membrane permeability, increased intracellular reactive oxygen species and induction of apoptosis through DNA fragmentation.[21] Monoterpenes found abundantly in essential oils include geranial, geraniol, citral and citronellol are suggested to mediate their antifungal activity through inhibition of spore production, and binding of ergosterol resulting in fungal cell membrane destabilisation.[57, 59]

In vivo anti-inflammatory and immunomodulatory actions of anti-dermatophytic compounds

In addition to direct anti-dermatophytic effects, plant compounds have anti-inflammatory and immunomodulatory effects, which can enhance treatment effects *in vivo*. Extract of *Salacia senegalensis* (Lam.) DC had inhibitory effects against soybean 5-lipoxygenase, an important enzyme in the inflammatory and oxidative response in humans.[60] Immunomodulation has also been proposed in studies where *in vivo* anti-dermatophytic efficacy were observed, but *in vitro* efficacy is not, by enhancement of immune self-resolution. For instance, in guinea-pigs with induced *M. canis* dermatophytosis, *U. dioica* extract at 100 mg ml^{-1} concentration did not achieve *in vitro* activity, but an *in vivo* assay demonstrated efficacy comparable to terbinafine, and was significantly better than negative control.[61] In another *in vivo* study of *Astragalus verus* Olivier in *T. verrucosum* infected guinea-pigs, 40% concentration was comparable to positive control terbinafine. However, lesions improved in the negative control at 13 days suggesting resolution is partially contributed by immune response. Immunomodulatory mechanisms of *Astralagus* were studied by Guo *et al*., (2016), showing dose-dependent inhibition of over-production of cytokines TNF-α, IL-1β, IL-6 and IFN-γ in LPS-stimulated macrophages.[62]

Current limitations in research, and future prospects

Despite numerous *in vitro* studies demonstrating antifungal activity, only a small proportion utilise rigorous methodology as required for robust evidence. Cos *et al*. (2006) outline a set of parameters and

efficacy criteria for high quality evaluation of anti-infective potentials of natural products.[63] These include use of reference strains, *in vitro* models on the whole organism, cytotoxicity testing, dose ranges reflecting a practical inhibitory concentration, and use of positive and negative controls. An endpoint criteria for all anti-infective bioassays was recommended as having usual IC50 values (the minimum drug concentration inhibiting 50% of fungal growth), below 100 μg ml^{-1}, and below 25 μM for pure compounds.[63] Most anti-fungal studies have reported MIC (the lowest drug concentration inhibiting visible fungal growth). However, endpoint criteria for antifungal efficacy and cytotoxicity assays are rarely stated in *in vitro* studies. Considering this, the number of plants having significant anti-dermatophytic properties may be exaggerated. Commonly utilised antimicrobial susceptibility testing methods include standardised protocols by CLSI or EUCAST, whilst some studies use agar well diffusion methods or poisoned food techniques.[64]

In vitro efficacy also may not translate into *in vivo* efficacy, particularly in humans. Many fungal, host factors such as immunosuppression and compliance, suboptimal absorption and penetration are all clinically relevant.[6] Importantly, penetration of antifungals into the stratum corneum and its persistence is important in achieving cure. Most *in vivo* data is based on animal models rather than clinical trials, which may undermine clinical applicability given differences in virulence factors and immune responses.[65] For example, *T. mentagrophytes,* a zoophilic species, tends to cause an acute inflammatory response whereas *T. rubrum,* an anthropophilic species, causes minimal inflammation.[65] Clinical trials show some encouraging results but generally constitute insubstantial evidence, given limitations of small sample size and methodological flaws.

Possible adverse outcomes of plant therapies such as contact dermatitis[17] should also be considered, in case of indiscriminate use for commercial purposes. For example, plants belonging to Rutaceae, the citrus family, or Moraceae, can cause phytophotodermatitis owing to photosensitising compounds furocoumarins and psoralens.[66] This can be clinically overlooked if the extract is not applied to sun-exposed areas, or are only momentarily used to prevent colonisation.[67] Alternatively, these undesirable components can be removed, such as furocoumarin-free extracts of bergamot oil, which retained their anti-dermatophytic activity in *in vitro* studies.[68] In most animal and human studies of anti-dermatophytic plants, minimal to no serious adverse effects have been reported from topical application, noting small sample sizes. For example, in one study evaluating 5% *Melaleuca alternifolia* oil in cream for toenail onychomycoses, four of 40 patients reported only mild inflammation.[30] Nonetheless, the risk of adverse cutaneous effects to topical plant extracts in general should be acknowledged. In one multi-centre study of 1274 patients using topical botanical products, 11% of patients reported adverse cutaneous reactions based on a questionnaire, and 16% showed positive reactions when tested in a botanical series.[69] In studies using cell cytotoxicity assays, optimistic results may be seen with minimal reports of toxicity. For example, in a study evaluating *S. senegalensis* leaves, human epidermal HaCaT keratinocytes experienced no detrimental effects at concentrations displaying anti-dermatophytic and anti-inflammatory properties. Cytotoxic activity was also evaluated for 2.5 μL ml^{-1} *A. major* oil, β-ocimene and α-pinene using a haemolytic activity assay and found 10%, 10% and 15% haemolysis respectively.[70] This shows promise for natural agents with high antifungal activity and low toxicity. However, significantly more cytotoxic studies are required to establish stronger safety evidence for plant-based treatments.

Antifungal resistance is a key global health problem and innovation and testing of natural products holds enormous potential. However, the mechanisms of plant extracts responsible for activity against drug resistance are not well investigated. Inhibition of multidrug (MDR) efflux pumps, and normal cell communication (quorum sensing) have been described as some of the key mechanisms against bacterial MDR pathogens.[71] Synergy between diverse plant constituents is suggested to play a key role in the effectiveness of herbal medicine in anti-microbial resistance.[72] As previously discussed, the anti-fungal mode of action of plants is due to numerous potential bioactive compounds and therefore multiple targets. Unlike agents with single constituents and targets, the presumed chances of development of resistance are lower with multiple targets. However, there is little research on whether fungi or other microbes will develop resistance against plants similar to the mechanisms of resistance evolved against current pharmaceuticals. Scientific evaluation of complex synergistic interactions is furthermore a likely challenging and costly process, without fully developed technology to study these mechanisms.[71]

Significant synergistic antifungal activity with conventional antifungals highlights the potential for combined drug therapy. For instance, the combination of essential oils with topical drugs can enhance penetration to deeper skin layers via abundant low molecular weight terpenes.[73] Combined therapy may also reduce therapeutic doses, improving side effect

profiles, mycological clearance and development of resistance.[74]

Despite the comprehensive search strategy used here it is likely many plant extracts with antifungal potential were not located, due to the innumerable diversity of the plant kingdom and ethnomedical studies not published in electronic databases.

Conclusions

Superficial fungal infections are highly prevalent and the prevalence of chronic and resistant cases is increasing, highlighting the need for novel anti-dermatophytic compounds. Based on numerous *in vitro* studies, many plant extracts demonstrate anti-dermatophytic efficacy. The plant extracts or isolated compounds have significant potential to be used in many contexts: directly applied alone - particularly in third world countries without readily accessible commercial antifungal drugs; incorporated into existing antifungal drugs for synergistic action; or further explored as novel antifungal drugs. Despite this, very few of these studies have had *in vivo* testing or clinical trials to assess their safety and effectiveness, although those conducted so far have been promising. Further *in vivo* studies will enable exploration and investment into new clinically effective anti-dermatophytic compounds, which is essential in the face of emerging dermatophyte resistance.

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Angela Mei *et al*, Plant-based therapies for dermophyte infections, Appendix 1: *In vitro* studies of anti-dermatophytic plants and phytochemical composition, methodology and results, by family. ND: Not determined. NCCLS: National Committee for Clinical Laboratory Standards.

Ref	Family and Scientific name	Phytochemical composition	Antimicrobial susceptibility method	Anti-dermatophytic activity
Lamiaceae				
56	*Rosmarinus officinalis L.*	Terpinen-4-ol, 1,8-cineole	Broth microdilution	Hydroalcoholic extract MIC 62.5 – 250 μgml^{-1} Amphotericin B MIC 1.9 – 15.6 μgml^{-1}
75		ND	Disc diffusion (NCCLS)	Essential oils did not inhibit tested fungi. Alcoholic plant extracts may confer antifungal activity.
75	*Thymus vulgaris L.*	O-cymene, γ-terpinene, thymol, carvacrol	Disc diffusion	Two-fractions of oil complete growth inhibition of all six dermatophytes
19		ND	Broth macrodilution	Essential oil MIC 72-288 μgml^{-1} Thymol MIC 192 μgml^{-1}
27		ND	Broth microdilution	Essential oil MIC 0·5 – 1 $mgml^{-1}$ Thymol MIC 0.25 – 1 $mgml^{-1}$
56	*Tetradenia riparia (Hochst.) Codd*	ND	Broth microdilution	Hydroalcoholic extract MIC 62.5-25 µg/ml
25	*Thymus pulegioides L.*	Carvacrol, thymol, p-cymene and c-terpinene	Broth microdilution (NCCLS)	Essential oil MIC 0.16 – 0.32 μlml^{-1} Thymol MIC 0.08 – 0.16 μlml^{-1} Carvacrol MIC 0.04 – 0.08 μlml^{-1} Fluconazole MIC 16 – 128 μgml^{-1}
76	*Ocimum gratissimum L.*	1 -Methoxy-4-(2-propenyl) benzene, 2-methoxy-4-(I-propenyl) fenol, 1,8-cineole, β-pinene, transcaryophyllene	Disc diffusion	Essential oil inhibition zone 30mm diameter
54		Eugenol	Agar dilution	Eugenol 250 μgml^{-1}, essential oil 250 μgml^{-1} and hexane extract at 125 μgml^{-1}inhibited 100% of dermatophyte growth Itraconazole MIC 0.97-125 μgml^{-1} for all isolates.
77		Thymol, p-cymene and γ-terpinene	Agar dilution	Essential oil MIC 80 - 150 $\mu l\ L^{-1}$
78		ND	Poisoned food technique	Essential oil MIC 50ppm 100% inhibition at 500ppm
79	*Ocimum sanctum L.*	Steroids, terpenoids, flavonoids, phenolic compounds, lignin proteins and carbohydrates.	Broth microdilution	Benzene fraction of leaf MIC 200 μgml^{-1}
18		Eugenol, β-elemene, methyl eugenol, trans caryophyllene	Broth microdilution	Essential oil MIC 0.4 – 0.8 μgml^{-1}
80	*Mentha piperita L.*	ND	Poisoned food technique	Essential oil MIC 0.1 to 1.5 $\mu l\ ml^{-1}$ Inhibition zone 36 – 44mm diameter
26		Menthone and Menthol	Broth microdilution	Essential oil MIC 0.125 – 0.5% v/v Synergism with Itraconazole observed Menthol MIC 0.06% v/v
81	*Thymus serpyllum L.*	p-cymene, carvacrol, y-terpinene	Microdilution	Essential oil MIC 0.1% (v/v)
75	*Origanum majorana L.*	terpinen-4-ol and thymol	Disc diffusion	Essential oil (colourless and yellow) inhibition zones of 36 - 47mm
81	*Origanum vulgare L.*	Thymol, carvacrol, y-terpinene	Broth microdilution	Essential oil MIC 0.5% (v/v)
Myrtaceae				
82	*Syzygium aromaticum (L.) Merr. & L.M.Perry*	Eugenol	Agar diffusion	Essential oil at 0.2 $mgml^{-1}$ inhibited greater than 60% hyphal growth
53		Eugenol	Broth microdilution	Essential oil MIC 0.16ml ml^{-1}
83		Eugenol. β-caryophyllene, eugenyl acetate	Agar well diffusion	Essential oil MIC 9 $\mu l\ ml^{-1}$
19		Eugenol	Broth microdilution and disc diffusion	Essential oil MIC 160 μgml^{-1} Synergistic effects observed with fluconazole decreasing to MIC 1.25 μgml^{-1} with fluconazole Eugenol MIC 80 μgml^{-1}, decreasing to MIC 2.5 μgml^{-1} with fluconazole
18		Eugenol, trans-caryophyllene	Broth microdilution	Essential oil MIC 0.2-0.4 μgml^{-1}
84	*Melaleuca alternifolia (Maiden & Betche) Cheel*	ND	Agar dilution	Essential oil MIC 1431 μgml^{-1} Miconazole of 0.2 μgml^{-1}
85		ND	Broth microdilution	Essential oil MICs 0.004% - 0.06%
86		ND	Nanocapsule suspensions against onychomycosis model	After 1 week of treatment a significant decrease in cell count was observed
87		ND	Agar dilution	Essential oil MIC 0.1%
23		Terpinen-4-ol, g-teripinene, α - terpinine, 1,8-cineole, terpinolene	Broth microdilution	Essential oil 0.12% to 1% Terpinen-4-ol MIC 0.03-0.2% MIC 1,8-cineole higher than that of tea tree oil
27	*Eucalyptus globulus Labill.*	ND	Broth microdilution	Oil fraction MIC 0·125–0·25 $mgml^{-1}$
46		ND	Microdilution	Essential oil activity better than 1% Nystatin against T mentagrophytes but much lower than 1% clotrimazole
21		Macrocarpal C	Microbroth dilution	Macrocarpal C MIC 1.95 $\mu g\ ml^{-1}$ Terbinafine hydrochloride 0.625 $\mu g\ ml^{-1}$ Nystatin 1.25 $\mu g\ ml^{-1}$

Ref	Family and Scientific name	Phytochemical composition	Antimicrobial susceptibility method	Anti-dermatophytic activity
Myrtaceae (continued)				
88	*Eucalyptus globulus Labill.*	ND	Agar well diffusion	Ethanol and water extract mean inhibition zone 26mm
76	*Eugenia uniflora L.*	Furanodiene, y-elemene, transocirnene, 2-β-pinene, y-terpinene	Disc diffusion	Essential oil inhibition zone 20mm
89		ND	Broth microdilution	Leaf ethanolic extract MIC 31.3 – 62.5 μgml^{-1}
36	*Eucalyptus pauciflora Sieber ex Spreng.*	ND	Poisoned food technique	Essential oil MIC 1.0 $\mu l\ ml^{-1}$
46	*Eucalyptus citriodora Hook.*	n-hexyl acetate, butyl formate, allyl valerate, n-dodecane.	Broth microdilution	Essential oil at 100% concentration caused 100% inhibition growth of dermatophytes
90	*Eucalyptus camaldulensis Dehnh.*	ND	Broth macrodilution	Hydro-alcoholic extract of leaves MIC 0.6 - 0.9 $mgml^{-1}$
91		ND	Agar diffusion	Distilled water extract MIC 0.2 - 0.4 $mgml^{-1}$
92		ND	Broth microdilution and Agar dilution	Methanol extracts of leaves MIC $0.4^{-1}.6\ mgml^{-1}$ using agar dilution and 0.2 to 1.6 $mgml^{-1}$ using broth dilution Griseofulvin MIC 0.001 - 0.008 $mgml^{-1}$
93	*Gully gum Eucalyptus smithii F.Muell. ex R.T.Baker*	1,8-cineole, α-terpineol	Broth microdilution (NCCLS)	Essential oil MIC 62.5 μgml^{-1} to >1,000 μgml^{-1}
Fabaceae				
89	*Libidibia ferrea (Mart. ex Tul.) L.P.Queiroz*	ND	Broth microdilution	Ethanolic extract of leaves MIC 31.3 μgml^{-1}
94	*Mimosa tenuiflora Benth.*	DMT (hallucinogenic) Indole alkaloids, chalcones, tannins	Agar dilution	Ethanol extract MIC 10ug ml^{-1}
95	*Cassia occidentalis (L.) Rose*	ND	Agar and broth dilution	Alcohol and acetone extract MIC 5000 μgml^{-1}
95	*Cassia tora L.*	ND	Agar and broth dilution	Methanol, alcohol, acetone, chloroform and petroleum ether extracts MIC 5 - 20 $mgml^{-1}$
96	*Acacia nilotica (L.) Delile*	ND	Poisoned food technique	10% ethyl acetate extract 22 - 29mm diameter of inhibition
Rutaceae				
57	*Agathosma betulina (P.J.Bergius) Pillans*	Limonene, menthone, isomenthone, disophenol, geranial	Diameter of mycelia growth inhibition on agar	40µL of essential oil inhibited *T. rubrum* growth with a fungal growth index of 2.3%.
68	*Citrus bergamia Risso*	ND	Broth microdilution	Natural essence MIC (v/v) 0.156% - 2.5% Distilled extract MIC 0.02% - 2.5% Furocoumarin-free extract MIC 0.08% - 1.25%
97		ND	Agar dilution	Bergamot oil 1.25% dilution - inhibition zone of 28mm 2.5% dilution - inhibition zone 32 mm diameter
98	*Aegle marmelos (L.) Correa*	Trace amounts of triterpenoids, phenolic compounds, tannins and flavonoids	Broth microdilution	Methanol, ethanol and water extract MIC 200 $\mu g\ ml^{-1}$
67	*Citrus acida Pers.*	ND	Agar diffusion	Fresh lime juice yielded zones of inhibition surrounding lime, miconazole and econazole
Zingiberaceae				
99	*Curcuma longa L.*	ND	Agar well diffusion	Essential oil in ethylene glycol MIC 114.9 – 919.2 μgml^{-1}
100		ND	Disc diffusion and microdilution	Essential oil MIC 1.4 – 1.6 $\mu l\ ml^{-1}$
101		Ar-turmerone, atlantone, zingiberone	Broth dilution	Turmeric oil cream MIC 312 μgml^{-1} Ar-turmerone MIC 1.56 – 6.25 μgml^{-1} Ketoconazole MIC 4.9 – 7.81 μgml^{-1}
76	*Alpinia speciosa (Blume) D.Dietr.*	1 ,E-Cineol, α-terpinene, P-pinene, α-pinene, transcaryophyllene	Disc diffusion	Essential oil up to 20mm diameter of inhibition
100	*Zingiber officinale Roscoe*	ND	Microdilution and disc diffusion	Essential oil MIC 0.05 – 0.06 $\mu l\ ml^{-1}$ with a 36 - 69mm diameter inhibition zone Clotrimazole 36mm inhibition zone Ketoconazole 60mm inhibition zone Essential oil combined with *Curcuma longa* MIC 0.02µl ml^{-1} and 82mm inhibition zone
Piperaceae				
28	*Pothomorphe umbellata (L.) Miq.*	Octadecanoic acid, pentasocanoic acid, tetracosanoic acid, tricosanoic acid, eicosanoic acid, b-stigmasterol and nerolidol	Plate microdilution	Ethanol extract MIC 156.25 μgml^{-1} and methanol extract 78.13 μgml^{-1} against multi-drug resistant strains of *T. rubrum*
18	*Piper betle L.*	Eugenol, eugenol acetate	Broth microdilution	Essential oil MIC 0.2-0.4 μgml^{-1}
58	*Piper regnellii (Miq.) C.DC.*	Eupomatenoid-3 & eupomatenoid-5	Broth microdilution (NCCLS)	Hydroalcoholic leaf extract MIC 15.6 - 62.5 μgml^{-1} Pure eupomatenoid-3 and eupomatenoid-5 MIC 50 μgml^{-1} and 6.2 μgml^{-1} respectively

Ref	Family and Scientific name	Phytochemical composition	Antimicrobial susceptibility method	Anti-dermatophytic activity
Apiaceae				
24	*Thapsia villosa L.*	limonene and methyleugenol	Macrodilution	Essential oil MIC 0.64^{-1}.25 µl ml^{-1} Methyleugenol MIC 0.32 µl ml^{-1} Limonene MIC 0.08-0.16 µl ml^{-1} Fluconazole MIC 16^{-1}28 µgml^{-1}
102	*Foeniculum vulgare Mill.*	Trans-anethoe, pinene, fenchone	Broth microdilution	Essential oil MIC 0.039-0.078 µgml^{-1} Fluconazole MIC 25^{-1}00 µgml^{-1} Amphotericin B MIC 0.156-0.132 µgml^{-1}
70	*Angelica major Gilib.*	α-pinene and cis-β-ocimene	Broth microdilution (NCCLS)	Essential oil from aerial parts MIC 0.32–0.64 µL ml^{-1}
Lauraceae				
76	*Cinnamomum zeylanicum Blume*	α-Pinene, 1,8-cineole, L-limonene, L-P-pinene	Disc diffusion	Essential oil inhibition zone 30mm diameter
19	*Cinnamomum verum J.Presl*	Cinnamaldehyde	Broth microdilution, disc diffusion assay	Essential oil MIC 80—160 µgml^{-1} Cinnamaldehyde MIC 40-80µg/ml against resistant strains of dermatophytes to fluconazole and itraconazole. Zone of inhibition 42.66 mm. Synergism is also exhibited as cinnamaldehyde reduced the MIC fluconazole from 200 µgml^{-1} to 25 µgml^{-1}.
Euphorbiaceae				
103	*Croton urucurana Baill.*	ND	Tube dilution and paper disk diffusion assay	Dragon's blood in sterile water MIC 1.25-2.5 mgml^{-1}, and inhibition zone range of 7.6-26.9mm Griseofulvin 25.2-34mm diameter inhibition
88	*Croton macrostachyus Hochst. Ex Delile*	ND	Agar well diffusion	Ethanol and water extract mean inhibition zone 23mm diameter
Poaceae				
29	*Cymbopogon citratus (DC.) Stapf.*	Citral	Disc diffusion, broth macrodilution, time-kill methods and checkerboard microtiter tests	Essential oil MIC 288 µgml^{-1} Citral MIC 72 µgml^{-1} Fluconazole and Itraconazole MIC 200 µgml^{-1} Citral, but not *C citratus* also had synergistic antifungal effect with fluconazole.
76		E-Citral, Z-citral, 2-P-pinene	Agar Diffusion	Essential oil *C. citratus* and Citral inhibited zones of diameter 24.7-32.6mm against azole resistant strains of T rubrum. All tested combinations had synergistic activity with fluconazole against T. rubrum.
104	*Cymbopogon martini (Roxb.) W. Watson*	trans-geraniol, b-elemene, E-citral and linalool	Poisoned food technique	Essential oil MIC 150 - 200ppm
105		ND	Broth microdilution	Volatile oil MIC 1-2µl ml^{-1}
Berberidaceae				
106	*Nandina domestica Thunb.*	ND	Disc diffusion	Essential oil, n-hexane, chloroform, ethyl acetate, and methanol extracts 31.1-68.6% and 19.2-55.1% anti-dermatophytic effect and MIC 62.5-2000 µgml^{-1}
Meliaceae				
107	*Azadirachta indica A.Juss.*	ND	Broth microdilution	Ethanol, ethyl acetate and hexane extract MIC 31 µgml^{-1}
108		ND	Broth microdilution	Methanol and hexane extract leaves MIC 50 – 200 µgml^{-1} Seed oil MIC 625 µg/ml – 2500 µgml^{-1} Terbinafine MIC 0.0019 – 0.0313 µgml^{-1}
109		Terpenoids	Broth microdilution	Ethanolic, ethyl acetate and hexane extract MIC 125 – 500 µgml^{-1}
Moringaceae				
110	*Moringa oleifera Lam.*	pentacosane, hexacosane, (E)-phytol and 1-[2,3,6-trimethylphenyl]-2-butanone	Broth microdilution	Crude essential oils MIC 0.2 - 1.6 mgml^{-1} Essential oils MIC80 43.5^{-1}33 µgml^{-1} Ketoconazole was 0.125 µgml^{-1}
111		alkaloids flavonoids, glycosides, tannins, triterpenoids and steroids.	Disc diffusion and broth dilution	Methanol and ethyl acetate extracts MIC 1.56 - 6.25 mgml^{-1}
Vitaceae				
48	*Vitis vinifera L.*	monomeric and polymeric flavan-3-ols.	Broth dilution	Ethanol seed extracts MIC 20 - 97 µgml^{-1} MICs inversely correlated with polymeric fraction of flavan-3-ols but weakly correlated with the monomeric fraction.
Lythraceae				
112	*Lawsonia inermis L.*	ND	Agar dilution	Hexane extract MIC 625ug ml^{-1}
113		ND	Broth dilution assay	Chloroform, methanol and aqueous extracts MIC 3.12 $^{-1}$2.5 mgml^{-1}
114		ND	Macroscopic broth dilution and agar disc diffusion	Crude methanol and aqueous extract MIC 25 mgml^{-1} Methanol diameter zones of inhibition 8.0 mm - 18.8. Aqueous extract diameter zones inhibition 9.0 mm - 14.5 mm.
115		ND	Broth microdilution	Ethanol and petroleum extracts MIC 5 – 10 mgml^{-1}
116		ND	Broth microdilution	Ethanol, ethyl acetate and hexane extracts MIC 31.25-62.5 µgml^{-1}
95		ND	Agar and broth dilution	Acetone, ethyl acetate, chloroform and pet. ether extracts 100% inhibition growth *T. rubrum* at 40 mgml^{-1}
47	*Punica granatum L.*	Gallic acid	Gallic acid (NCCLS)	Gallic acid MIC 43.75 µgml^{-1} Comparable to fluconazole 10.4 µgml^{-1}
117		Tannins (ellagic acid, gallic acid), polyphenols , punicalagin	Broth microdilution and disc diffusion (NCCLS)	Crude hydroalcoholic plant extract MIC 125 – 250 µgml^{-1} Punicalagin MIC 62.5 µgml^{-1} Nystatin 0.78 µgml^{-1}

Ref	Family and Scientific name	Phytochemical composition	Antimicrobial susceptibility method	Anti-dermatophytic activity
Asteraceae				
95	*Xanthium indicum Klatt*	ND	Agar and broth dilution	Methanol, alcohol, acetone, ethyl acetate and chloroform MIC 20 $mgml^{-1}$
118	*Ageratina pichinchensis (Kunth) R.M.King & H.Rob.*	Chromenes	Agar dilution	Active components of the n-hexane extract: Encecalin MIC 6.2 – 12.5 μgml^{-1} Taraxerol MIC 12.5 μgml^{-1} β-eudesmol MIC 25 μgml^{-1} Miconazole 0.004 μgml^{-1}
Phytolaccaceae				
88	*Phytolacca dodecandra L'Hér.*	ND	Agar well diffusion	Ethanol and water extract mean inhibition zone 20mm
Araliaceae				
50	*Polyscias fulvaI (Hiern) Harms*	Crude extract: ethyl acetate, n-butanol and residue fractions: saponins, tannins, alkaloids, anthraquinones and phenols. Hexane fraction: only alkaloids.	Broth microdilution and well diffusion	The ethyl-acetate, n-butanol and residue fractions displayed the highest anti-fungal activity. MIC 0.125-0.5 $mgml^{-1}$
119		phenolics, steroids, triterpene and terpenoid saponins	Broth microdilution	Dichloromethane-methanol extract from the stem bark MIC 0.78 $^{-1}$00 μgml^{-1}. Saponins most active against tested dermatophytes
Faboideae				
120	*Astragalus verus Olivier*	ND	Disc diffusion	Aqueous extract MIC 160 $mgml^{-1}$ Acetone and methanol extracts MIC 320 $mgml^{-1}$
Pinaceae				
121	*Picea abies (L.) H. Karst.*	p-coumaric acid, resin acids and ligans	Agar diffusion tests	Salve with a resin concentration at 20% caused a fungicidal effect with a significant inhibition zone for all dermatophytes.
Combretaceae				
122	*Terminalia chebula Retz.*	phenolic, tri-terpinoid and tannin	Broth microdilution (NCCLS)	Ethanolic and aqueous extract MIC 3.125-6.25 μgml^{-1} Itraconazole MIC 3.125 μgml^{-1} Fluconazole 50 μgml^{-1}
123		ND	Microbroth dilution	Stem and stem bark MIC 0.039 $mgml^{-1}$ and inhibition zone 41.5+-0.5mm diameter. Apigenin (flavonoid) most active antifungal component
Amaranthaceae				
104	*Chenopodium ambrosioides L.*	m-Cymene, myrtenol, alpha-terpene	Poisoned food technique	Essential oil MIC 350 - 700ppm Griseofulvin, ketoconazole and fluconazole MIC 1000-5500ppm
124		Absence of phenols	Poisoned food technique	Essential oil MIC 50ppm
Moraceae				
125	*Ficus exasperate Roxb.*	ND	Well-in-agar diffusion	Ethanolic extract MIC 25.12 – 44.67 $mgml^{-1}$ Diameter zones of inhibition between 13.00 and 22.45mm.
Ranunculaceae				
126	*Nigella sativa L.*	Thymoquinone; p-cymene	Broth macrodilution	Essential oil MIC 4 $mgml^{-1}$
127		ND	Agar diffusion	Ether extract MIC 40mg/ml Thymoquinone MIC 0.125-0.25 $mgml^{-1}$ Griseofulvin: 0.00095 – 0.0155 $mgml^{-1}$
128		ND	Broth microdilution	Essential oil MIC 2-4 $mgml^{-1}$
129		ND	Disc diffusion	Essential oil inhibition zones 20-35mm diameter
Caprifoliaceae				
130	*Lonicera japonica Thunb.*	ND	Poisoned food technique	Essential oil MIC 62.5 – 500 μgml^{-1}
Hypericacacea				
131	*Hypericum perforatum L.*	Terpinen-4-ol, terpinolene, 1,8-cineole, c-terpinene, a-terpinene, qcymene, a-terpineol and b-myrcene	Broth microdilution	Essential oil MIC 0.01% - 0.06% v/v T. tonsurans Griseofulvin 0.5 – 1 μgml^{-1}
132		Xanthones	Broth microdilution	Control roots MIC 125-500 μgml^{-1} Chitosan treated roots to enhance xanthone content MIC 64–125 μgml^{-1} Amphotericin MIC 0.125-8 μgml^{-1} Fluconazole MIC 1-32 μgml^{-1}
133		Glycosides, flavonoids, oils, phenols, amino acids, tannins and terpenoids	Broth microdilution	Methanol extract MIC 0.1-0.6 $mgml^{-1}$
Primulaceae				
134	*Anagallis arvensis L.*	ND	Serial agar dilution plate technique	Aqueous extract 15 μgml^{-1} fully inhibited *T. violaceum* growth, and inhibited the growth of *M. canis* and *T. mentagrophytes* to a great extent
133		Glycosides, saponins, Alkaloids, phenols, terpenoids	Broth microdilution	Methanol extract MIC 0.4^{-1}.9 $mgml^{-1}$
Araceae				
135	*Pistia stratiotes L.*	ND	Broth microdilution	Methanolic extract MIC 250 µg ml^{-1}
Polypodiacaeae				
136	*Drynaria quercifolia (L.) J. Sm.*	Coumarins and triterpenes	Agar disc diffusion	Di-ethyl ether extract 25mm inhibition zone Ethanol extract did not show inhibitory activity up to concentration of 20 $mgml^{-1}$
Convolvulaceae				
137	*Argyreia nervosa (Burm. F.) Bojer*	Flavonoids and glycosides, steroids and tannins	Disc diffusion	Ethyl acetate of leaves extract MIC 70-80 μgml^{-1}

Ref	Family and Scientific name	Phytochemical composition	Antimicrobial susceptibility method	Anti-dermatophytic activity
Geraniaceae				
27	*Pelargonium graveolens L.'Her.*	Citronellol and geraniol	Broth microdilution, disc diffusion assay and checkerboard microtitre assay	Essential oil MIC 0·25–2 $mgml^{-1}$ Essential oil fraction and main components exhibited synergistic activity with ketoconazole (MIC 0.18-0.38 $mgml^{-1}$)
Celastraceae				
60	*Salacia senegalensis (Lam.) DC.*	Quercetin, myricitrin (flavonoids)	Broth microdilution	Hydroethanolic extract MIC <1 – 4 $mgml^{-1}$
Caesalpiniaceae				
95	*Caesalpinia bonducella (L.) Fleming*	ND	Agar and broth dilution	Ethyl acetate extract 100% inhibition growth dermatophytes at 10 $mgml^{-1}$
Urticaceae				
61	*Urtica dioica L.*	Coumarins, saponins, tannins, moderate content of triterpenes, steroids and cardiac glycosides	Disc diffusion	No antifungal activity at 100 $mgml^{-1}$for both aqueous and hydroalcoholic extracts
138		ND	Agar dilution	Cold methanol extract of whole plant inhibited growth at rate of 4.5mm per day against 15.4mm for control
Rubiaceae				
139	*Borreria verticillate (L.) G. Mey.*	Flavonoids, terpenes, saponins, glycosides. Absence of phenols	Agar well diffusion and broth dilution	Crude extract MIC 512 $mgml^{-1}$ Terbinafine MIC 64 $mgml^{-1}$
Apocynacea				
140	*Calotropis procera (Aiton) Dryand*	Alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone, and triterpenoids.	Agar incorporation method	100% undiluted latex inhibited mycelial spread diameter significantly less than negative control
141		ND	Agar dilution	Ethanolic leaves extract MIC 25 0 μgml^{-1}
142		ND	Paper disc diffusion	Ethanolic extract MIC 125ppm and maximum inhibitory zone 9.3 - 12.5mm.
143		Saponins, tannins, sesquiterpenes and alkaloids	Agar diffusion	Aqueous extract, MIC 0.5-0.9 $mgml^{-1}$ Zones of inhibition were comparable to griseofulvin

Mei *et al*, Plant-based therapies for dermophyte infections, Appendix 2: *In vivo* animal studies of anti-dermatophytic plants.

Ref	Scientific name	Study animals	Intervention	Control	Main results
99	*Curcuma longa* L.	Guinea pigs with induced *T. rubrum* infection	Turmeric oil (1: 80 dilution)	Canesten, Nil treatment	Turmeric oil reduced erythema and scale by 2-3 days, and lesions disappeared by day 7. Canesten decreased the erythema on the sixth day. Control group had increasing scale over 3 weeks.
50	*Polyscias fulva* (Hiern) Harms	12 guinea pigs with induced *T. menta-grophytes* infection	Plant extract-oil at concentrations (1.25, 2.5 and 5%)	5% Griseofulvin-oil, negative control oil vehicle	At 14 days, 5% formulated extract-oil was comparable to griseofulvin-oil (5%) in microbial eradication.
119	*Astragalus verus* Olivier	25 male albino guinea pigs with induced *T. verrucosum* infection	10%, 20% and 40% concentrations of aqueous extract of *A. verus*	Negative control, Vehicle group, 1% terbinafine	At 7 days, significant reduction in lesion scores was seen in 20%, 50% aqueous extract and terbinafine groups compared to negative control group. Terbinafine was significantly better than 10% and 20% aqueous extract groups in lesion scores.
61	*Urtica dioica* L.	Guinea pigs with induced *M. canis* infection	Stinging nettle 10%, 20% and 30% hydroalcoholic extracts	No treatment, 1% terbinafine, 10% DMSO (Vehicle)	At 30 days, only 30% stinging nettle and terbinafine had significant net clinical efficacy compared to negative control. No *in-vitro* activity recorded.
144	*Thymus vulgaris* L.	25 Wistar rats with induced *Trichophyton* infection	1% essential oil of *T. vulgaris*	Bifonazole cream, No treatment	Rats were cured after 24- 37 days of treatment with essential oil and 14^{-1}5 days with bifonazole. Untreated rats were symptomatic throughout 37 days of observation.
123	*Terminalia chebula* Retz.	30 mice with induced *T. mentagrophytes* infection	Isolated apigenin at 2.5mg g^{-1} and 5mg/g	Terbinafine 5mg g^{-1} , No treatment	After 12 days, complete cure was recorded for 5mg g^{-1} of apigenin and terbinafine with no significant difference. Apigenin 2.5 mg g^{-1} achieved cure on the 16^{th} day of treatment.
112	*Lawsonia inermis* L.	15 naturally infected goats with ringworm	Henna paste, Aqueous extract Ethanolic extract	Clotrimazole cream Negative control	Disappearance of the lesion at 30 days for henna paste, aqueous extract and ethanol extract, 27 days for clotrimazole, and 70 days for control group. Significant differences were between treated groups and negative control group only.
104	*Cymbopogon martini* (Roxb.) W.Watson *Chenopodium ambrosioides* L.	Guinea pigs with induced dermatophytosis with *T. rubrum* and *M. gypseum*	Essential oils of *C. martini, C. ambrosioides* and a combination of essential oils	Negative control (petroleum jelly)	*C. martini* achieved complete cure of infection (by culture) at day 17 and 21 for *T. rubrum* and *M. gypseum,* respectively. *C ambrosioides* and oil combination treated the disease at day 21. The control had 100% culture recovery throughout the observation period. No statistical analysis.
124	*Chenopodium ambrosioides* L.	Guinea pigs with induced dermatophytosis.	Essential oil ointment	None	*Chenopodium* ointment cured the infection on day 15 for *T. mentagrophytes* and day 13 for *M. andouinii* infection with zero percent culture recovery.
81	*Thymus serpyllum* L., *Origanum vulgare* L. and *Rosmarinus officinalis* L.	21 sheep infected with *T mentagrophytes* in an outbreak	13 sheep with mixture of 5% *O. vulgare,* 5% *R. officinalis* and 2% *T. serpillum* in swe*et al*mond oil 6 sheep with 5% iodine	2 sheep with no treatment	All treated animals with the plant extract and iodine were clinically cured with negative cultures after 6 weeks. Untreated animals remained clinically affected and culture positive.
78	*Ocimum gratissimum* L., *Trachyspermum ammi* (L.) Sprague	16 guinea pigs with induced *M. gypseum* and *E. floccosum* infection	Essential oil ointment of 1000ppm *O gratissimum* or *T. ammi* and polyethylene glycol	No treatment control	*Ocimum* essential oil treatment cured infections by day 9^{-1}1. With *T. ammi* oil, dermatophytosis was cured by day 11-13 Control had 100% culture recovery at day 15.
22	*Nigella sativa* L.	24 cattle with *T. verrucosum* dermatophytosis	Oil of *N. sativa* Combined treatment of *N. sativa* and enilconazole	10% enilconazole	At 42 days, clinical healing occurred completely in six animals treated with enilconazole, five animals treated with *N. sativa* and all animals treated with a combination of enilconazole and *N. sativa*.