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Full Length Research Paper

Control of dermatophyte-causing agents (*Trichophyton mentagrophytes* and *Trichophyton rubrum*) using six medicinal plants

T. O. Adejumo* and B. S. Bamidele

Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Nigeria.

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The efficacy of ethanol and distilled water extracts of *Azadirachta indica*, *Jatropha curcas, Jatropha gossypifolia, Cassia alata, Anacardium occidentale* and *Aloe vera* was determined against *Trichophyton mentagrophytes* and *Trichophyton rubrum* isolated from the skin of ringworm-infected patients. Highest antifungal activity was obtained from ethanol extracts where complete inhibition of *T. rubrum* was observed in all the extracts at 2, 5 and 10 mgl^{-1}, while partial growth of *T. mentagrophytes* was observed on *J. curcas*, *A. vera* and *C. alata* extracts. The water extracts of *A. vera*, followed by *A. occidentale* had the highest activity on *T. mentagrophytes* at 2 mgl^{-1}, while those of *C. alata*, *A. vera* and *J. curcas* were effective at 5 mgl^{-1}. At 10 mgl^{-1}, extracts of *A. vera*, *A. occidentale* and *J. gossypifolia* appeared best on *T. mentagrophytes*. Also, extracts of *C. alata*, *A. vera* and *J. curcas* performed well on *T. rubrum* at 2 mgl^{-1}, while those of *J. gossypifolia*, *C. alata* and *A. vera* were effective at 5 mgl^{-1}. At 10 mgl^{-1}, water extracts of *J. gossypifolia, C. alata* and *A. vera* appeared best. *A. vera*, *C. alata, J. gossypifolia* and *A. occidentale* compared favourably with mycoten, therefore they can be used as an alternative for treating ringworm infections on man caused by *Trichophyton* species.

Key words: medicinal plants, antifungal, *Trichophyton mentagrophytes, Trichophyton rubrum,* ringworm.

INTRODUCTION

Dermatophytes are the most important microorganisms that cause superficial mycoses on man; the lesions are characterized by circular disposition, desquamation, alopecia and erythma of the edges (Gallardo et al., 2004). The infections include the athlete's foot, jockey itch and ringworm occurs through direct contact with the spores or hyphae of any of the genera of *Microsporum*, *Trichophyton* or *Epidermophyton*. *Trichophyton* species (*T. mentagrophytes, T. rubrum and T. schoenleinii*) can grow on hair, skin and nails (Dubey and Maheshwari, 2004; Kayser et al., 2004).

Herbal medicine has been widely formulated and used as an integral part of primary health care in Nigeria, China, Ethiopia and Argentina. A variety of herbal preparations are being used to treat different kind of microbial diseases (Akinyemi et al., 2005). Antimicrobials of plant origin are efficient in the treatment of infectious diseases mitigating simultaneously many of the side effects that are often associated with synthetic ones (Iwu et al., 1999). Plants have limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen substituted derivatives. Most are secondary metabolites of which at least 12,000 have been isolated (Schutles, 1978). In this investigation, water and ethanolic leaf extracts of *Azadirachta indica*, *Jatropha curcas*, *Jatropha gossypifolia*, *Cassia alata, Aloe vera* and *Anacardium occidentale* were assessed *in vitro* for their efficacy in the treatment of dermatophyte-causing agents.

*Corresponding author. E -mail: toadejumo@yahoo.com.

MATERIALS AND METHODS

Collection and isolation of *Trichophyton* species

Suspected lesions from infected human skins in Akungba Akoko were cleaned with 70% alcohol to remove dirt and contaminants; they were scraped with sterile scalpel and brought to the laboratory. The sample was initially mounted on a slide in 10% potassium hydroxide solution and stained with 1% methylene blue for 1 min for direct microscopic examination. Later, it was inoculated

Table 1. Identity of medicinal plants used as extracts.

S/N	Botanical name	Common name	Local name (Yoruba)	Family
1.	*Azadirachta indica*	Neem	Dongoyaro	Meliaceae
2.	*Jatropha curcas*	Physic nut	Botuje/Lapalapa	Euphorbiaceae
3.	*Jatropha gossypifolia*	Coral plant	Ogege/Lapalapa pupa	Euphorbiaceae
4.	*Cassia alata*	Candle stick plant	Asurun Oyinbo	Fabeceae
5.	*Anacardium occidentale*	Cashew	Kaju	Anacardiaceae
6.	*Aloe barbadensis*	*Aloe vera*/Burn plant	-Eti erin	Liliaceae

into the centre of the Saboraud Dextrose Agar (SDA) and incubated at 25°C for 2 weeks. It was sub-cultured unto newly prepared SDA plates, identified and maintained on SDA.

Plant materials used and preparations of extracts

The plants used were fresh leaves of *A. indica, J. curcas, J. gossypifolia, C. alata, A. occidentale* and *A. vera* (Table 1). They were collected at a garden near Adekunle Ajasin University, Akungba-Akoko. Mycoten, a synthetic antifungal ointment was obtained at a pharmaceutical store in Ikare-Akoko.

The leaves were thoroughly rinsed with clean water and ground in the laboratory using mortal and pestle. Each sample was weighed into 3 portions: 2, 5 and 10g and were put into different 250 ml conical flasks. One hundred milliliters of sterile distilled water and 95% ethanol were used for extraction (2, 5 and 10 mgl^{-1}) and were shaken on a shaker for 3 h. Mycoten was also dissolved in water and ethanol at the same concentrations as above.

Antifungal tests

This test was to determine the radial growth inhibition of *T. mentagrophytes* and *T. rubrum* by the plant extracts. Two millilitres of each extract was incorporated into 15 ml of molten SDA inside a Petri dish. Three plates were used for each treatment. The media were swirled front, backward and in a circular fashion for thorough diffusion and were allowed to solidify.

Two week-old *T. mentagrophytes* and *T. rubrum* were inoculated separately into the media and were incubated at 30°C for 15 days. Plates were examined and radial growth was measured daily, treatments without the plant extracts served as control. Fungitoxicity was also expressed in terms of percentage of mycelial growth inhibition at 5, 10 and 15 days and calculated according to the formula of Pander et al. (1982): (dc-dt)/dc x 100, where dc = average diameter of fungal colony with control and dt = average diameter of fungal colony with treatment.

RESULTS

Identification of *Trichophyton* species

The *Trichophyton* species mounted on KOH and stained were observed as branched threads with presence of cross walls running through the cells. The identification of the organisms was confirmed as *Trichophyton* species using the colony morphology, hyphal septation and type of spores. *T. mentagrophytes*: On Sabouraud Dextrose Agar (SDA), the colonies were pale to puff in colour and appeared fluffy, while the reverse of the plates appeared yellow-orange (Kayser et al., 2004). *T. rubrum*: The colonies were whitish and appeared cottony, while the reverse showed deep red colour (Haley et al., 2000).

The microscopic identification of *T. mentagrophytes* appeared as thin-walled, cylindrical macroconidia with smooth surface, with numerous microconidia often in clumps on hyphae (Kayser et al., 2004). *T. rubrum* were observed as smooth-walled macro- and microconidia, mostly borne laterally directly on the hyphae on short pedicels, thin and thick-walled. Macroconidia were few and spherical, microconidia were observed and ranged from 2 to 4 um. The two fungi identified belong to the Dermatophytes of the genera *Trichophyton*. They were identified as *T. mentagrophytes* and *T. rubrum* according to Haley et al. (2000) and Robert and Pihet (2008).

In vitro antimicrobial assays

The results of the antifungal sensitivity test of the plant extracts are represented in Tables 2, 3 and Figures 1 - 6. For 2 mgl^{-1} tested extracts in water, Mycoten had the highest antifungal effect on the growth of *T. mentagrophytes* by inhibiting the growth of the fungus completely followed by *A. vera* (61%), while *J. curcas* had the lowest antifungal activity of 13% (Table 2 and Figure 1). At 5 and 10 mgl^{-1} mycoten also had the highest antifungal effect followed by *C. alata* and *Aloe vera* respectively, *A. occidentale* and *A. indica* had the lowest activity against the organism respectively (Figures 2 and 3). On *T. rubrum* water extracts at 2 and 10 mgl^{-1}, *C. alata* and *J. gossypifolia* had the highest antifungal activity, while *A. occidentale* had the lowest effect (Figures 4 and 6). At 5 mgl^{-1} mycoten and *J. gossypifolia* had the highest antifungal activity while *A. indica*, *C. alata* and *J. curcas* had the lowest effect (Figure 5).

Ethanol extracts at 2 mgl^{-1}, *Anacardium occidentale, Azadirachta indica, C. alata* and *J. gossypifolia* completely inhibited the growth of *T. mentagrophytes*, while there was growth on the plates containing *J. curcas, Aloe vera* and mycoten after 5 days of incubation (Table 3). The plate containing mycoten started growing on 8^{th} day of incubation, while those of *A. vera* started growth on 11^{th} day. At 5 mgl^{-1}, there was inhibition on the growth of *T. mentagrophytes* in all the plates except the plates containing *C. alata* extracts which started to grow on the 13^{th}

Table 2. Fungitoxicity of aqueous extracts of six medicinal plants on *Trichophyton* species.

	Trichophyton mentagrophytes			***Trichophyton rubrum***		
	Concentrations (mgl^{-1})					
	2	**5**	**10**	**2**	**5**	**10**
Aloe vera	61	66	47	80	77	72
Anacardium occidentale	29	5	34	15	52	29
Azadirachta indica	29	16	29	46	49	43
Cassia alata	24	71	24	100	49	100
Jatropha curcas	13	40	26	55	49	58
Jatropha gossypifolia	26	5	37	100	100	100
Mycoten	100**	100	100	100	100	100

*Fungitoxicity was calculated as % reduction in radial growth in 5days of incubation. An inhibition of > 60% was considered effective.
**100% inhibition indicates no growth of the fungus on agar plate.

Table 3. Fungitoxicity* of ethanolic extracts of six medicinal plants on *Trichophyton* species.

	Trichophyton mentagrophytes			***Trichophyton rubrum***		
	Concentrations (mgl^{-1})					
	2	**5**	**10**	**2**	**5**	**10**
Aloe vera	100**	100	100	100	100	100
Anacardium occidentale	100	100	100	100	100	100
Azadirachta indica	100	100	100	100	100	100
Cassia alata	100	100	100	100	100	100
Jatropha curcas	16	100	66	100	100	100
Jatropha gossypifolia	100	100	100	100	100	100
Mycoten	100	100	100	100	100	100

*Fungitoxicity was calculated as % reduction in radial growth in 5 days of incubation. An inhibition of > 60% was considered effective.
**100% inhibition indicates no growth of the fungus on agar plate.

day. There was growth of *T. mentagrophytes* at concentration 10 mgl^{-1} only in plates containing the control and extracts of *J. curcas* and *Aloe vera,* while other extracts showed growth inhibition. All the concentrations (2, 5 and 10 mgl^{-1}) of the extracts showed no growth with *T. rubrum* (Table 3). Ethanol extracts appeared to show higher antifungal activities on the microorganisms, while water extracts was observed with lesser antifungal activity.

DISCUSSION

The results of the antifungal sensitivity test showed that the antimicrobial potential of the extracts in ethanol on *T. mentagrophytes* and *T. rubrum* was higher than those of distilled water. Many reports have indicated that the ethanolic extracts of plants parts were more inhibitory than the aqueous extracts, which suggests that ethanol may be a better extracting solvent (Ke-Qiang et al., 2001). All the extracts in ethanol were very effective on *T. rubrum*. The high inhibitory activity in these extracts could be due to the concentration of the antimicrobial constituents of the solvent. Although, ethanol has the highest ability to extract the phytochemical than water, the effectiveness of solvent extraction is ranked as best for methylene chloride, methanol and least for water.

Generally, in all the extracts, the growth of the organism increased gradually, while in most of the plates containing mycoten, there was no fungal growth; this may be due to the component of the ointment like the azole group that has been found to be effective drugs for treating fungal diseases. The inhibitory activity of *C. alata* and *J. gossypifolia* extracts on *T. rubrum* may be as a result of the phytochemical components of the plants on the organism.

The juice expressed from the young leaves of *C. alata* is commonly used in the treatment of skin infections and healing of wounds in many parts of Nigeria. The active constituents involved in their use as antiseptics in certain skin diseases are the anthranols (and anthrones) present in the leaves (Benjamin and Lamikanra, 1981). Other classes of compounds from the plant material are hydroxyanthraquinones, glycosides, chrysophanic acid, kampferin and sannoxide A and B (Abo et al., 1998; Kochar, 1981). The results from this work support the

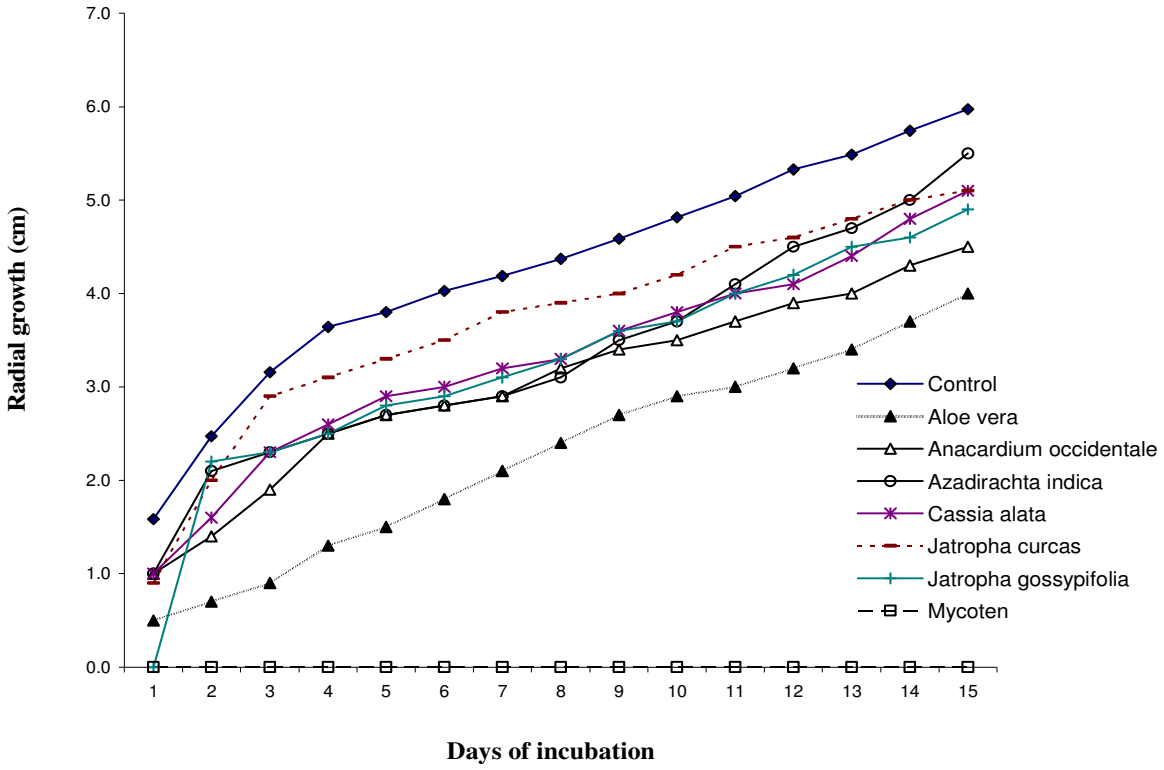

Figure 1. Radial growth of *Trichophyton mentagrophytes* with 2 mg/l of medicinal plants in distilled water.

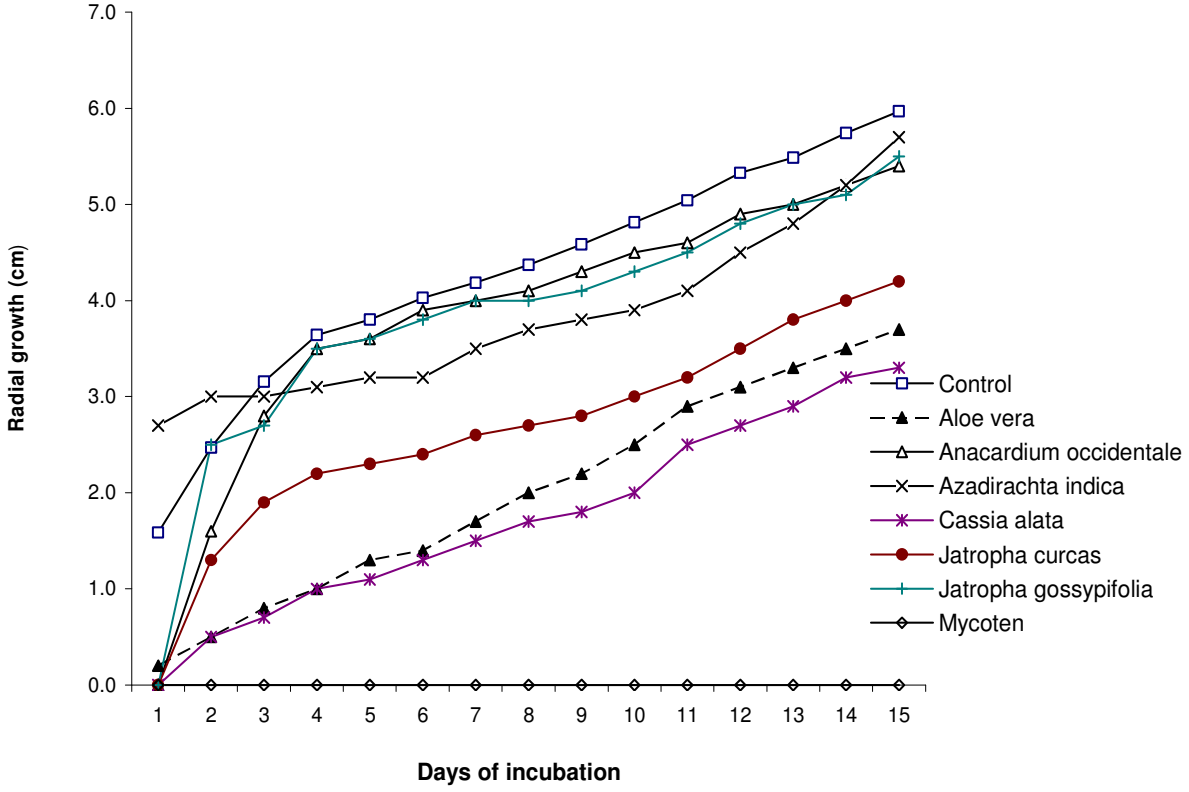

Figure 2. Radial growth of *Trichophyton mentagrophytes* with 5 mg/l of medicinal plants in distilled water.

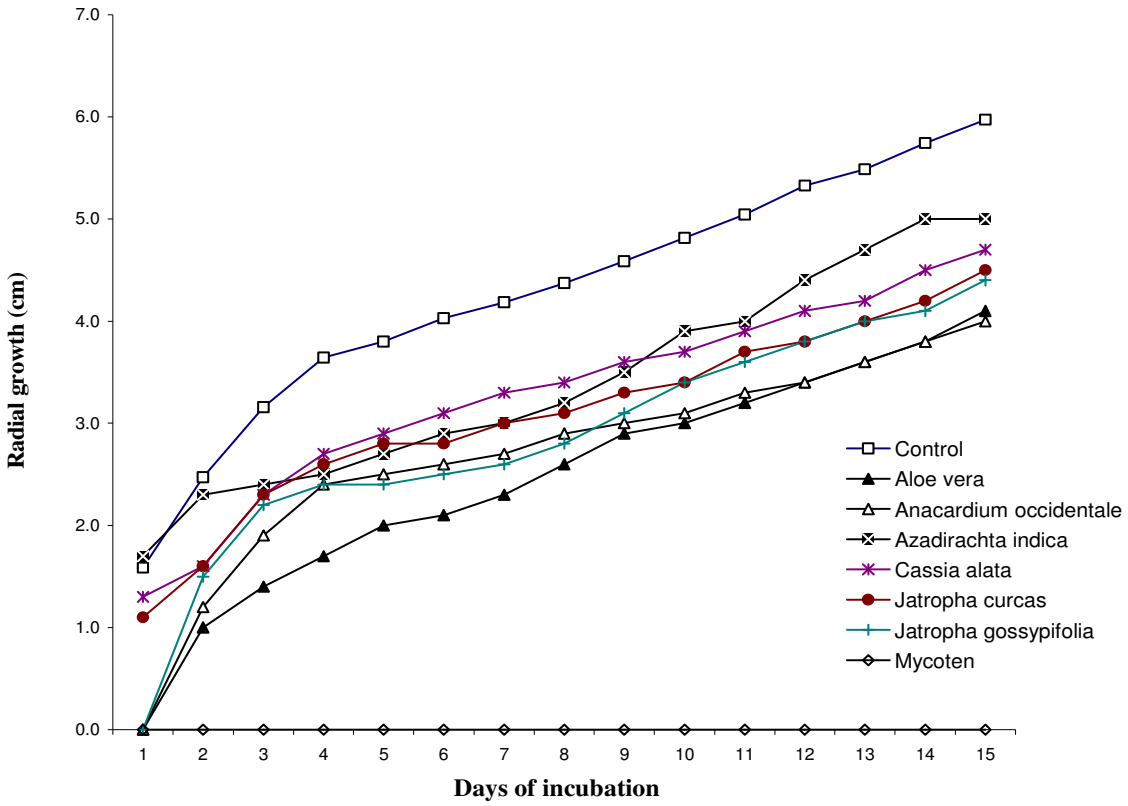

Figure 3. Radial growth of *Trichophyton mentagrophytes* with 10 mg/l of medicinal plants in distilled water.

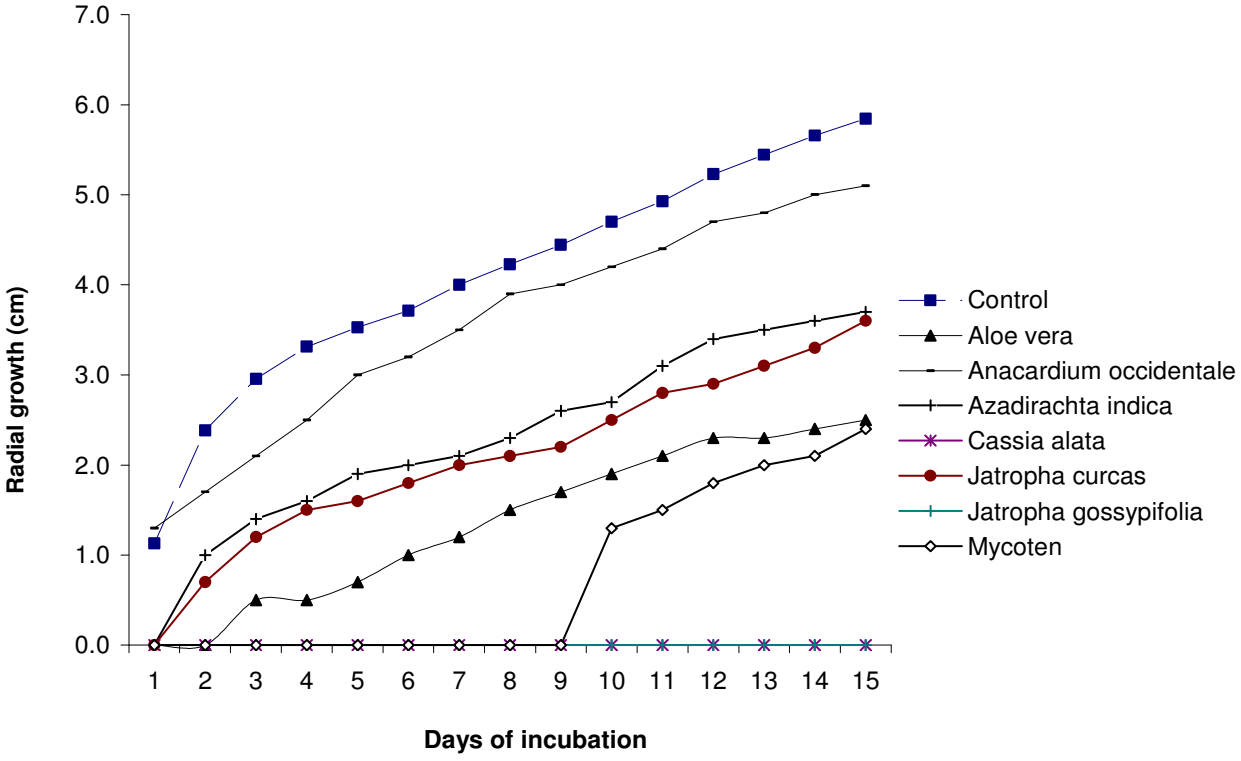

Figure 4. Radial growth of *Trichophyton rubrum* with 2 mg/l of medicinal plants in distilled water.

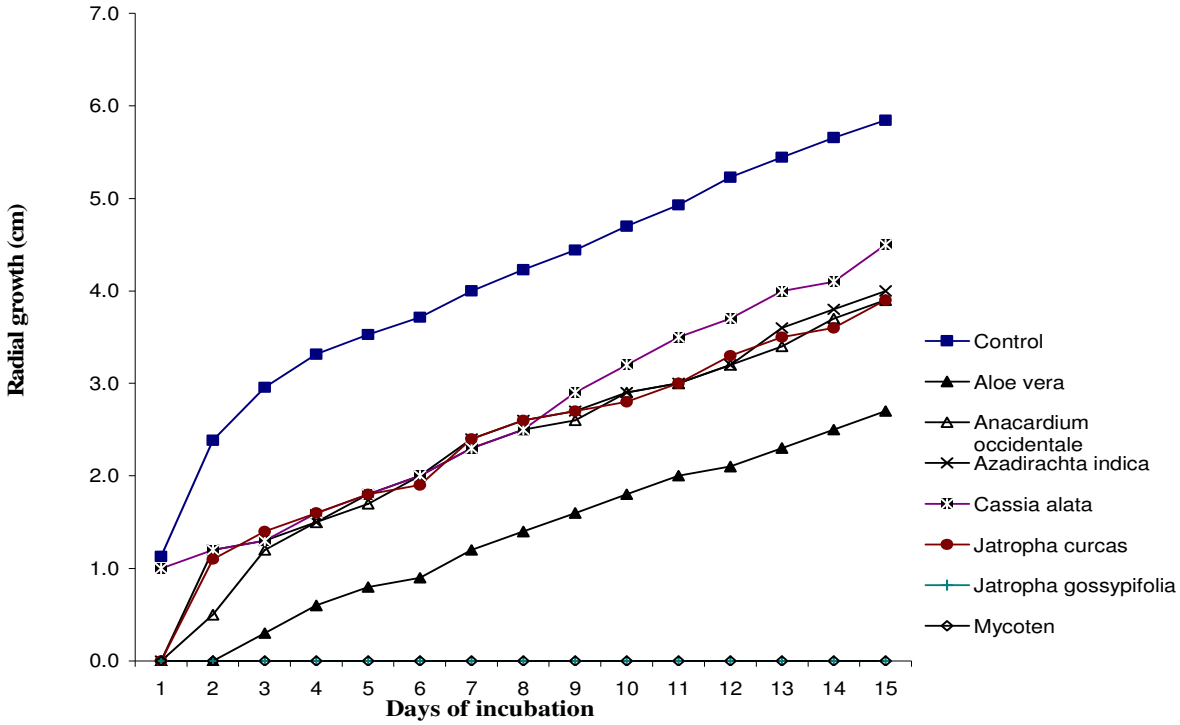

Figure 5. Radial growth of *Trichophyton rubrum* with 5 mg/l of medicinal plants in distilled water.

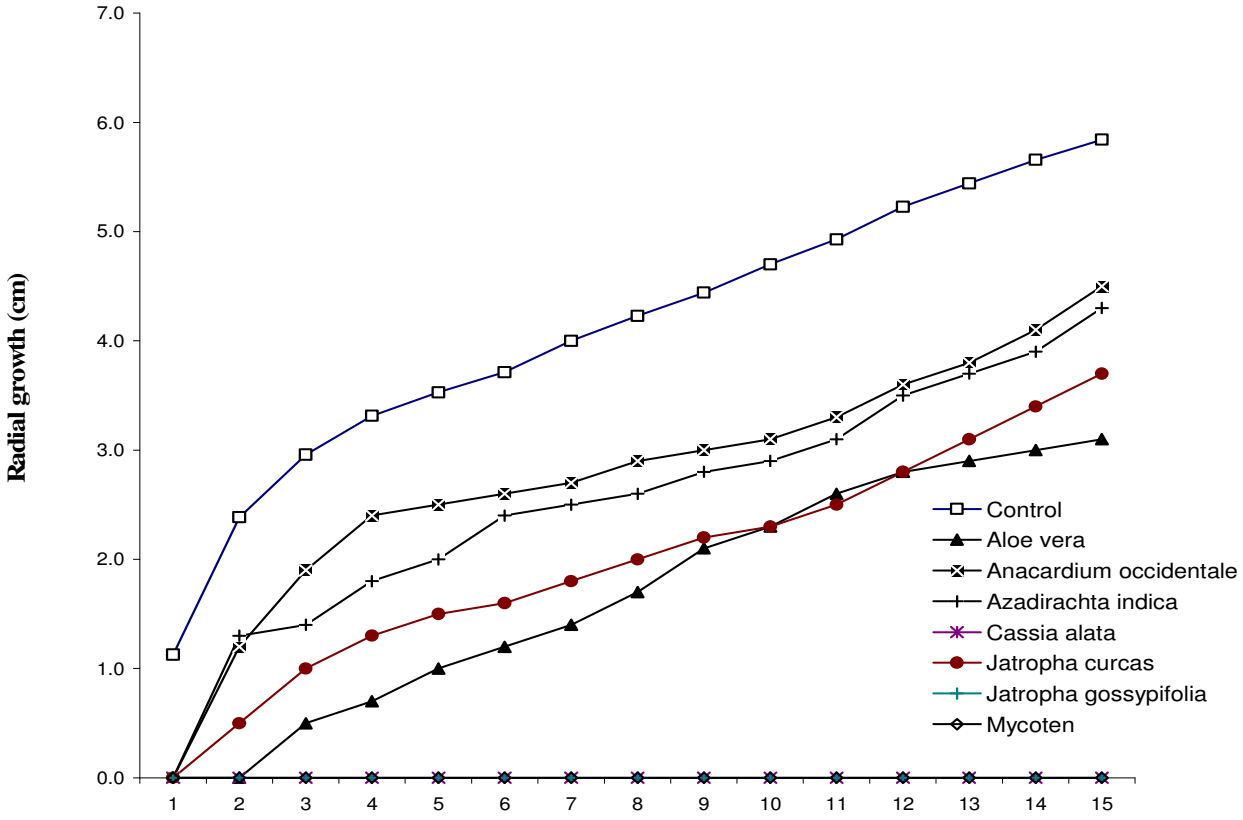

Figure 6. Radial growth of *Trichophyton rubrum* with 10 mg/l of medicinal plants in distilled water.

findings of Makinde et al., 2007 who reported that *C. alata* leaf extracts showed a range of activity against all the tested bacteria and fungi including *T. mentagrophytes and* this lend support to the traditional use of the plant (flower and leaf) for the treatment of fungal skin diseases.

In this study, *A. vera* extracts showed consistent inhibitory activities on *T. mentagrophytes* and *T. rubrum.* However, Agarry et al. (2005) who worked on the comparative antimicrobial activities of the gel and leaf of *Aloe vera*, reported that only the gel inhibited the growth of *T. mentagrophytes*, while the ethanolic extracts of the leaf possesses inhibitory effects on both *Pseudomonas aeruginosa* and *Candida albicans*. The authors stated that the gel and the leaf are useful and that they can complement one another in their medicinal capabilities. The gel has been found to promote wound healing due to the presence of some components like anthraquinones and hormones (Davis, 1997), which posses antibacterial antifungal and antiviral activities. Most of the constituents are found in the gel and not in the leaf; hence the gel is likely to be more active than the leaf. The fact that *A. vera* extracts on microorganisms gave credence to the popular use of both *Aloe vera* gel and leaf.

Other plants and more solvents can also be used in further research with different concentrations. The use of medicinal plants in the treatment of dermatomycoses will help to reduce the dependence on the use of microbial or chemically synthesized antimicrobials and thus overcome the problem of the emergence of fungi being resistant to antifungal chemicals on various etiological agents of dermatophyte infections.

The choice of proper treatment for dermatophytoses is determined by the site and extent of the infection and the species involved, as well as by the efficacy, safety profile and pharmacokinetics of the available drugs (Aydan et al., 2006).

RECOMMENDATIONS

Further research is needed. This work has indicated that extracts have the potential application and that there is justification for the use of these plants as antiseptics and herbal soaps in the treatment of dermatophyte-causing agents especially *T. mentagrophytes* and *T. rubrum.* The future for using plant extracts and plant products is promising, because they are lee expensive and less hazardous to the environment.

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