Acta Scientific Microbiology (ISSN: 2581-3226)
Volume 1 Issue 9 September 2018
Research Article

In Vitro Inhibition of Tinea Corporis from Various Extracts of *Aloe vera* and *Azadirachta indica*

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Received: July 13, 2018; **Published:** August 10, 2018

Abstract

The current study evaluated and established the activity of various extracts from *Aloe barbadensis* Mill and *Azadirachta indica* against Tinea Corporis, a fungus causative agent of ringworm disease. The disease is widely distributed all over the world with various degrees. The aqueous, ethanol and acetone extracts of these plants were tested. The aim of this study was to examine the antifungal effect of these extracts against Tinea Corporis. The *in vitro* antifungal activity was discovered by observing and measuring the zone of inhibition formed on selective nutrient media. The results suggest that most effective inhibition of the fungus was obtained from acetone extracts of both the plants. In addition, *Aloe vera* extract showed more potent antifungal activity as compared to the extract of *Azadirachta indica*. This finding supports the use of *Aloe vera* and *Azadirachta indica* acetone extracts in the treatment of ringworm infections.

Keywords: Tinea Corporis; Aloe vera barbadensis; Azadirachta indica; Antifungal Activity

Introduction

Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to public health, despite the tremendous progress in human medicine. Their impact is particularly significant in developing countries due to the relative unavailability of medicines and the emergence of widespread multiple drug resistance as a result of indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases [1]. Moreover, antimicrobial drugs are sometimes associated with adverse effects on the host including hypersensitivity, immunesuppression and allergic reactions [2]. Research on new antimicrobial substances must therefore be continued and all possible strategies should be explored. Besides synthetic molecules from medicinal chemistry, natural products are still major sources of innovative therapeutic agents for various conditions, including infectious diseases [3]. Current research on natural molecules and products primarily focuses on plants, since they can be sourced more easily and be selected on the basis of their ethno-medicinal use [4].

Medicinal plants, which form the backbone of traditional medicine have in the last few decades been the subject of very intense pharmacological studies [5-8]. In this connection, higher plants continue to be a rich source of therapeutic agents since they produce hundreds to thousands of diverse chemical compounds as secondary metabolites with different biological activities [9]. The compounds produced by the plants are active against plant and human pathogenic microorganisms [10]. The system of medicine for the treatment of pathogenic fungi as well as other ailments depends on natural products or components which are obtained from plants such as 'herbal extract'. A herbal extract is a liquid solution of herbs and it does not contain any synthetic or toxic substance which is hazardous or harmful for human as well as other aquatic animal.

Aloe vera-called "Pharmacy of Nature" has been associated with myth, magic and medicine since pre-biblical times [11], but its antifungal trait has not been worked out extensively. *Azadirachta indica* (Neem) is a tree which has been used for a long time in

agriculture and medicine. *Azadirachta indica* is an indigenous plant widely distributed in India. The medicinal properties of the plant *Azadirachta indica* have been studied by several research groups.

Dermatophytosis, also called as tinea or ringworm is infection caused by a group of keratinophylic fungi called dermatophytes. The three major genera causing tinea are Trichophyton, Microsporum and Epidermophyton [12,13]. Dermatophytes colonize the skin, nails and hair of human population. It affects the keratinous tissues of humans and other vertebrates and thus causes superficial infections [14]. Contagiousness among animal communities, high cost of treatment, difficulty of control and the public health consequences explain their great importance [15]. A wide variety of dermatophytes have been isolated from animals, but a few zoophilic species are responsible for the majority of the cases, viz. *Microsporum canis, Trichophyton mentagrophytes, Trichophyton equinum* and *Trichophyton verrucosum*, as also the geophilic species Microsporum gypseum [16,17]. The Tinea Corporis fungus infects damaged areas and appears as tiny white threads which multiply into tuffs. The other symptom like cloudiness of the arms, legs, nails may also be caused by fungus. Disease generally occurs as a result of the interaction among a host organism, a causative agent and the environment. Fungi thrive in moist, warm areas, such as locker rooms, tanning beds, swimming pools and in skin folds. It can be spread by sharing sport goods, towels, and clothing.

A few antifungal agents are available and licensed for use in veterinary practice or human being treatment. The use of systemic drugs is limited to treatment or animal due to their high toxicity and problems of residues in products intended for human consumption [18]. Different treatments have been recommended to control dermatophytes. In general, pharmacological treatment option includes antifungal agents [19,20], but recently the use of some natural plant products has been emerged to inhibit the causative organisms. The antimicrobial and antitoxin properties of some plants, herbs, and their components have been documented since the late 19th century [21]. Many of the existing synthetic drugs cause various side effects. Hence, drug development plant based compounds could be useful in meeting this demand for newer drugs with minimal side effects [22]. Medicinal plants are a rich source of antimicrobial agents and normally produce bioactive secondary metabolites and many of them exhibit activity, hence can be used in antimicrobial drugs.

Hence, the current study was aimed to investigate the antifungal effects of different extracts of *Aloe vera barbadensis* (Miller) and *Azadirachta indica* (Neem) against Tinea Corporis, a fungus-causative agent of ringworm disease of human skin. The basis of selection of these plants was their abundance in Rajasthan so that if their possible use as antifungal is detected, they could be duly utilized for the treatment of the mycological disease.

Materials and Methods

Identification and collection of plants

Plants were identified and collected from various areas of Dungarpur district of Rajasthan, India, after proper verification by ayurvedic medical professional. *Aloe vera barbadensis* species was selected for this study (Figure 1A). *Aloe vera barbadensis* can grow up to 100 cm high although mostly to 30 to 60 cm and it has thick leaves in rosette pattern. The parenchyma cells of the leaves contain large quantities of pulp. The fleshy leaves with serrated edges that arise from a central base and grow to nearly 30 - 50 cm long. *Aloe vera* (*Aloe barbadensis* Miller) is a perennial plant of liliacea family with turgid green leaves joined at the stem in a rosette pattern. *Aloe vera* leaves are formed by a thick epidermis (skin) covered with cuticle surrounding the mesophyll, which can be differentiated into chlorenchyma cells and thinner walled cells forming the parenchyma (fillet). The parenchyma cells contain a transparent mucilaginous jelly which is referred to as *Aloe vera* gel. *Aloe vera* leaves are normally sensitive to subfreezing temperature. *Azadirachta indica* (Figure 1B) was also identified and collected after the proper verification by botanist from the same place described above.

Figure 1: (A) Aloe barbadensis (Miller) plant and its leaf sample. (B) Azadirachta indica (Neem) tree and its leaves.

Preparation of Crude Extract of *Aloe vera*

Fresh *Aloe vera* whole leaves (Figure 1A) were washed with distilled water, chopped or crush into small pieces, air-dried and grinded into powder. The dried powder was extracted with 95% ethanol for one week and 500 ml of acetone then it was filtrated through filter paper. The entire extract was then evaporated at 90°C in oven to get a paste form. This concentrated leaf extract was used for further experiments.

Preparation of Leaf extract of *Azadirachta indica* (Neem)

The dried Neem leaves available in yellowish green color were crushed to coarse powder and then extracted in vertical extractors with 20% ethyl acetate. Four extractors are generally found to be sufficient for extraction of medicinally active compound. All extraction washings were pooled and distilled under vacuum till maximum alcohol is removed.

This concentration containing 12 - 15% TDS was extracted with n-hexane by stirring a hot solution for 1 hour. The solution was allowed to stand for layer separation. The upper hexane layer mainly contains fatty matter while aqueous layer constitute all active principles. The lower aqueous layer was drained in to a storage tank and concentrated to about 30% TDS in a falling evaporator. This operation can also be done in a centrifugal separator. The extract in powder form can be obtained by spray drying the concentrate of 30% TDS. It was then dried in a vacuum tray or rotary drier. The dried extract obtained as flakes or lumps is micro-pulverized to desired particle size.

Aqueous extract: 10 gm of powder dissolved in 250 ml distilled water was added and shaking then the filtrate was collected for experiment.

Acetone Extract: 10 gm of dried leaf powder was taken to a separate container and 250 ml of acetone was added and kept for 24h with periodic shaking then filtered and the filtrate was collected. The procedure was repeated three times with fresh volume of acetone. The filtrates were pooled.

Ethanol Extract: 10 gm of dried leaf powder of *Azadirachta indica* was taken to a separate container. To this 250 ml of ethanol was added and kept for 24h with periodic shaking. It was filtered and the filtrate was collected. The procedure was repeated three times. The collected filtrates were pooled.

Disease Sample Collection and Identification

Skin scrapings from patients with superficial lesions were collected from Department of Dermatology, Venerology and Leprology, E.S.I.C. hospital, Jaipur, Rajasthan, India and some ideal sample were procured from IMTECH, Chandigarh. All the samples were collected in sterilized plastic bags. The preliminary microscopic examination of the material was done immediately. Small portions of infected skin sample were examined under microscope for presence of yeast cells or hyphal fragments. Following to this, demographic characteristics of patients were also recorded including, gender (male or female), age of patients, nature of infection, occupation, symptoms etc. In addition, climatic influence, clothing and condition of personal hygiene were recorded for Para-clinical data. Remaining infected skin samples were then transferred in triplicates on Sabouraud's dextrose agar slants. These cultures were maintained at 28 ± 20C temperature in B.O.D incubator for further growth. After 7 days, biochemical tests (e.g. catalase test and urease test) were performed for confirmation of Tinea Corporis.

Antifungal susceptibility testing

Aqueous and organic solvents like methanol or ethanol and acetone extracts were tested for the antifungal property according to the method described by Khan., *et al* [23]. Sterile sabouraud dextrose agar media was poured into sterile 90 mm Petri dish and then inoculated with the test organism. The 1:10, 1:50 and 1:100 dilutions from each extract were prepared. Each of various type of extract concentration was poured in 6mm wells and incubated at 37^0C for 48 hrs. The presence of zone of inhibition was considered as the presence of antifungal action. The antifungal activity was recorded by measuring the diameter of the zone of inhibition. The experiment was performed under strict aseptic conditions and repeated for three times to minimize errors along with control group.

Results

Assessment of antifungal activity of Aloe vera extracts

The antifungal activity of three different extracts of *Aloe vera* is shown in the table 1. The zone of inhibition was noted for each of the three extracts (Figure 2). The acetone extract showed the maximum zone of inhibition against the fungus as compared to the alcoholic and the aqueous extracts. The 1:100 dilution of the crude acetone extract showed the highest antifungal activity compared to the other lower dilutions (1:10, 1:50). Moreover, antifungal activity of the acetone extract was enhanced when incubation was extended for more than 2h hrs (i.e. 48 hrs) as demonstrated by the larger zone of inhibition (Figure 2C). There was no significant change in the zone of inhibition for the other two extracts upon extending the incubation time (data not shown).

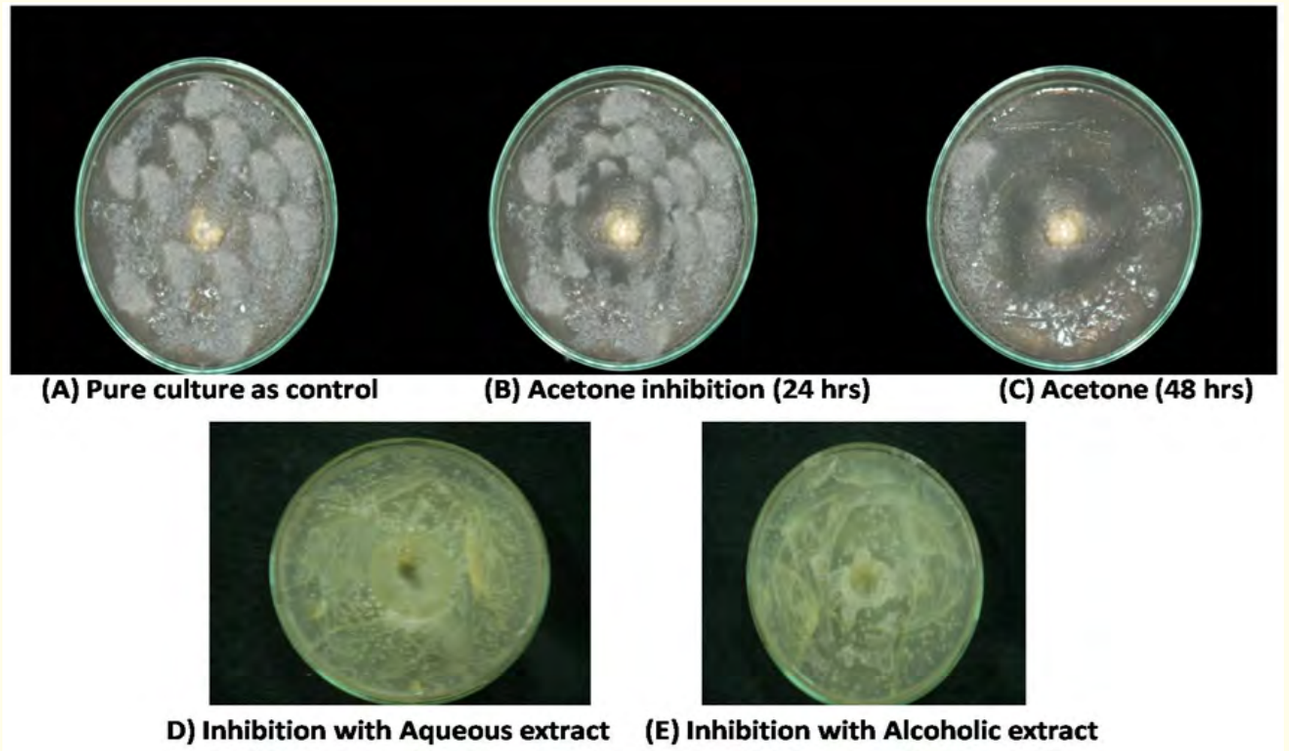

Figure 2: Antifungal effect of Aloe Vera extracts: (A) Pure culture as control; (B) Acetone inhibition (24 hrs); (C) Acetone (48 hrs); (D) Inhibition with Aqueous extract; (E) Inhibition with Alcoholic extract.

Extracts	Control (untreated) (Mean ± SD)	Dose (crude extract) and Zone of Inhibition (diameter in mm; Mean ± SD)		
		1 ml (1:10 dilution)	1 ml (1:50 dilution)	1 ml (1:100 dilution)
Aqueous	5.3 ± 1.2	0.7 ± 0.1	1.8 ± 0.6	1.5 ± 0.5
Alcoholic	5.7 ± 1.1	1.9 ± 0.5	2.00 ± 0.5	2.8 ± 0.8
Acetone	5.8 ± 1.0	3.5 ± 0.8	3.8 ± 0.2	4.0 ± 0.7

Table 1: Antifungal effect of *Aloe vera* extracts.

Assessment of antifungal activity of Aloe vera extracts

The antifungal activity of three different extracts of *Azadirachta indica* is shown in the table 2. The zone of inhibition was noted for each of the three extracts (Figure 3). The antifungal activity did not show significant difference between aqueous and alcoholic extracts; however, acetone extract showed the highest antifungal activity against the fungus as compared to the other two extracts. The antifungal activity of acetone extract was best observed with dilution of 1:100 for 24 hrs incubation.

Extract	Control (Mean ± SD)	Dose and Zone of Inhibition (diameter in mm; Mean ± SD)		
		Dose 1 ml (1:10)	Dose 1 ml (1:50)	Dose 1 (1:100)
Aqueous	5.0 ± 0.2	1.0 ± 0.1	1.2 ± 0.1	1.5 ± 0.2
Alcoholic	5.5 ± 0.4	1.5 ± 0.2	1.8 ± 0.1	2.3 ± 0.1
Acetone	5.2 ± 0.1	2.0 ± 0.1	2.5 ± 0.1	3.5 ± 0.1

Table 2: Antifungal effect of *Azadirachta indica* extracts.

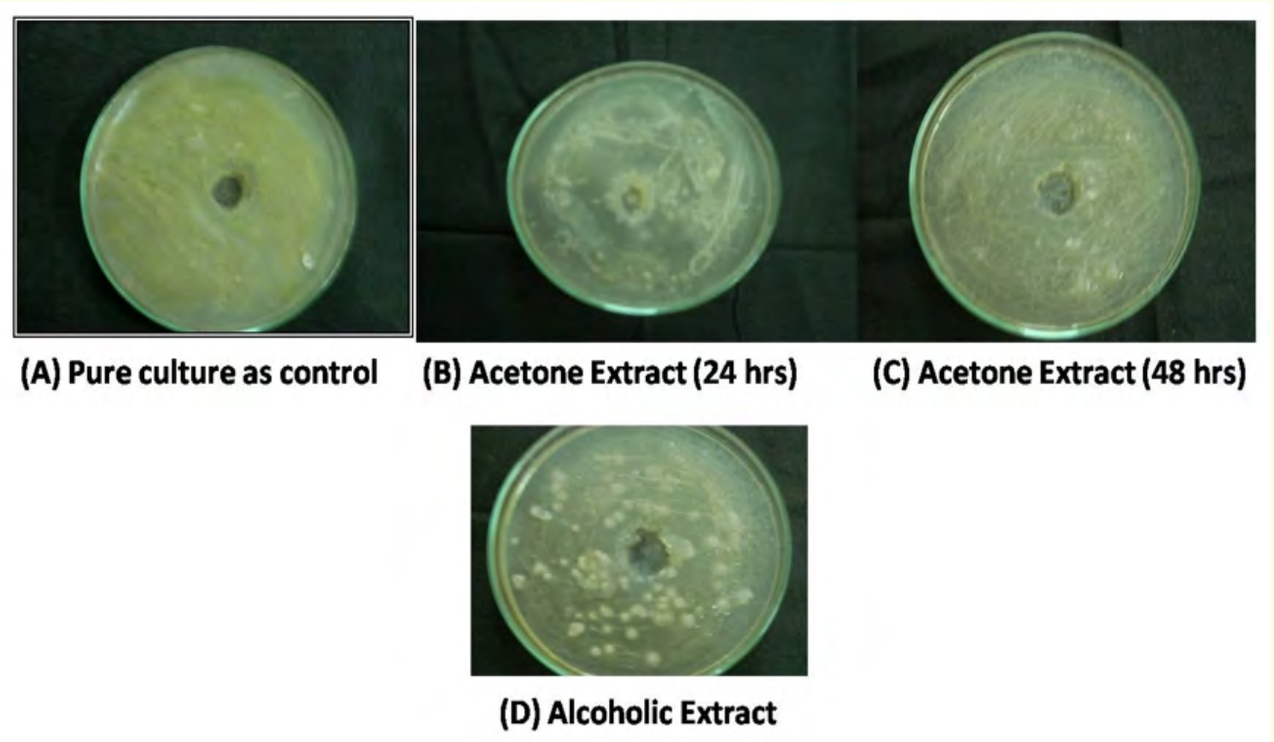

Figure 3: Antifungal effect of Azadirachta indica extracts: (A) Pure culture as control; (B) Acetone Extract (24 hrs); (C) Acetone Extract (48 hrs); (D) Alcoholic Extract.

Discussion

Initial screening of plants for possible antimicrobial activities typically begins by using crude aqueous or alcohol extraction and can be followed by various organic extraction methods. Since nearly all of the identified components from plants, active against microorganism are aromatic or saturated organic compounds, they are often obtained through initial ethanol or methanol extraction [24,25]. The present work encompasses the screening of aqueous, acetonic and alcoholic extracts of the two plant species, namely *Aloe vera barbadensis* Miller and *Azadirachta indica*, for their antifungal property against ringworm fungus, Tenia corporis. This is in pursuance of the efforts to search for drugs from plants and the verification of the scientific basis of some known practices in traditional medicine.

Aloe vera is a stemless or very short-stemmed succulent plant belongs to family Liliaceae. The leaves are hard edges, thick, fleshy and green to grey green in color with some varieties showing white flecks on the upper and lower stem surfaces. *Aloe vera* contains various components including phenol, saponin, anthraquinones, which are classified as anti-bacterial, antiviral and antifungal agents. *A. barbadensis* Miller (*A. vera*) possessed a number of therapeutic uses viz., anti-inflammatory [26,27], immunostimulatory [28], antibacterial [29], antiviral [30], antifungal [31] and cell growth stimulatory activity [32,33]. Ibrahim., *et al.* [34] investigated the phytoconstituents and antimicrobial activity of aqueous, ethanol and acetone extracts of the *A. vera* gel against some human and plant pathogens by disc diffusion method. Among the three extracts, ethanol and acetone extracts recorded significant antimicrobial activity against all test pathogens. Antibacterial and antifungal activity of the acetone extract was found to be quite impressive as compared to ethanol and aqueous extracts. The results of the present study are in concordance with the above said study and suggested that acetone extract of *A. vera* contains highly effective antifungal activity. Agarry., *et al*. [35] compared the antimicrobial activities of ethanolic extracts of A. vera gel and leaf against *Staphylococcus aureus, Pseudomonas aeruginosa, Trichophyton mentagrophytes, Trichophyton schoenleinii, Microsporum canis* and *Candida albicans*. Interestingly, only the gel inhibited the growth of T. mentagrophytes; while the leaf possesses inhibitory effects on C. albicans. Renisheya Joy Jeba Malar., *et al.* [36] also showed antifun-

gal activity of *Aloe vera* against *C. albicans* and *Penicillium* spp. Additionally, in a study on ringworm in animals, it has been concluded that *Aloe vera* can be used for treating ringworm infection [37].

Azadirachta indica (Neem) is used in traditional medicine as a source of many therapeutic agents in the Indian culture and grows well in the tropical countries. Earlier studies on Neem have showed that it contains active substances with multiple medicinal properties [38]. *Azadirachta indic*a in folklore medicine for the treatment of diabetes shows the potential role of antidiabetic activity [39]. The great potential of Neem aqueous extract has been suggested as powerful chemotherapeutic and viral agent [40]. *Azadirachta indica* leaves possessed good anti-bacterial activity, confirming the great potential of bioactive compounds and is useful for rationalizing the use of this plant in primary health care [41,42]. The phytoconstituents alkaloids, glycosides, flavonoids and saponins are antibiotic principles of plants which are actually the defensive mechanism of the plants against different pathogens [43].

In addition, flavonoids have been reported to possess many useful properties, including anti-inflammatory, estrogenic, antimicrobial, antiallergic, antioxidant, vascular, and cytotoxic antitumor activities and enzyme inhibition [44]. Owing to the widespread ability of flavonoids to inhibit spore germination of plant pathogens, they have been proposed for use against fungal pathogens of human beings [45]. One of the study showed that, a novel quercetin derivative present in Neem showed antifungal activity. The results indicated a remarkable increase in the biological activity of this compound as compared to rutin. Rutin is known as one of the common and naturally occurring flavonoids with a variety of biochemical, antimicrobial activity [46] and pharmacological activities.

Antimicrobial properties of *Azadirachta indica* were studied by several authors. Rao., *et al.* [47] reported the antimicrobial activity of the seed oil against a variety of pathogens. The antifungal effects were reported of gedunin against polyporous wood rot [48] of a leaf extract against *Alternaria alternate* [49] and of a mixture of sulphurous compounds from the steam distillate of fresh matured leaves against *Trichophyton mentagrophytes* [50]. In a study on *Azadirachta indica*, it was observed that the ethyl acetate extract of neem leaf was effective against *T. violaceum* and *E. floccosum* [51]. Venugopal and venugopal [52] worked on 88 clinical isolates of dermatophytes and showed antidermatophytic activity with ethanolic and aqueous extracts of neem leaves. In contrast, our study suggested that acetone extract of *A. indica* has more effective antifungal activity.

Conclusion

The results of the study showed that most effective fungal inhibition being from acetone extract of both plants. The zone of inhibition of acetone extract was greater than aqueous and alcoholic extracts. This suggests that plants extracts in organic solvent proved to be more active. In addition, *Aloe vera* extract showed more potent antifungal activity as compared to the extract of *Azadirachta indica*. These results further confirm the therapeutic potency of these plants which are being used as traditional medicine. Overall, the present study analyzed the antifungal effect of *Aloe vera* and *Azadirachta indica* against Tinea Corporis and suggests its use for preparation of economic, natural and safe antifungal drugs, which can be used as an alternative for expensive allopathic medicine in treatment of ringworm disease.

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