Comparative Efficacy of Aloe vera and Tamarix aphylla against Cutaneous Leishmaniasis

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Abstract – The pharmacological screening of methanolic extract of Aloe vera leaf and Tamarix aphylla bark were assessed to investigate the in vitro antileishmanial activity of the medicinal plants against cutaneous leishmaniasis and to develop a patent on confirmation of their activities. Different concentrations ranging from (25-100) µg/ml of methanolic extract from Aloe vera and Tamarix aphylla were tested for their effect on the growth of leishmania promastigotes cultured in vitro from leishmania tropica inoculated in Novy-Mcnicelle-Nicolle medium (NNN) and then maintained in RPMI 1640 medium. Numbers of promastigotes grown in treated culture tubes were statistically compared to the non-treated culture tubes after time interval of 48 and 96 hrs and percent growth inhibition was observed as Mean±SD in proportion to concentrations and incubation time. Maximum percent growth inhibition was observed in Aloe vera and Tamarix aphylla at concentrations of 100µg/ml in T4 (A1=66% and A2=84%) followed by T3 at 75µg/ml (B1=43% and B2=54%) after the incubation period of 96hrs respectively, while the concentration of 50µg/ml was considerable less effective in T2 (C1=27% and C2=28%) for Aloe vera and Tamarix aphylla respectively, whereas the lowest concentration of 25µg/ml fail to produce any significant effect in the parasite inhibition in both medicinal plants. In this study, Tamarix aphylla was also shown to have a statistically highly significant effect on motility of the parasites as compared of Aloe vera (P<0.05). Aloe vera and Tamarix aphylla had a significant dose dependant antipromatigote activity against L. tropica as that suggest promising phytotherapeutic agents for cutaneous leishmaniasis.

Keywords – Aloe vera, Tamarix aphylla, Leishmania tropica

1. Introduction

Leishmaniasis is a disease caused by single cellular, hemoflagellate protozoan parasites of the genus Leishmania. The parasites are transmitted by the bite of an infected female sand fly [1]. It is estimated that 350 million people are at risk for leishmaniasis. Twelve million people are currently infected with 1.5-2 million new cases being reported annually and 70,000 deaths occurring annually [2]. Leishmaniasis comprises a group of diseases with distinct clinical manifestations caused by different species of Leishmania parasites and causes one of four clinical forms of the disease: cutaneous leishmaniasis, diffuse cutaneous leishmaniasis, mucocutaneous leishmaniasis and visceral leishmaniasis [3]. Although cutaneous leishmaniasis disease is not lethal, it can cause significant morbidity and the course of the disease is often accompanied by psychological and social repercussions [4]. Due to the limited availability and high incidence of serious side effects of pharmaceutical products, most people in areas where leishmaniasis is prevalent depend largely on popular treatments and traditional medicines to alleviate and relieve the symptoms [5]. Recently, the emergence of multiple drug resistance to human pathogenic organisms, a search for new antimicrobial substances from other sources including plants has become necessary. Plants are an important source for drug candidates, particularly against parasites. Over 100 plants have been reported to be active against various forms of leishmanial parasites [6]. Different parts of medicinal plants are used for extracts as antileishmanial agents [7]. The selection of medicinal plants like Aloe vera and Tamarix aphylla is based on the fact that these plants were not previously screened as antileishmanial agents. Aloe vera and Tamarix aphylla are commonly used medicinal plants and are well established for different medicinal uses ranging from treating skin burns to antimicrobial activities [8]. A number of biological activities such as antiseptic (saponins and anthraquinones), antitumoral (mucopolysaccharides), anti-inflammatory (steroids and salicylic acid), anti-oxidant (Vitamins) and immune regulator (glucocannons) have been reported [9]. The bark of Tamarix aphylla is an astringent, tonic and is commonly used for the treatment of hepatitis, eczema and skin diseases like capitis, syphilis and scaly skin conditions [10]. Keeping in view this knowledge and as part of its search for new and better pharmaceuticals of high availability and low toxicity, the Tropical Diseases Programme of the World Health Organization has considered the investigation of plants used in traditional medicine practices for the treatment of leishmaniasis as essential and of high priority [11]. In an ongoing search for better and cheaper leishmanicidal agents,
plant-derived products are an attractive option and indeed a need to explore the therapeutic potential of higher plants to get new, less toxic, less expensive and more effective drugs [12].

2. Materials and Methods

2.1. Collection of Plant Material

Fresh plants samples of Aloe vera leaf and Tamarix aphylla bark were collected randomly from District Kohat, (Khyber Pakhtunkhwa) Pakistan and authenticated by the herbarium staff of botany department, Kohat University of Science and Technology and were kept in the sterile environment of the laboratory for further process. After collection, the desired plants were first cleaned from extra weeds and seeds and washed with distilled water; air-dried and were chopped and ground into fine powder and passed through sieved of 0.5 mm mesh screen and kept separately in clean polythene bags.

2.2. Extraction of Aloe Vera Leaf and Tamarix Aphylla Bark

The respective powder-plant parts were soaked in 70% methanol (1g/10mL) for at least two weeks at room temperature in a conical flask and kept on rotary shaker at 120 rpm for occasional stirring. The methanolic extracts of powdered drugs were filtered through whatman filter paper #42 and evaporated in a rotary flask apparatus under reduced pressure and a temperature not exceeding than 42°C temperature leaving behind their respective syrup residues and were air dried again to get the powder form. The values of methanolic plant extract were analyzed accordingly [13].

2.3. Collection of Sample Specimen

The lesions and the adjacent normal-looking skin around the infected area were cleaned, sterilized with 70% ethanol and allow it to dry. The skin scrapings were made with the help of sterilized surgical blades or lancet by making a small incision on the periphery in one direction till the blood oozes out of the lesion and an incision was made mostly in the inflamed border region of the lesion. Sample specimens were stored in the sterile labeled eppindorf containing 0.9 percent normal saline solution.

2.4. Parasite Culture

To obtain the culture of leishmania parasites from the samples, 10.43g/1000ml of RPMI 1640 was dissolved in sterile distilled water and distributed amongst the 20 vials of culture tubes each having 5ml of the dissolved media supplemented with 10% fetal calf serum [14]. The antibiotics consisting of Penicillin G and Kanamycin were added to the culture medium to avoid bacterial and fungal contaminations.

The skin scrapings were directly mixed from the lesions of infected patients to each culture tube containing 0.9% normal saline placed in ice jar and were brought to the laboratory of Zoology department, Kohat University of Science and Technology (KUST) where they were kept in an incubator (memmert type Inb 500, Germany) at 26±1°C to avoid contamination. After 4-6 days of incubation, culture was observed with Giemsa stained smears for the growth of leishmania promastigotes under Olympus microscope at 10X, 40X and 100X.

2.5. Dilution of the Crude Extracts

Serial dilutions of the increasing concentrations 25-100µg/ml of methanolic extracts of Aloe vera and Tamarix aphylla dissolved in 50% ethanol diluted in 1% Dimethyl Sulfoxide (DMSO) were exposed to culture medium of RPMI 1640 contained in screwed caped tubes aiming to reach the concentration that would produce to decrease the cell growth of leishmania parasites by 50 percent using percent of growth inhibition method.

2.6. Leishmanicidal Assay

The antileishmanial activity was assessed using promastigotes of Leishmania tropica, cultivated in bulk and was grown in modified NNN biphasic medium using normal saline. Parasites at log phase were centrifuged at 3000rpm for 10mins, washed with normal saline at same speed and time. Parasites were diluted to a final density of 1×10⁶cells/ml with a fresh culture medium of RPMI 1640. Eventually 100µl of the culture was added in all screwed caped culture tubes. The tubes were manually shaken for 2mins. The drug activity values of methanolic extracts were analyzed and assessed to determine the number of leishmanial parasites in non-treated control (1% DMSO without plant extracts) to control culture tubes after different time intervals of 48 and 96 hrs microscopically using improved Neubauer–counting chamber programme with the associated 95% confidence interval [15]. All the in vitro experiments were run in duplicate and the results were expressed as the percent inhibition in parasites number.

2.7. Statistical Analysis

Data management and analysis were made using SPSS version 16.0 for windows. The numbers of parasites were counted with a haemocytometer under a light microscope after 48 and 96 hours. P values < 0.05 were considered as statistically significant.

In order to keep in line with previous works in the same field, 48 and 96hours were chosen to express the effect of the extract [16, 17 and 18].

3. Results and Discussion

Concentrations of methanolic extract of Aloe vera leaf and Tamarix aphylla bark tested against Leishmania tropica for their antileishmanial activity and the results are given in Table 1.

The currently used treatment regimens for leishmaniasis is primarily based on chemotherapy [19] followed by the administration of available drugs such as Diamidine, Pentamidine, Amphoterin-B, and Glucantime [20] are usually avoided because of the association with severe side effects, high cost, variable degree of efficacy under same doses, ineffective and the emergence of drug-resistant parasites [21]. All of these factors emphasized the urgent need to develop for a new, safe, inexpensive and easily administered new drugs of herbal origin will have useful and interesting consequences [22, 23]. Studies focusing on herbal remedies useful for the treatment of leishmaniasis have been emerged world widely [24].
Table 1 Antileishmanial Activity of Aloe vera leaf and Tamarix aphylla bark extract against Leishmania tropica

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Concentrations (µg/ml)</th>
<th>Percent growth inhibition</th>
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<tr>
<td></td>
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<td>Aloe vera</td>
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<td>After 40hrs</td>
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<td>T₀</td>
<td>Non treated control (NTC)</td>
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</tr>
<tr>
<td>T₁</td>
<td>25</td>
<td>12</td>
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<tr>
<td>T₂</td>
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<td>75</td>
<td>37</td>
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<tr>
<td>T₄</td>
<td>100</td>
<td>53</td>
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P <0.05: Significant statistical difference in comparison to NTC at same time interval. Numbers of parasites in non-treated control cultures and treated cultures after different time intervals were calculated as Mean±SD and percent of growth inhibition. The mean numbers were compared at the same time interval using student’s t test.

of work to be carried out on the antileishmanial effects of these plants so far. The aim of this work was to identify a new leishmanicidal agent from the Aloe vera leaf and the bark of Tamarix aphylla.

Figure 1. Activity of Aloe vera leaf extract against Leishmania tropica

Figure 2. Activity of Tamarix aphylla bark extract against Leishmania tropica

The effect of concentrated methanolic extracts at concentrations ranging from 25-100µg/ml of Aloe vera leaf and Tamarix aphylla bark, was evaluated on the viability of L. tropica, a causative agent of cutaneous leishmaniasis and the percent growth inhibition concentration was determined to assess the number of leishmanial parasites in non-treated/control culture tubes after different time intervals of 48 and 96 hrs. Inhibition of parasite multiplication was noticed in proportion to concentration of the drug and incubation time. In the present study, we found the antipromastigote activity of the methanolic extracts of Aloe vera leaf and Tamarix aphylla bark which was much higher and is responsible for antileishmanial activity of this plant. This is a first report

Figure 3. Comparative Efficacy of Aloe vera and Tamarix aphylla against Leishmania tropica

The results tabulated in table 1 showed that the growth of Leishmania tropica was significantly inhibited with the increase in dose of Aloe vera leaf extract. The maximum percent growth inhibition was recorded in T₄ (A₂ = 66%) at 100µg/ml, followed by T₃ (B₂ = 43%) at 75µg/ml and minimum growth inhibition was observed in T₂ where it was (C₁ = 27%) and almost no significant percent growth inhibition was noted in T₁ and non-treated control (T₀) where it was observed the same (E=13%) for both the plant extracts.

Figure 1 shows, the time course of the viability of L. tropica in the presence of different concentrations of Aloe vera leaf extract. 93µg/ml and 75µg/ml concentrations of Aloe vera extract were able to kill 50% of the parasite after 48 and 96hrs respectively.

Similar observations with more potent activity were observed for the bark of Tamarix aphylla extract (Fig. 2) and same doses of concentrations were able to inhibit the growth of L. tropica, a causative agent of cutaneous leishmaniasis and showed a dose-dependent activity. The maximum percent growth inhibition was recorded in T₄ (A₂=84%), followed by T₃ (B₂=54%) and minimum growth inhibition was observed in T₂ where it was (C₂=28%) at various concentrations of 100, 75 and 50µg/ml respectively as shown in Table 1.

Figure 2 shows, the percent growth inhibition
observed in Tamarix aphylla bark. The values of which were recorded as 80µg/ml and 60µg/ml of concentrations after 48 and 96hrs respectively. In this study, Tamarix aphylla were also shown to have a statistically highly significant effect on motility of the parasites as compared of Aloe vera (P < 0.05). At concentration of 50µg/ml of methanolic extract, both the plant samples showed nearly same antileishmanial activity (Figure 3) as the numbers of parasites inhibited at the said concentrations were equal. However at concentrations exceeding than 50µg/ml, Tamarix aphylla showed more promising results as the number of parasites inhibited (Mean ±SD) were more as compared to Aloe vera.

Our decision was based on the observations for their well-known medicinal role in health sciences and products including topical treatment of burns, abrasions, an ulcer remedy and an adjuvant cancer treatment and other epithelial injuries, swellings and itching as well as their antiinflammatory properties might be explained in the light of these results.

Most of the studies have been done using the promastigote form of the parasite because it is easier to maintain these under in vitro conditions. However, since the promastigote is not the infective form of the parasite in vertebrate hosts, evaluations done with promastigotes have only an indicative value of the possible leishmanicidal activity [25]. The results of this study reveal antileishmanial activity against L. tropica by new medicinal plants and suggest that these methanolic extracts have the potential to be used as topically with CL patients. Further studies will be necessary to investigate the effect of these active plant extracts when combined with antileishmanial agents are commonly used. We report that Aloe vera and Tamarix aphylla extracts have direct parasiticidal effects on promastigotes. Aloe vera leaf and Tamarix aphylla bark can be considered as potential herbal remedies against CL that need to be tested in vivo. It can be therefore assigned that Aloe vera leaf and Tamarix aphylla possess a direct parasiticidal effect on Leishmania promastigotes.

4. Conclusion

These data reveal that Aloe vera leaf and the bark of Tamarix aphylla extracts contain active compounds, which could serve as an alternative agents as the drug therapy in the control of leishmaniasis. Thus, more investigations should be done, first in vitro assay for finding anti-leishmanial effect by using Leishmania amastigote and then to investigate in vivo activity in laboratory infected animals. This would help us in obtaining a novel drug that could potentially be less toxic and more cost-effective against the leishmania parasite.

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References


