Antimicrobial Activity of *Aloe sinkatana*

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*Aloe sinkatana* is a plant belonging to the family Xanthorrhoeaceae, subfamily Asphodeloideae, genus *Aloe*. It is cultivated in the Red Sea Mountains eastern of the Sudan. In the present study, the extract of *A. sinkatana* leaves was screened for its antibacterial and antifungal activity. The phytochemical screening of *A. sinkatana* extracts was carried out using Thin Layer Chromatography (TLC) technique. Four extracts of *A. sinkatana* were prepared using chloroform, ethanol, methanol, and water. Antibacterial activity of the extract was performed following the cup-plate agar diffusion method. Also, the antifungal activity of the extract was tested. The result showed that the extracts of *A. sinkatana* leaves revealed antimicrobial activities greater than the commercial antifungal (Nystatin) which can be used for treatment of Candidiasis and ketoconazole that is used for treatment of fungal infection. The antimicrobial activity might be due to specific plant compounds, which was found to be more effective than commercial antifungal compounds. However, the commercial antibiotic used for treatment of bacterial infection, displayed better antimicrobial activity than the *A. sinkatana* extracts. In conclusion *A. sinkatana* extract can be a useful treatment against fungal infections.

Key words: *Aloe sinkatana*, antibacterial, antifungal

*Aloe sinkatana* adalah tanaman yang termasuk keluarga Xanthorrhoeaceae, subfamili Asphodeloideae, genus *Aloe*. Itu ditanam di daerah pegunungan timur Sahara. Dalam penelitian ini, dilakukan skrining aktivitas antibakteri dan antijamur dari ekstrak daun *A. sinkatana*. Skrining secara fitokimia dari ekstrak *A. sinkatana* dilakukan dengan menggunakan teknik kromatografi lapis tipis (KLT). Enam jenis ekstrak *A. sinkatana* dipersiapkan dalam larutan chloroform, etanol, methanol, dan air. Aktivitas antibakteri dari ekstrak dilakukan mengikuti metode cup-plate agar diffusion. Demikian pula dilakukan uji aktivitas antijamur telah dilakukan. Hasil penelitian menunjukkan bahwa ekstrak dari *A. sinkatana* mengungkapkan kegiatan antimikroba lebih besar daripada antibakteri komersial, nistatin, yang digunakan untuk pengobatan *Candidiasis*, dan ketoconazole yang digunakan untuk pengobatan infeksi jamur. Aktivitas anti mikroba mungkin disebabkan karena senyawa tertentu, yang lebih efektif daripada senyawa anti jamur komersial. Akan tetapi, antibiotik komersial untuk pengobatan infeksi bakteri, mempunyai aktivitas anti mikroba lebih baik daripada ekstrak *A. sinkatana*. Sebagai kesimpulan ekstrak *A. sinkatana* berpotensi sebagai obat infeksi jamur.

Kata kunci: *Aloe sinkatana*, anti bakteri, anti jamur

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*Aloe sinkatana* is a clumping rosettes plant belonging to family Xanthorrhoeaceae, subfamily Asphodeloideae, genus *Aloe*. It is found in Eastern Sudan in Red Sea Mountains mainly in Arkavit area. The plant is up to 60 cm tall and 60-90 cm in diameter (Reynolds 1957).

The active component in *Aloe vera* is Anthraquinone glycosides; which is an aromatic organic compound with the formula C₁₄H₁₂O₂ with several isomers, each of them is known as a quinone derivative. Three different solvents such as water, ethanol and acetone were used to extract the bioactive compounds from the leaves of *A. vera* to screen the antimicrobial activity of selected human pathogens by agar diffusion method. The antifungal activity of *A. vera* was analysed against *Aspergillus flavus* and *Aspergillus niger*. The maximum antifungal activity was observed in acetone extracts when compared with other extracts. *A. vera* plant extract with acetone can be used as antimicrobial agents (Arunkumar and Muthuselvar 2009).

The comparative antimicrobial activities of the gel and leaf of *A. vera* were tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Trichophyton menthae*, *T. schoeleinii*, *Microsporium canis*, and *Candida albicans* (Pandey and Mishra 2010). Ethanol was used for the extraction of the leaf after obtaining the gel from it. Antimicrobial effect was measured by the appearance of zones of inhibition (Pandey and Mishra 2010; du Plessis and Hamman 2013).

Antimicrobial susceptibility test showed that both the gel and the leaf inhibited the growth of *S. aureus*.
Only the gel inhibited the growth of T. mentagrophytes while the leaf possesses inhibitory effects on both P. aeruginosa and C. albicans. The results of this stimulate the use of both A. vera gel and leaf (O et al. 2005; Pandey and Mishra 2010). The aim of this study is to test the antimicrobial activities of the A. sinkatana. There is no work done on A. sinkatana so far and accordingly, this is the first report on investigation on its antimicrobial activity.

**MATERIALS AND METHODS**

**Plant Material.** A. sinkatana plant was obtained from faculty of Pharmacy collection at the University of Medical Science and Technology, Khartoum, Sudan.

**Preparation of the Crude Extracts.** Coarsely powdered A. sinkatana leaves (80 g) were extracted for twenty hours with chloroform in soxhlet apparatus. The chloroform extract was filtered and evaporated under reduced pressure. The extracted leaves were air-dried, re-packed in soxhlet till exhaustively extracted with methanol. The methanolic extract was filtered and evaporated under reduced pressure. The residue of chloroform was re-dissolved in a mixture of petroleum ether and methanol in the ratio of (1:2 v/v) and the methanol extract was re-dissolved in methanol. The final product was kept in a refrigerator till used. Simultaneously, water extract was prepared by adding 10 mL of boiled distilled water to a sample of 10 g of the coarsely powdered plant materials in a beaker, with occasional shaking for four hours and the final volume was adjusted to 10 mL with boiled distilled water at a temperature of 25 °C. The aqueous extract was filtered and the precipitate was washed in distilled water and the filtrate was used immediately (Almagboul, 1992).

**Phytochemical Screening of A. sinkatana Extracts.** The phytochemical screening was carried out according to qualitative methods described. Six extracts have been obtained such as chloroform extract, methanol extract, and aqueous extract. The powdered dried samples of the leaves of A. sinkatana were separately screened for the following constituents: carbohydrates and/or glycosides, tannins, flavonoids, saponins, alkaloids and/or nitrogenous bases, anthraquinones, unsaturated sterols and/or triterpenes, and coumarins using water and organic solvents according to the required material.

**Preparation of the Test Organisms.** One mL of a 24 h broth culture of the test organism was aseptically distributed onto nutrient agar slopes and incubated at 37 °C for 24 h. The bacterial were harvested and washed in sterile normal saline, and suspended in a small volume of normal saline to produce a suspension containing about 10^5-10^6 colony forming units per mL. The suspension was stored in a refrigerator at 4 °C till used. The average number of viable organism per ml of the stock suspension was determined by means of the surface viable counting technique (Miles and Amyes, 1996). Serial dilutions of the stock suspension were made in sterile normal saline, and dropping pipettes to the surface of dried nutrient agar plates transferred 0.02 mL of the appropriate dilutions. The plates were allowed to stand for 2 h at room temperature, following incubation at 37 °C for 24 h. After incubation the number of colonies in each drop was counted. The average number of colonies per drop (0.02 mL) was multiplied by 50 and by the dilution factor to give the viable count of stock suspension, expressed as the number of colony forming units per mL of suspension. Each time a fresh stock suspension was prepared.

**Antibacterial Activity of the Extract.** The cup-plate agar diffusion method (Kavanagh, 1972), was adopted with some minor modification to assess the antibacterial activity of the prepared extracts. One mL of the standardized bacterial stock suspension (10^5-10^6 colony forming units per mL) was thoroughly mixed with 100 mL of sterile molten Muller-Hinton agar, which was maintained at 45 °C. 20 mL aliquots of the inoculated Muller and Hinton agar were distributed into sterile Petri dishes. The agar was left to set. On each of these plates, four cups (10 mm in diameter) were cut using a sterile cork borer and agar discs were removed. The cups were filled with 0.1 mL sample of each of the extract using standard fine pipette adjustable volume digital pipette, and allowed to diffuse at room temperature for two hours. The plates were incubated in the upright position, at 37 °C for 18 h. Three replicates were made for each extract against each of the tested organisms. Simultaneously, positive control was inoculated using respective solvents. After incubation, the diameters of the resultant growth inhibition zones were measured. The average mean values were tabulated. To determine whether the plant extracts were bacteriostatic or bactericidal subcultures were made from within the zones of inhibition onto Muller and Hinton agar and incubated at 37 °C for 24 h. Then the plates were examined for bacterial growth. Growth of the organism indicates the bacteriostatic activity of extract and no growth indicates its bactericidal effect.

**Antifungal Activity of The Extracts.** The fungal tested organism A. flavus and A. niger were spread over the Sabouraud’s dextrose agar plates after the
microbial, four cups (10 mm in diameter) were cut using a sterile cork borer and agar discs were removed. The cups were filled with 0.1 mL samples of each of the extract, and allowed to diffuse at room temperature for 2 h. The plates were incubated in the upright position, at 30 °C for 72 h. Three replicates were made out for each extract against each of the tested organisms. Simultaneously, positive control was involved by adding respective solvents instead of the extracts. After incubation, the diameters of the resultant growth inhibition zones were measured in mm, the average mean values were tabulated.

For other fungal tested organisms, one mL of the extract was thoroughly mixed with 100 mL of sterile molten Sabouraud’s dextrose agar which was maintained at 45 °C. Twenty mL aliquots of the inoculated Sabouraud’s dextrose agar were distributed into sterile petri-dishes. The agar plates were left to set; the test organisms were inoculated in the center of plate. Positive control was inoculated in the same manner as above.

Antimicrobial Susceptibility-Test Susceptibility Test Procedure. Plates with Mueller and Hinton agar were prepared according to a method previously described (Bauer and Driesen 1966). For rapidly growing aerobic organisms, 3-4 similar colonies from pure cultures as inoculum were selected and transferred into 5 mL of Tryptone Soya Broth. Incubation was performed at 35 °C for 2-8 h until moderate turbidity developed. A sterile non-toxic cotton swab on a wooden applicator was dipped into the standarized inoculum and the soaked swab was firmly rotated against the upper inside wall of the tube angle between each streaking. The inoculum was allowed to dry for 5-15 min with lid in place. Using aseptic technique the discs were applied. The discs with centers at least 24 mm apart were deposited. For Penicillin and Cephalosporin, the discs were deposited with centers 30 mm apart. The plates were incubated immediately at 37 °C and examined after 14-19 h. Zones showing complete inhibition was measured (Bauer and Driesen 1966).

RESULT

The Antimicrobial Activity against S. arues, E. coli, and P. aerginosa. The antimicrobial activity of different antibiotic and A. sinkatana in different solvent systems against S. arues, E. coli, and P. aerginosa was estimated and revealed inhibition zones (Table 1). In comparison virtually no difference could be seen between antibiotic tested and extracts from A. sinkatana tested.

The antimicrobial activity of different antibiotic and A. sinkatana in different solvent systems against C. albicans, A. niger, A. flavus, T. mentaegraphytes, and Phialophorarichardsiae revealed inhibition zones (Table 2). All dermatophytes tested revealed sensitivity to all extracts. Furthermore, no growth was observed on Sabouraud dextrose agar after A. sinkatana extracts treatment. Thus, the extracts of A. sinkatana tested against dermatophytes included in experiments.

Table 1 Effect of Aloe sinkatanain in different solvent systems and antibiotic against against Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa

| Solvent      | Inhibition zone (mm) |          |          |          |
|--------------|----------------------|----------|----------|----------|
|              |                      | S. aureus | P. aeruginosa | E. coli  |
| Solvent system | Chloroform           | 19       | 18       | 17       |
|               | Methanol             | 22       | 20       | 20       |
|               | Water                | 0        | 14       | 14       |
| Antibiotic    | Co-trimexazole       | 30       | -        | 32       |
|               | Gentamycin           | 20       | 19       | 21       |
|               | Ciprofloxacin        | 35       | 35       | 38       |
|               | Amoxycillin          | -        | -        | 30       |
|               | Ceftriazone          | -        | 26       | 30       |
|               | Cephalothin          | 34       | -        | -        |
|               | Ampicillin           | 34       | -        | -        |
|               | Amikacin             | -        | 30       | -        |
|               | Imipenem             | -        | 32       | -        |
flavonoids, tannins, saponins, carbohydrates, steroids, and reduced sugars. These results are similar to previous results (Arunkumar and Muthuselvam 2009) showing that *A. vera* contains tannin, saponin, and flavonoids and there are findings reported for *A. vera* to have mono- and polysaccharides, tannins, sterols, organic acids, enzymes, saponins, vitamins, and minerals (Rodriguez et al. 2010; Nejatzadeh-Barandozi 2013). Thus, this shows that *A. sinkatana* and *A. vera* share almost the same constituents.

**Antibacterial activity of** *A. sinkatana* **against** *S. aureus*, *P. aeruginosa*, and *E. coli* revealed antibacterial activity in the methanol extract compared to the other extracts. Among the three bacterial organisms tested the maximum growth suppression was observed with *S. aureus* (22 mm) compared with *E. coli* and *P. aeruginosa*. This finding harmonise to a previous finding, (Arunkumar and Muthuselvam 2009) in which it was shown that *A. vera* leafs inhibited growth of *S. aureus*, *S. pyogen*, *P. aeruginosa*, and *E. coli*. This might be due to the anthraquinones compound which disclosed to have an antimicrobial activity (Ernst 2000). According to available literature there is no work done on antimicrobial activity of *A. sinkatana* and performed show antimicrobial activity. Two fungi were tested on the antimicrobial activity of *A. sinkatana*, *T. verrucosum*, and *M. canis* were tested in different solvent system of *A. sinkatana* and revealed growth inhibition (Table 3). Clearly the effect of *A. sinkatana* extracts against *T. verrucosum* was indisputable since all extracts rendered no growth detectable. *M. canis* on the other hand showed growth after treatment of one extract which was chloroform. All dermatophytes tested revealed high sensitivity to all extracts. No growth was observed when culturing them on Sabouraoud’s dextrose agar.

### DISCUSSION

It can safely be presumed that the major part of a traditional medicine involves the use of plants and their derived active principles, although their use is not always verified by the scientific means, very little and scattered investigations have been carried out searching for plants with antimicrobial activity.

In the present study the phytochemical screening of *A. sinkatana* showed that the plant contains same ingredients as in *A. vera* such as anthrancene, alkaloids, flavonoids, tannins, saponins, carbohydrates, steroids, and reduced sugars. These results are similar to previous results (Arunkumar and Muthuselvam 2009) showing that *A. vera* contains tannin, saponin, and flavonoids and there are findings reported for *A. vera* to have mono- and polysaccharides, tannins, sterols, organic acids, enzymes, saponins, vitamins, and minerals (Rodriguez et al. 2010; Nejatzadeh-Barandozi 2013). Thus, this shows that *A. sinkatana* and *A. vera* share almost the same constituents.

**Table 2** Inhibition zones of *Aloe sinkatana* against *Candida albicans*, *Aspergillus niger*, *Aspergillus flavus*, *Trichophyton mentagrophytes*, and *Phialophora richardiae* in different solvent systems

| Solvent     | *C. albicans* | *A. niger* | *A. flavus* | *T. mentagrophytes* | *P. richardiae* |
|-------------|---------------|------------|-------------|---------------------|-----------------|
| Solvent system | Chloroform | 23 | 18 | 20 | 22 | 20 |
|             | Methanol     | 22 | 20 | 21 | 18 | 15 |
|             | Water        | 19 | 0  | 0  | 0  | 0  |
| Antifungal agents | Nystatin | 17 | -  | -  | -  | -  |
|             | Ketoconazole 100 mg | - | 27 | 25 | -  | -  |
|             | Ketoconazole 50 mg | - | 24 | 20 | -  | -  |
|             | Ketoconazole 25 mg | - | 18 | 18 | -  | -  |

**Table 3** Inhibition of growth by *Aloe sinkatana* against *Microsporum canis*, and *Trichophyton verrucosum* in different solvent

| Fungi            | Growth inhibition |
|------------------|--------------------|
|                  | Chloroform | Methanol | Water |

| *Trichophyton verrucosum* | No Growth | No growth | No growth |
|----------------------------|-----------|-----------|-----------|
| *Microsporum canis*        | Growth    | No growth | Growth    |

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Moreover, *A. sinkatana* extracts have shown to inhibit the growth of fungi that cause tinea since complete inhibition of growth of *M. canis* and *T. verrucosum* was observed.

In the present study, the extracts of *A. sinkatana* leaves revealed antimicrobial properties greater than commercial antifungal agent (nystatin) used for treatment of *C. albicans* and ketoconazole used for treatment of *A. niger* and *A. flavus*.

In conclusion present study revealed the presence of secondary metabolites in the leaves of *A. sinkatana*. It was further shown that the plant extracts may be used for the treatment of fungal infections such as ringworm and aspergillosis. The results lend credence to the folkloric use of this plant in treating microbial infection and shows that *A. sinkatana* could be exploited for new potent antimicrobial agents especially antifungal agents. This becomes more relevant as current antimicrobial agents in use are fast loosing effectiveness due to emergence of resistant microorganisms.

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