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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 Mountainseastern 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* . Tanaman ini dibudidayakan di Red Sea Mountainseastern, Sudan . 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 kloroform, etanol, metanol, dan air. Aktivitas anti bakteri 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 anti jamur komersial, nistatin, yang digunakan untuk pengobatan *Candidiasis*, dan *ketoconazolethat* 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

Aloe sinkatana is a clumping rosettes plant belonging to family *Xanthorrhoeaceae*, subfamily *Asphodeloideae*, genus *Aloe*. It is found in Eastern of Sudan in Red Sea Mountains mainly in Arkawit 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 $C_{14}H_8O_2$ 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 Muthuselvam 2009).

The comparative antimicrobial activities of the gel and leaf of *A. vera* were tested against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Trichophytonmenta graphytes*, *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.*

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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, anthraquinons, 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 10^8-10^9 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 (10^8-10^9 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 antibiotica 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 antibiotica tested and extracts from *A. sinkatana* tested.

The antimicrobial activity of different antibiotica 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 Sabouraoud 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
	Ceftriaxone	-	26	30
	Cephalothin	34	-	-
	Ampicillin	34	-	-
	Amikacin	-	30	-
	Imipenem	-	32	-

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

	Solvent	Inhibition zone (mm)				
		C. albicans	*A. niger*	*A. flavus*	*T. mentaegraphytes*	*P. richardsiae*
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 cains*, and *Tricophyton verrucosum* in different solvent

Fungi	Growth inhibition		
	Solvent system		
	Chloroform	Methanol	Water
Tricophyton verrucosum	No Growth	No growth	No growth
Microsporum canis	Growth	No growth	Growth

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. cains* 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 anthracene, 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 *A. vera* to have mono- and polysaccharides, tannins, sterols, organicacids, 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

accordingly this is the first report on such work.

Antifungal activity of *A. sinkatana* against *C. albicans*, *A. flavus*, *A. niger*, *Microsporum canis*, *Trichophyton mentagraphytes*, *T. verrcosum spp.*, and *Phialophorarichardsiae* could be estimated and the revealed maximum antifungal activity with ethyle acetate extract was observed against *A. niger* (28 mm). This finding is similar to previous findings, where it has been shown that, the maximum antifungal activity of *A. vera* was observed in *A. flavus* (15 mm) (Arunkumar and Muthuselvam 2009).

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.

REFERENCE

Almagboul AZ. 1992. Antimicrobial and Phytochemical investigation of Vernonia and other Sudanese medicinal plants. PhD, University of Khartoum.

Arunkumar S, Muthuselvam M. 2009. Analysis of Phytochemical Constituents and Antimicrobial Activities of *Aloe vera* L. Against Clinical Pathogens. World J Agr Sci. 5(5):572-576.

Bauer KM, Driesen D. 1966. [Cerebral aneurysm and seminoma as a combined malformation. Report on 2 clinical cases]. Med Welt. 46:2492-2494.

Du Plessis L.H, Hamman J H. 2013. In vitro evaluation of the cytotoxic and apoptogenic properties of *Aloe* whole leaf and gel materials. Drug Chem Toxicol. doi:10.3109/01480545.2013.83435.

Ernst E. 2000. Adverse effects of herbal drugs in dermatology. Br J Dermatol. 143(5):923-929. doi:10.1046/j.1365-2133.2000.03822.x.

Farnsworth NR, Akerele O, Bingel AS, Soejarto DD, Guo Z. 1985. Medicinal plants in therapy. Bull World Health Organ. 63(6):965-981.

Miles RS, Amyes SB. 1996. Laboratory control of antimicrobial therapy. In practical Medical Microbiology, New York, Churchill Livingstone.

Nejatzadeh-Barandozi F. 2013. Antibacterial activities and antioxidant capacity of *Aloe vera.* Org Med Chem Lett. 3(1):5. doi:10.1186/2191-2858-3-5.

Newall CA, Anderson LA, Phillipson JD. 1996. Herbal medicines: A Guide for Health-Care Professionals. London, England: Pharmaceutical Press.

Agarry OO, Olaleye MT, Bello-Michael CO. 2005. Comparative antimicrobial activities of *Aloe vera* gel and leaf. Afr J Biotechnol. 4(12):1413-1414.

Pandey R, Mishra A. 2010. Antibacterial activities of *Aloe barbadensis* to clinically isolated bacterial pathogens. Appl Biochem Biotechnol. 160(5):1356-1361. doi:10.1007/s12010-009-8577-0.

Reynolds. 1957. Classification of *Aloe sinkatana*. J African Botany. 23-29.

Rodriguez ER, Martin JD, Romero CD. 2010. *Aloe vera* as a functional ingredient in foods. Crit Rev Food Sci Nutr. 50(4):305-326. doi:10.1080/10408390802544454.

Sharma D, Lavania AA, Sharma A. 2009. In vitro comparative screening of antibacterial and antifungal activities of some commo plants and weeds axtracts. Asian J Exp Sci. 23:169-172.

Van Gorkom BA, De Vries EG, Karrenbeld A, Kleibeuker J H. 1999. Review article: anthranoid laxatives and their potential carcinogenic effects. Aliment Pharmacol Ther. 13(4):443-452. doi:10.1046/j.1365-2036.1999.00468.x.