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Antimicrobial activity and minimum inhibitory concentration of *Aloe vera* sap and leaves using different extracts

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Abstract
Many of the health benefits associated with *Aloe vera* have been attributed to the polysaccharides contained in the sap of the leaves. The main objectives of this study is to evaluate the antimicrobial activity for both sap and leaves extracts of *A. vera* using different extraction methods. *Aloe vera* sap and leaves extracts were investigated using agar-well diffusion technique with different concentrations. Both types of extract revealed antimicrobial inhibitory effect ranged from pronounced, intermediate, and low. The sap extract showed more effective than the leaves extract against all test microorganisms. The highest mean diameter of zone (MDIZ) showed with sap water extract (100 µg/ml) against *Pseudomonas aeruginosa* and *Bacillus subtilis* (47 mm). While the lowest one showed with sap and leaves saponins (100 µg/ml) against all test microorganisms (12 – 14 mm) with exception of *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Staphylococcus aureus* whom showed resist (0.0 mm). The minimum inhibitory concentration (MIC) showed ≤ 6.25 µg/ml for almost all test microorganisms and different types of extracts except acetone extract from leaves which exhibited MIC≥ 50 µg/ml. The present study concludes that the *A. vera* sap and leaves could be used as medicinal plant with further investigation to determine its toxicity and side effects if any.

Keywords: *Aloe vera*, antimicrobial activity, sap, leaves, minimum inhibitory concentration

Introduction
Aloe vera (L.) Burm.f. (*Aloe barbadensis* Miller) is a perennial succulent xerophyte, which develops water storage tissue in the leaves to survive in dry areas of low or erratic rainfall [1]. Commonly referred to as *Aloe vera*, is one of more than 400 species of *Aloe* belonging to family Liliaceae [2]. The species does not have any naturally occurring populations, although closely related aloes do occur in Northern Africa [3]. It is a cactus-like plant that grows readily in hot, dry climates and currently because of demand; it is cultivated in large quantities [4]. *Aloe vera* plant almost sessile perennial herb, has leaves 30-35cmm long and 10cm broad at the base, colour pea-green (when young), bright yellow tubular flowers 25-35cm in length arranged in a slender loose spike, stamens frequently projected beyond the perianth tube [5].
Plant extracts represent a continuous effort to find new compound against pathogens. Approximately 20µg/ml of the plants found in the world have been submitted to pharmacological or biological test, and a substantial number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources [6]. The skin plays an important role in protection from the body internal environment and it is the largest organ in human's body so exertion of serious damage to this organ may cause several problems in its survival [7]. Medicinal plants according to the World Health Organization (WHO) defines them as herbal preparations made by introducing plant materials to extraction, fractionation, purification, concentration, or other physical or biological processes, which may be produced as a basis for herbal products or for immediate consumption [8]. *Aloe vera* has modified thick fleshy leaves, it not only has cell wall carbohydrates such as cellulose and hemicellulose but also storage carbohydrates such as acetylated mannans the polysaccharides found in the inner leaves parenchymatous tissue have medicinal importance and also the biological activities are due to presence of large number of compounds [9]. The herb is used internally to combat most digestive problems, including constipation, poor appetite, colitis, irritable bowel syndrome as well as, asthma, diabetes, immune system enhancement, peptic ulcers. *Aloe* is used externally for the treatment of skin irritation, burns, scalds, sunburn wounds, eczema, psoriasis, acne, dermatitis, ulcers, to stimulate cell regeneration. The plant is also used in the treatment of healing properties, effects on skin exposure to UV and gamma radiation, anti-inflammatory, antiviral and antitumor, moisturizing, anti-aging effect, antiseptic, enhance immune system,

hypoglycemic, cytotoxic, and anti-diabetic effects, antibacterial effect, antioxidant, cardiovascular effect [10]. Its juice may help some people with ulcerative colitis, an inflammatory bowel disease [11].

The main objectives of this study is to evaluate the antimicrobial activity for both sap and leaves extracts of *A. vera* using different chemical extractors.

Materials and Methods

Chemicals

Acetone	Methanol	*n*-Butanol
Ethanol	Acetic acid	Sodium chloride
Calcium hydroxide	Ammonium hydroxide	
Hydrochloric acid	Diethyl ether	

Methods

Aloe vera chemical components were extracted according to the Harborne [12].

Preparation of methanol extract from leaves

An amount of 15g of dried powdered *A. vera* leaves were placed into 300cm³ conical flask, 100cm³ of 80µg/ml methanol were added. The conical flask was stoppered. The mixture was filtered after two days and the marc was watched with more solvent and the volume was adjusted to 150 cm³ with 80µg/ml ethanol.

Preparation of methanol extracts from sap

Six hundred (600) ml of sap were dried in oven (60-80 °C) and the dried sap was weighed. An amount of 5 g of dried powdered sap were placed into 500 ml beaker and 100 ml of 80µg/ml ethanol were added and allowed to stand to 2 days the mixture, was filtered and the marc was watched with more solvent and volume was adjusted to 150 cm³ with 80µg/ml methanol.

Preparation of water extract from leaves

An amount of 15g of dried powdered *A. vera* leaves were placed into 300cm³ conical flask 100cm³ of distilled water were added. 1ml of 10µg/ml acetic acid was added. The conical flask was stoppered and after two days the mixture was filtered and the marc was watched with more distilled water, the volume was adjusted to 150 cm³ with distilled water.

Preparation of water extract from sap

Six hundred (600) ml of sap were dried in oven (60-80 °C) and the dried sap was weighed. An amount of 5 g of dried powdered sap were placed into 600 ml beaker and 100 ml distilled water were added and 1ml of 10µg/ml acetic acid was added and allowed to stand to 2 days, the mixture was filtered and the marc was watched with more solvent and the volume was adjusted to 150 cm³ with distilled water.

Preparation of acetone extracts from leaves and sap

An amount of 10gms of sample were placed into 300cm beaker and 100 ml of acetone were added the mixture was stirred to 4 hrs. The extract was filtered and dried.

Alkaloid determination

An amount of 5gms of the plant sample were placed into a 250ml beaker. 10µg/ml acetic acid in ethanol were added, the mixture was covered and allowed to stand for 4hrs. The extract was filtered and concentrated on a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with diluted ammonium hydroxide and then filtered the residual alkaloid was dried and weighed.

Saponin determination

An amount of 10gms of plant sample were put into a conical flask and 50ml of 20µg/ml aqueous ethanol solution were added. The sample was heated over a hot water-bath for 4hrs with continuous stirring at about 55. The mixture was filtered and the residue re-extract with another 200 ml of 20µg/ml ethanol. The combined extracts were reduced to 40ml over water-bath about 90 °C. The concentrate was transferred into a 250ml reparatory funnel and 20ml of diethyl ether were added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of *n*-butanol were added and the combined *n*-butanol extracts were washed twice with 10ml of 5µg/ml aqueous sodium chloride. The remaining solution was heated in water-bath. After evaporation, the sample was dried in the oven to a constant weight and the saponin content was calculated as percentage.

Flavonoid determination

An amount of 10gms of the plant sample was extracted repeatedly with 100ml of 80µg/ml aqueous methanol at room temp. The whole solution was filtered through whatmman filter paper No 42 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over water-bath and weighted to constant weight and the flavonoid content was calculated as percentage.

Acids determination

An amount of 5gms of dried sample were boiled for 30 minutes with 20 ml of distilled water. The mixture was cooled and filtered. The previous step was repeated twice using different solvents as mixture of distilled water and ethanol (1:1) and ethanol. The pH of above solutions was measured (water extract 1.3, alcohol extract 1.5, alcohol: water extract 1.4.).

The aqueous extract was neutralized at room temperature with suspension of calcium hydroxide giving yellow precipitate in sap and gray with leaves. Then filtered and dried the weight was recorded.

Preparation of the test microorganisms

Standard microorganisms

The microorganisms used in the present study were kindly provided by scientists at Khartoum National Health Laboratory and designated as follows:

Standard microorganisms

Bacterial organisms	Code number
Escherichia coli	ATCC/25923
Pseudomonas aeruginosa	ATCC/27853
Bacillus subtilis	NCTC/8236
Staphylococcus aureus	ATCC/25923
Candida albicans	ATCC/7596
Aspergillus niger	ATCC/9763

* American Type Culture Collection (ATCC) Rockville, Maryland, USA.

* NCTC: National Collection of Type Culture, Colindale, England.

Culture media

Nutrient broth (oxoid Ltd, London)

Composition: Peptone: 5 g, sodium chloride: 5 g, beef extracts 1g, yeast extract: 2g at pH: 7.4. The media were prepared according to the manufacture instructions and autoclaved at 121°C/15lbs for 15 minutes [13].

Mueller-Hinton agar (oxoid Ltd, London)

Composition: Nutrient broth: 300 ml, casein hydrolysate: 17.5 ml, starch: 1.5g, agar: 10 g and distilled water: 1000 ml, at pH: 7.6. The media were prepared according to the manufacture instructions and autoclaved at 121°C/15lbs for 15 minutes. The medium is used for all antibiotic sensitivity tests as standard drug as well as for plant extracts evaluation [13].

Preparation of the standard microbial suspensions

One ml aliquots of a 24-hours broth culture of the test organisms will be aseptically distributed onto nutrient agar slopes and incubated at 37 ºC for 24-hours.The bacterial growth was harvested and washed off with 100 ml sterile normal saline, to produce a suspension containing about 10^8 - 10^9 colony forming units per ml. The suspension was stored in the refrigerator at 4ºC till used. The average number of viable organisms per ml of the stock suspension was determined by means of the surface viable counting technique [14]. Serial dilution of the stock suspensions were made in sterile normal saline solution and 0.02 ml volumes of the appropriate dilution were transferred by micropipette to the surface of dried nutrient agar plates. The plates were allowed to stand for 2-hours at room temperature for the drops to dry, and then incubated at 37 ºC for 24 hours. After incubation, the number of developed colonies in each drop was counted. The average number of the colonies per drop (0.02ml) will be multiplied by 50 and by the dilution factor to give the viable count of stock suspensions, expressed as the number of colony forming units per ml of suspension. Each time a fresh stock suspension will be prepared, all the above experimental condition will be maintained constant so that suspensions with very close viable count would be obtained.

Determination of antimicrobial activity of plant extracts

At the time of testing, the extracts were reconstituted to concentration of 100, 50, 25, 12.5, and 6.25 percent in dimethyl sulphoxide (DMSO). Antimicrobial activity was assessed by the agar–well diffusion method [15]. The inoculums size of each tested bacterium was adjusted to a suspension of 10^6 cells. The inoculums suspension was spread over a Mueller Hinton agar (MHA) plate, to achieve confluent growth, and allowed to dry. 10 mm- diameter wells were bored in the agar using a sterile cork borer (NO.4) and the agar discs were removed. A 100µl - aliquot of the reconstituted extract was placed into a well with standard Pasteur pipette and the plate was held for 1 hr at room temperature for diffusion of extract into the agar. Subsequently, the plate was incubated for 18 hr at 37 ºC. After incubation, the diameters of the zones of inhibition were measured to the nearest mm. Three replicates were performed and results were recorded [15].

Results

Evaluation of anti-microbial Activity of acids Extracts from sap of *A. vera*

As can be seen in table 1, the test microorganisms were subjected to different solvent extracts of leaves and sap of *Aloe vera*. The microorganisms exhibited different susceptibility responses against those extracts. The pronounced effect was showed when sap acid used to inhibit the growth of all test microorganisms. The mean diameter of inhibition zone (MDIZ) was recorded as 36 mm, 31 mm, 30 mm, 27 mm, and 21 mm for *E.coli*, *C.albicans* and *A. niger*, *B.subtilis*, *S.aureus*, and *Pseu. aeruginosa*, respectively. The leaves acids showed inhibitory effect with highly susceptibility against *B.subtilis* (33 mm), *S.aureus* (31 mm), *C.albicans* (30 mm), and *E.coli* (26 mm). The leaves acids also showed intermediate inhibitory effect *Pseu. aeruginosa* (19 mm) and low inhibitory effect against *A. niger* (17 mm).

In comparison between leaves and sap methanolic extract, the sap methanolic extract showed higher inhibitory effect against *S.aureus* (41 mm), *B.subtilis* (40 mm), and *E.coli* (37 mm). Also it showed high to intermediate inhibitory effect against *Pseu. aeruginosa* (30 mm), *A. niger* (21 mm), and *C.albicans* (18 mm). while the leaves methanolic extract showed high to intermediate inhibitory effect against all test microorganisms as *S.aureus* (25 mm), *A. niger* (24 mm), *C.albicans* (22 mm), *B.subtilis* (20 mm), and 19 mm for both *E.coli* and *Pseu. aeruginosa*.

The water extract of sap and leaves of *A. vera* exhibited variable inhibitory effects. The sap water extract was determined as potent substance to inhibit the growth of all test microorganisms with exceptional inhibition zones against the bacterial species as *B.subtilis* (47 mm), *S.aureus* and *Pseu. aeruginosa* (45 mm), and *E.coli* (40 mm); while the rest test microorganisms (the fungal species) exhibit intermediate susceptibility as *A. niger* (19 mm), and *C.albicans* (17 mm).

The leaves water extract showed intermediate inhibitory effects against almost all test microorganisms with exception of the mould *A. niger* which showed resist to its action. The diameter of inhibition zone of leaves water extract was recorded as *Pseu. aeruginosa* and *S.aureus* (21 mm), *E.coli* and *B.subtilis* (20 mm), and *C.albicans* (18 mm).

Sap acetone extract showed high to intermediate inhibitory effect against all test microorganisms. The pronounced effect was with *Pseu. aeruginosa* (46 mm).The highly inhibitory effect was showed against *S.aureus* (41 mm), *B.subtilis* (40 mm), and *E.coli* (39 mm). An intermediate effect was determined against both fungal species as *A. niger* (21 mm), and *C.albicans* (19 mm).

The leaves acetone extract showed low inhibitory effect against almost all test microorganisms with exception of yeast-like fungus *C.albicans* which exhibited no susceptibility to it. The other test microorganisms showed an intermediate inhibitory effect as *S.aureus* (27 mm) and low inhibitory effect for *E.coli* and *A. niger* (15 mm), *B.subtilis* (14 mm), and *Pseu. aeruginosa* (13 mm).

Two main chemical constituents (alkaloids and saponins) of *A. vera* were determined and investigated for their antimicrobial activities. The sap saponins showed low inhibitory effect against *S.aureus* (15 mm), *E.coli* and *C.albicans* (14 mm), *B.subtilis* and *Pseu. aeruginosa* (13 mm); while *A. niger* showed more sensitive to its action (17 mm). In contrast, the leaves saponins showed low inhibitory effect only against three test microorganisms, namely, *A. niger*, *C.albicans*, and *E.coli* with similar MDIZ (14 mm).

The sap alkaloids also showed low to intermediate inhibitory effect for five out of six test microorganisms. The microorganisms organized according to the MDIZ as *E.coli* (20 mm), *Pseu. aeruginosa* and *A. niger* (16 mm), *B.subtilis* and *C.albicans* (14 mm). The leaves alkaloids showed low to intermediate inhibitory effect for four out of six test

microorganisms as *A. niger* (18 mm), *B.subtilis* (15 mm), *E.coli* (14 mm), and *C.albicans* (13 mm).
Staphylococcus aureus showed no susceptibility for both sap alkaloids and leaves alkaloids, while *Pseu. aeruginosa* showed no susceptibility for leaves alkaloids with low susceptibility to sap alkaloids.

Table 1: Evaluation of anti-microbial Activity of Different Solvents Extracts of leaves and sap of *A. vera*

Extract Concentration (100µg/ml)	Mean Diameter of Inhibition zone (MDIZ)/mm					
	E.c	***Pseu.a***	***B.S***	***S.a***	***C.a***	***A.n***
leaves methanolic extract	19	19	20	25	22	24
Sap saponins	14	13	13	15	14	17
leaves saponins	14	0	0	0	14	14
leaves Alkaloids	14	0	15	0	13	18
Sap alkaloids	20	16	14	0	14	16
leaves acetone extract	15	13	14	27	0	15
Sap acetone extract	39	46	40	41	19	21
Sap water extract	40	47	47	45	17	19
Sap methanolic extract	37	30	40	41	18	21
leaves water extract	20	21	20	21	18	0
Sap acids	36	21	30	27	31	31
leaves Acids	26	19	33	31	30	17

Key: E.c ≡ *Escherichia coli*, Pseu.a ≡ *Pseudomonas aeruginosa*, B.s ≡*Bacillus subtilis*, S.a ≡ *Staphylococcus aureus* C.a ≡ *Candida albicans*, A.n ≡ *Aspergillus niger*

Minimum Inhibitory Concentrations (MICs)

As can be seen in table 2, different concentrations (50, 25, 12.5, and 6.25 µg/ml) of different extracts were investigated to determine their MICs against test microorganisms.
All sap extracts (methanolic, acetone, water and acids from sap) showed MIC less than 6.25 µg/ml against all test microorganisms. Acids and methanolic extract from leaves also showed MIC less than 6.25 µg/ml against all test microorganisms but less inhibitory effects than those of sap extracts and acids from sap.
Water extract from leaves showed MIC less than 6.25 µg/ml almost all test microorganisms except *C. albicans* which exhibit MIC of 25 µg/ml or less but more than 12.5 µg/ml.
Acetone extract from leaves showed minimum inhibitory concentration (MIC) above 50 µg/ml for both test bacterial and fungal species except *S.aureus* which exhibit MIC less than 6.25 µg/ml.

Table 2: Evaluation the minimum inhibitory concentration (M.I.C.) of anti-microbial Activity of Different Solvents *A. vera* leaves and sap Extracts

Extract	Concentration (µg/ml)	Mean Diameter of Inhibition zone (MDIZ)/mm					
		E.c	***Pseu.a***	***B.S***	***S.a***	***C.a***	***A.n***
methanol extract from leaves	50	17	15	17	22	20	22
	25	15	14	16	19	18	20
	12.5	14	12	14	17	17	19
	6.25	12	11	13	16	15	16
acetone extract from leaves	50	0	0	0	20	0	0
	25	0	0	0	19	0	0
	12.5	0	0	0	18	0	0
	6.25	0	0	0	17	0	0
acetone extract from sap	50	36	39	36	38	27	30
	25	35	35	35	35	25	25
	12.5	32	33	33	32	23	20
	6.25	31	31	30	30	20	18
water extract from sap	50	35	37	35	42	18	29
	25	33	35	34	40	17	27
	12.5	30	30	30	35	16	25
	6.25	29	28	28	32	15	23
methanol extract from sap	50	36	35	30	36	30	28
	25	35	31	29	33	26	27
	12.5	34	26	27	30	22	25
	6.25	32	20	26	29	20	23
water extract from leaves	50	20	21	18	23	18	24
	25	17	18	17	20	17	20
	12.5	16	17	17	15	0	17
	6.25	16	16	16	14	0	16
acids from sap	50	33	18	28	35	27	31
	25	31	16	28	33	25	33
	12.5	29	15	32	31	23	34
	6.25	29	13	35	28	22	35
acids from leaves	50	29	17	32	35	26	32
	25	30	15	35	33	24	30
	12.5	32	14	35	30	23	27
	6.25	34	12	36	31	22	25

Key: E.c ≡ *Escherichia coli*, Pseu.a ≡ *Pseudomonas aeruginosa*, B.s ≡*Bacillus subtilis*, S.a ≡ *Staphylococcus aureus* C.a ≡ *Candida albicans*, A.n ≡ *Aspergillus niger*

Discussion

In current study, the present findings of antimicrobial susceptibility for both *A. vera* leaves and sap extracts at 25µg/ml concentration were showed in disagreement with the findings of Agarry *et al*., [16] who reported that the zones of inhibitory were 18 mm (gel) and 4 mm (leaves) against *S.aureus*, 4 mm (leaves) and no inhibitory effect of gel against *Pseu. aeruginosa*, and 3 mm (leaves) and also no effect against *C.albicans*. The same author stated that the both leaves and gel extracts showed no inhibition zones against two out of three test fungal species *Trichophyton schoeleini* and *Microsporum canins*, while the gel extract showed intermediate inhibition effect (20 mm) against *Trichphyton mentagrophytes*. The sap (gel) of *A. vera* showed more inhibitory effects than those of leaves extracts because the most plant constituents were found in sap rather than leaves, this findings were in agreement with Davis [17] who stated that the gel of *A. vera* can promote wound healing due to the presence of some components like anthraquinones and hormones which posses antibacterial, antifungal and antiviral activities. Davis [17] also said that the most constituents are found in the gel and not in the leaves; hence the gel is likely to be more active than the leaves.

The present findings were in disagreement with those of Mbajiuka *et al*., [18] who studied the antimicrobial effects of *A. vera* gel prepared by different solvents. The researcher and his colleagues found that the ethanol, aqueous, and methanol gel extracts had low inhibitory effects against *E.coli* (6, 6, and 3 mm), *S.aureus* (5, 4, and 0 mm), and *C.albicans* (4, 3, 0 mm) respectively. The same author found that MIC of both ethanol and aqueous extracts was 12.5 µg/ml for *E.coli*, 6.5 µg/ml or less for *S.aureus*, and 50 µg/ml for *C.albicans*; this is in agreement with the present findings.

Also the present findings were in contradiction to those reported by Cock, [19] who said *A. vera* juice showed antibacterial activity against only the Gram negative bacteria *Aermonas hydrophilia* and *E.coli*. Cock and his colleagues stated that the *A. vera* juice did not show any inhibitory activity against any of the fungi (*A. niger* and *C.albicans*) and yeast (*Saccharomyces cervisiae*) tested. The same author stated that the gel extract showed antifungal activity against *A. niger*.

Kedarnath *et al*., [20] conduct an investigation to determine the chemical composition and antimicrobial activity of *A.vera*. The findings of them study revealed that the plant contains mainly tannin, saponin, flavonoids and terpenoids which are resemble to the findings of the present study. Also reported that the *A. vera* gel has a antibacterial potentiality and among the three bacterial organisms maximum growth suppression was observed in *Escherichia coli* (24 mm) and *Staphylococcus aureus* (28 mm) when compared with *Klebsiella pnemoniae* (22 mm) and *Bacillus cereus* (16 mm). The same author stated that the maximum antifungal activity was observed in petroleum ether and ethanol extracts (22 mm and 22 mm) when compared to chloroform extracts. These findings almost resemble to the current study with different extractor.

Many previous studies such as that of Ferro *et al.,* [21] have focused on the antimicrobial activity of *Aloe vera* whole gel. *Aloe vera* extracts have been shown to inhibit the growth of fungi and bacteria. These studies were in agreement with the present study.

Conclusions

The present study conclude that the *A. vera* has potential antimicrobial activities for both test bacterial and fungal species whom subjected to different concentrations of different sap and leaves extracts.

The most effective extract was sap water extract followed by sap methanolic extract, sap acetone extract, and sap acids. The intermediate effects were showed in leaves acids followed by leaves methanolic extract, sap saponins, and sap alkaloids. The lowest antimicrobial activities showed with leaves alkaloids and leaves saponins.

The MICs of almost all different concentrations of different extracts studied were less than 6.25 µg/ml with exception of water extract from leaves which lied between 25 µg/ml or less but more than 12.5 µg/ml in *C.albicans* and more than 50 µg/ml for acetone extract from leaves for five out of six test microorganisms.

Finally, according to these findings *A. vera* could be used as a folkloric medicinal substance after further investigation to determine its toxicity and side effects.

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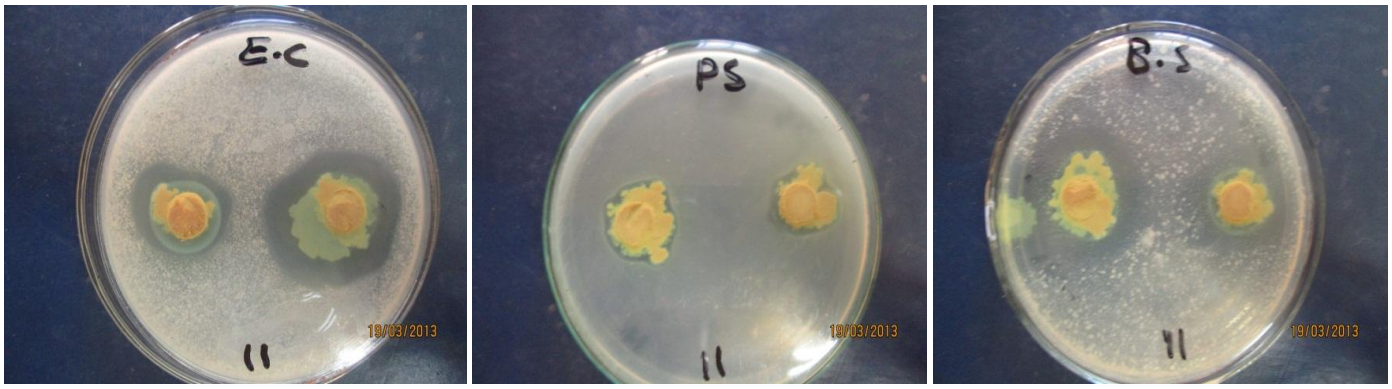

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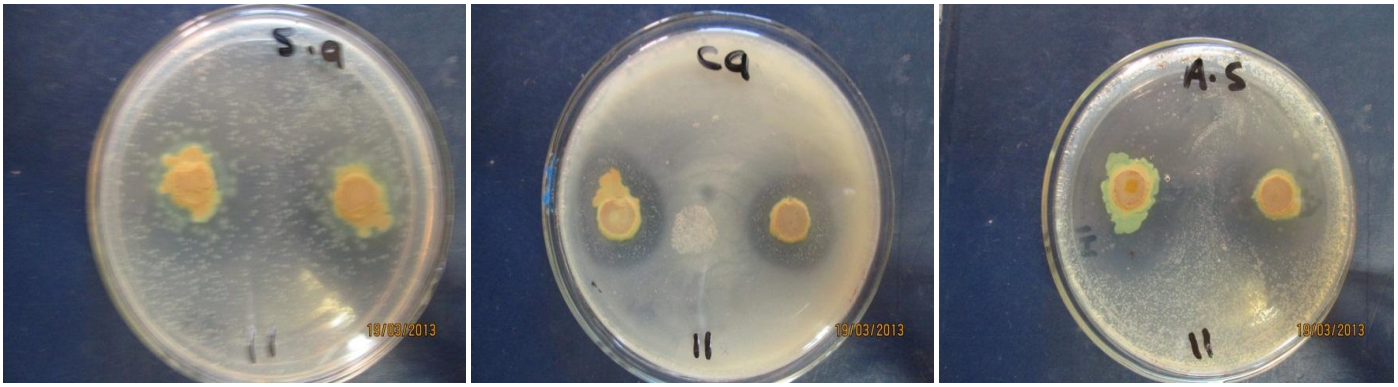

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Antimicrobial activity of *A. vera* sap on different test microorganisms: A= *E.coli*, B=*Pseu. aeruginsa*, C=*B.subtilis*, D=*S.aureus*, E= *C.albicans*, F= *A. niger*.

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