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In vitro antimicrobial activity of *Aloe vera* L. extracts against pathogenic bacteria and fungi

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Abstract

The current research aimed to evaluate *in vitro* antimicrobial activity of crude extract prepared from leaves of *Aloe vera* L. The extract was prepared in different solvents including methanol and ethanol. The inhibitory potential of extract was determined via agar well diffusion method against diverse range of microbes including *Erwinia carotovora, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Bacillus subtilis, Bacillus cereus, Staphylococcus aureus* and *Candida albicans*. The extracts were applied at 30 µL and 60 µL concentrations. The zone of inhibition was measured and compared with three standards i.e. Clotrimazole (50 µg mL^{-1}) for *Candida albicans*; Ciprofloxacin (30 µg mL^{-1}) for Gram negative bacteria and Azithromycin (50 µg mL^{-1}) for Gram positive bacteria. The antimicrobial susceptibility test showed that leaf and gel extracts inhibited the growth of all the tested microorganisms while the gel has significantly retarded the growth of *E. carotovora, E. coli, B. subtilis and S. aureus*. Furthermore, the gram positive strains were inhibited up-to larger extent as compared to gram negative one due to absence of lipopolysaccheride layer in cell wall. The higher inhibitory effect was observed for rotary dried gel methanol extract dissolved in water against *C. albiacans* at both 30 µL and 60 µL (78%, 104%), respectively. Our results suggest that these herbal extracts may be used against bacterial and fungal pathogens which possesses resistant to conventional antibiotics.

Keywords: *Aloe vera* L., antimicrobial, herbal extracts, medicinal plants.

Introduction

Most of plant species have medicinal value and have been characterized from ancient time, harboring no toxic effects on human body. According to World Health Organization (WHO) about 80% of the people in the developing countries still rely on medicinal plants for their primary health care and creating agricultural markets for those economies (Chatterjee *et al*., 2015). Approximately 20% of the plants are 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 semisynthetic resources (Mothana and Linclequist, 2005; Hamman and Viljoen, 2008). Many herbal based medicines are emerged possessing wide range of biomedical efficacy with higher treatment effects against inflammation, arteriosclerosis, hyperlipemia, bone resorption, osteoporosis and many of them useful against in cardiovascular ailment, immune deficiency, central nervous system disorders and cancer (Jin *et al*., 2006; Radad *et al*., 2006; Sajjad *et al*., 2015a) and also against different bacteria (Sajjad *et al*., 2015b). Among these medicinal plants, *A. vera* got importance because of its curative and therapeutic properties (Radha and Laxmipriya, 2015). *A. vera* is a perennial succulent plant belongs to the Liliaceae family (Suleyman and Sema, 2009) that probably originated in Northern Africa (Strickland *et al*., 2004; Akinyele and Odiyi, 2007). *A. vera* is extensively used for therapeutic possessions, such as injury treatments, diabetes, constipation, ulcers, cancers, arthritis and immune system deficiencies etc (Choi and Chung, 2003). Besides its therapeutic importance *A. vera* components are also used in manufacturing of cosmetics, nutraceuticals and in food industries. Recently many pathogenic bacteria have been reported for resistance to antibiotics (Seddik *et al*., 2010), most common resistant strains including gram-positive (*Staphylococcus aureus*), gram-negative (*Pseudomonas aeruginosa*) (El Solh and Alhajhusain, 2009) and *Mycobacterium tuberculosis* (Eloff *et al*., 2005). Similarly, resistance to antifungal compounds has been widely reported (Denning, 2003). Patel, (1998) reported the resistance of *Candida* species to azole and amphotericine. Synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often associated with adulterations and side effects (Chatterjee *et al*., 2015). To combat with the resistance problem, new antimicrobial and antifungal agents are desirable (Selitrennikov, 2001). Therefore, scientists are interested to search new antimicrobial and antifungal agents from natural sources or by design and synthesis of new agents that are effective, affordable and easily available (Adekunle and Adekunle, 2009). In this regards, *A. vera* has been reported to be antibacterial and antifungal (Johnson *et al*., 2012). Voluminous bodies of research works have been conducted on the medicinal plants uses, although, *A. vera* extract is still exigent. Therefore, present study was envisaged to evaluate the antimicrobial activity *in vitro* using organic solvents methanol and ethanol to identify the potentiality of *A. vera* plant against pathogenic bacteria and fungus.

Materials and Methods

Plant material

Fresh A. vera plants were collected from different fields of Pakistan Council of Scientific and Industrial Research (PCSIR) Complex Peshawar, Pakistan. Leaves were cut in fresh condition at the bottom near stem and were thoroughly washed with tape water to remove the soil particles, dirt and dust, after washing.

Extraction of gel

The clean leaves were chopped vertically and gel was removed and collected in a flask. The leaves (the greenish rind part) were air dried for 24 to 48 hours. The gel was divided into two parts; fresh gel and dried gel. Dried gels were further divided into two parts; Gel dried in oven at temperature 80 °C for 48 hours and Gel dried with the help of rotary evaporator at temperature 50 °C.

Crude leaf extraction

Dried leaves (the greenish rind part) were cut into small pieces and grinded into powder. About 1.5 gm of powder was mixed with 50 mL ethanol (99%) and methanol (80%) each in flask. Flasks were kept for four days at room temperature and shaken twice daily. The compounds that were soluble in solvents were filtered by using Whatman filter paper (No 42). The filtrate containing the leaves compounds in ethanol were dried by a rotary evaporator. The ethanol filtrate was taken in round bottom flask of rotary evaporator and ethanol was evaporated at 45ºC under vacuum pressure. The semi-solid extract was removed from the round bottom flask and dried in a china dish with water bath at 45ºC. Nutrient agar was used for the culturing microorganisms throughout the study. Nutrient broth was used for standardization and shaking incubation of these microorganisms.

Well diffusion susceptibility procedure

Pure bacterial and fungal isolates were obtained from different sources (Table 1). Microbial cultures (a standardized inoculums 2×10^7 CFU mL^{-1}, 0.5 McFarland Turbidity Standard) of 100 µL were spread on nutrient agar plates. Wells (8 mm in diameter) were made in the agar media with the help of sterilized cork borer

and plant extracts in concentrations of 5 and 10 mg well^{-1} in 30 and 60 µL volumes were poured into the wells. Antibiotics (30 µL well^{-1}), Dimethylformamide (DMF) and water (30 µL well^{-1}) were also poured into the wells as positive and negative controls, respectively. Inoculated plates were incubated at 37 ºC for 24 h and inhibitory zones were measured.

Positive Controls

Following species were used as positive control.

For *Candida albicans*; Clotrimazole 50 µg 6 µL^{-1}

For Gram negative bacteria; Ciprofloxacin 30 µg 6 µL^{-1}

For Gram positive bacteria; Azithromycin 50 µg 6 µL^{-1}

The whole procedure was repeated thrice for each microorganism in order to get the consistent results and overcome the possibility of any uncertainty.

Results and Discussion

A. vera has been used as a medicinal plant with many therapeutic applications from ancient times. The present study was carried out to evaluate antimicrobial activities of various extracts of *A. vera* against gram positive and gram negative bacteria as well as *C. albicans*. Biological activities of *A. vera* are due to the presence of some important polysaccharides present in the gel, but it is very difficult to attribute the therapeutic properties to an individual polysaccharide component (Adekunle and Adekunle, 2009). The composition and biological activities of *A. vera* gel vary with type of genotype, seasonal variation, type of separation and extraction method (Hamman and Viljoen, 2008). However, the heated gel (10-20 ºC) did not show any activity against most of the microorganisms. It might be due to the fact that at the given temperatures, most of enzymes are in active form and hydrolyze the biologically active components. The antimicrobial effect of ethanol extracts and methanol extracts was higher. Cowan *et al*. (1999) reveals that biologically active components against microorganisms identified from plants are aromatic or saturated organic compounds and mostly obtained through initial ethanol or methanol extraction. This argument explains the higher activity for ethanol and methanol extracts.

Our findings showed that different solvent extracts of *A. vera* have shown tremendous inhibitory effects against gram positive bacteria as compared to gram negative bacteria, it might be the additional lipopolysacchride layers in the cell wall of gram negative bacteria. The extracts like *A. vera* gel and heated *A. vera* gel not showed any obvious effect against *Pseudomonas aeruginosa* at any concentration. The highest activity was shown by *A. vera* leaf extracts (46%, 53%) and dried gel ethanol extract dissolved in DMF (43%, 55%) at both 30 µL and 60 µL concentrations. Among the extract showing effect the lowest inhibitory effect was observed for dried gel ethanol extracts dissolved in water (33%, 43%). Dried gel methanol extract dissolved in DMF (39%, 53%), rotary dried gel ethanol extract dissolved in water (36%,47%), dried gel methanol extract dissolved in water (42%, 50%) and rotary dried gel methanol extract dissolved in water (43%, 53%) respectively (Fig. 1 and Fig. 2a). Similar results were also reported by Agarry *et al*. (2005) who found that Aloe gel did not inhibit *Pseudomonas aeruginosa* growth while leaf extract inhibited the growth. Dried gel ethanol extracts dissolved in DMF have also shown utmost effect, while rotary dried gel methanol, ethanol extracts and dried gel methanol, ethanol extracts retard the bacterial growth.

A. vera gel inhibited the *Bacillus subtillus* growth at both concentrations of 30 µL (37%) and 60 µL (39%) respectively. *A. vera* leaf ethanol extracts inhibited its growth up to (57%) and (63%). Dried gel methanol extract dissolved in DMF (50%, 68%) and dried gel ethanol extract dissolved in DMF (63%, 68%) have shown moderate inhibitory effects on bacterial growth than dried gel methanol and ethanol extracts dissolved in water as well as rotary dried gel methanol, ethanol extracts dissolved in water (Fig. 1 and Fig. 2b). This might be possible that in water the biologically active components were not completely active, where in DMF certain components were completely active showing higher biological activities. Our findings are in commitment with those of Ke-Qiang *et al*. (2001) that recorded maximum *B. subtilis* growth inhibition with ethanol extracts.

Our results showed that *A. vera* gel has no significant effect on the growth of *C. albicans* and represent 0% inhibition, while ethanol extract of leaf inhibited the fungus growth at both concentrations (30µL, 54% and 60µl, 68%) (Fig. 1 and 2c). These findings were in line with Agarry *et al*. (2005) indicated that aloe gel had no effect on *C. albicans* and leaf extract inhibit the growth of fungus. *Candida* is responsible for various infections in human such as gastrointestinal diseases, urogenital and meningitis. *C. vaginitis* is

a common disease found during pregnancy. Mouth and esophagus infected by *Candida* are common in people with HIV disease (Cheeshrough, 1984). The inhibitory effect on *C. albicans* made it clear that both gel and leaf though share certain components but are different from each other at reaction (Foster, 1999). The highest activity was shown by rotary dried gel methanol extract in water, which showed that methanol extracted components were excellent inhibitors of *C. albicans*.

In order to assessed *A. vera* extract inhibition against *Bacillus cereus* the highest activity was obtained for rotary dried gel methanol extract dissolved in water at 30 µL (55%) and 60 µL (58%) as compared to control. Aloe gel and heated gel did not inhibit the pathogen at any concentration. *B. cereus* was found more susceptible to rotary dried gel methanol extracts dissolved in water followed by dried gel ethanol extract dissolved in DMF (47%, 54%). The dried gel methanol extract dissolved in DMF (43%, 53%), dried gel ethanol extract dissolved in water (42%, 46%), *A. vera* leaf extract (41%, 41%), rotary dried gel ethanol extract dissolved in water (38%, 51%) and dried gel methanol extract dissolved in water (37%, 42%) inhibition respectively (Fig. 1 and Fig. 2d). Among the extracts dried gel methanol extract dissolved in water showed the minimum inhibition. Lawrence *et al*. (2009) also found that ethanol extract had higher inhibitory effect than methanol. Ethanol was efficient in extracting the biologically active components which show lethal affects against *B. cereus.*

E. carotovora, a gram negative strain, commonly grown on nutrient agar medium, as compared to positive control (ciprofloxacin). The aloe fresh gel was found active against *E. carotovora*, while heated gel, dried gel methanol extract dissolved in DMF, dried gel ethanol extract dissolved in water and leaf extract in ethanol did not inhibit its growth. *E. carotovora* showed resistance to most of the extracts. Leaf (rind) extract in ethanol had no negative effect on its growth and development. Among all microbial strains used, *E. carotovora* showed inconsistency. Rotary dried gel methanol extract dissolved in water was the paramount anti-*E. carotovora* agent (68%, 73%) (Fig. 1 and 2e).

Dried gel methanol, ethanol extracts in DMF and water, rotary dried gel ethanol and methanol and *A. vera* leaf ethanol extract inhibited the bacterium *E. coli*. Dried gel ethanol extract in water had shown lower inhibitory effect than dried gel methanol extract in water (Fig. 1 and 2f) which is in accordance to the findings of Lawrence *et al*. (2009). Our results showed contradiction to the findings of Pandey and Mishra, (2010) that might be due to different genotypes used.

Dried gel methanol, ethanol extracts in DMF, water and aloe leaf extracts in ethanol retard the growth of *K. pneumoniae* considerably, while the other extracts did not show any inhibition. Dried gel methanol extract in water and dried gel ethanol extract in water inhibited the bacterium at both concentrations of 30 µL and 60 µL (methanol 50%, 61% and ethanol 54%, 54%) respectively, (Fig. 1 and Fig. 2g). These results were not in line with the findings of Lawrence *et al*. (2009). According to them ethanol extract has more active than methanol. This might be due to diversity existed in *A. vera.*

Table 1: Test microorganism used for checking antimicrobial efficiency of extracts.

Microbial species	Gram strain type	Details of the microbial strains
Bacillus subtilis	Positive	*Clinical isolate from the lab of QAU, Islamabad, Pakistan*
Bacillus cereus	Positive	*Clinical isolate from the lab of QAU, Islamabad, Pakistan*
Staphylococcus aureus	*Positive*	ATCC # 6538
Erwinia carotovora	*Negative*	Plant Pathology department of KPK AUP Pakistan
Escherichia coli	Negative	ATCC # 25922
Kleibsiella pneumoniae	Negative	*Clinical isolate from the lab of QAU, Islamabad, Pakistan*
Pseudomonas aeruginosa	Negative	ATCC # 9721
Salmonella typhi	Negative	*Clinical isolate from the lab of QAU, Islamabad, Pakistan*
Candida albicans	Fungus	Clinical isolate obtained from Hayatabad Medical Complex Peshawar KPK Pakistan

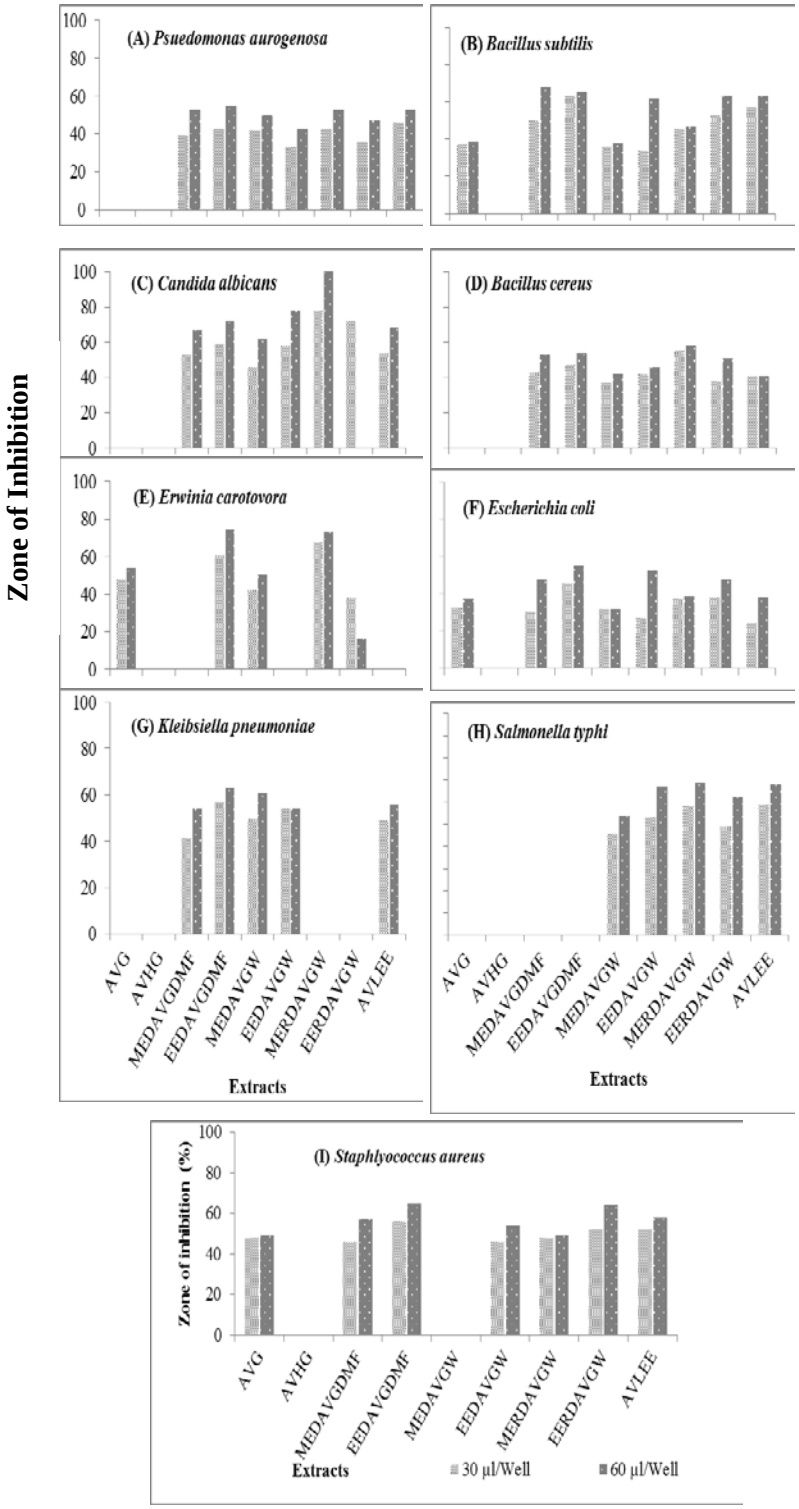

Fig. 1. Antibacterial activity of *Aloe vera* gel, heated gel, ethanol, methanol extracted samples of *Aloe vera* against tested microorganisms by well diffusion assay. AVG = *A. vera* gel, AVHG = *A. vera* heated gel, MEDAVGDMF = Methanol extracted dried *A. vera* gel in DMF, EEDAVGDMF = Ethanol extracted dried *A. vera* gel in DMF, MEDAVGW = Methanol extract of dried *A. vera* gel in water, EEDAVGW = Ethanol extract of dried *A. vera* gel in water, MERDAVW = Methanol extract of rotary dried *A. vera* gel in water, EERDAVGW = Ethanol extract of rotary dried *A. vera* gel in water, AVLEE = *A. vera* leaf ethanol extract.

S. typhi a gram negative bacterium was inhibited by *A. vera* leaf extract and some others dried gel extracts. However, the bacterium showed resistance to most of the extracts. It seems that these strains are either resistant or the biological active components are not present or active in different solvents (Fig. 1 and 2h). The dried gel ethanol extracts in water had shown higher activity than that of dried gel methanol extracts in water, which confirms the findings of Lawrence *et al*. (2009). *A. vera* contains compounds like anthraquinone, aloe emodin (Garcia-Sosa *et al*., 2006; Dabai, 2007) Saponins (Reynolds and Dwec, 1999), dihydroxyanthraquinones (Wu *et al*., 2006) and acemannan (Pugh *et al*., 2001), which have either direct or indirect influence. Furthermore studies are required to investigate the facts behind this mechanism.

The growth and development of *S. aureus* (a gram positive bacterium) is inhibited by different extracts of *A vera* on nutrient agar medium using well diffusion assay at a concentrations of 30 µL well^{-1} and 60 µL well^{-1}. Azithromycin was used as positive control; inhibited bacterium on agar plate. *A. vera* heated gel and dried gel methanol extract dissolved in water did not show any effect against *S. aureus*. Rotary dried gel methanol extracts dissolved in water (48%, 49%), dried gel methanol extracts dissolved in DMF (46%, 57%), dried gel ethanol extract dissolved in water (46%, 54%) and *A. vera* gel (48%, 49%) inhibited the bacterium moderately. The maximum effect showed by Aloe leaf ethanol (52%, 58%), rotary dried gel ethanol dissolved in water (52%, 64%) and dried gel ethanol extract dissolved in DMF (56%, 65%) at both 30 and 60 µL (Fig. 1 and 2i). The inhibitory effect of *A. vera* gel and leaf extract on *S. aureus* was also found by Agarry *et al*. (2005). The highest inhibitory effect was shown by dried gel ethanol extract dissolved in DMF (56%) and (65%). At high temperature the active enzymes were degraded. Biologically active components were more efficiently extracted in alcohol (ethanol and methanol). Aloe gel and leaf extracts are useful for gastrointestinal irritations (Foster, 1999). *S. aureus* also contributed in the normal microflora of skin, upper respiratory tract and intestinal tract. Gel is also responsible for promoting wound healing because it contains compounds like anthraquinone etc, which possesses antimicrobial activity (Cheesbrough, 1984).

Conclusion

It is concluded Aloe plants showed remarkable activity against gram negative as well as gram positive bacteria and fungi. The plants were more effective at low concentrations. Furthermore, the herbal extracts may be used for anti-bacterial and anti-fungal pathogens resistant to conventional antibiotics and severe infections remedy.

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