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Full Length Research Paper

Evaluation of *Aloe vera barbadensis* for its antimicrobial, phytochemical and ethnobotanical status

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Phytochemical studies of the plant *Aloe vera* (*barbadensis* Miller) were carried out in order to detect the presences of alkaloid and flavonoids in the gel of *Aloe vera* through certain detection test and Thin Layer Chromatography (TLC). The antimicrobial activity of *A. vera (barbadensis Miller)* was investigated on some test organisms using disc diffusion method. Ethanol, hot water and cold distilled water were the solvents used for extraction of the active ingredients from the plant leaf. The test organisms used were *Escherichia coli, Streptococcus sp., Staphylococcus aureus, Salmonella typhi and Bacillus subtilus.* The result showed that *A. vera* hot water extract had more antibacterial activity then ethanolic and cold water extract. The largest zone of inhibition (42.00 mm) was produced in case of hot water extracts against *S. typhi* and *E. coli*. The results also showed that all the organisms tested were susceptible to the extract except *E. coli*, which was resistant to the cold and ethanolic extract. For susceptible strains, the diameter of zone of inhibition ranged from 33 – 42 mm.

Key words: *Aloe vera*, antibacterial activity, thin layer chromatography, alkaloids, flavonoids.

INTRODUCTION

Herbs had been used by all the cultures throughout history for healthcare, and methodically collected information on medicinal plants developed well defined herbal pharmacopoeias. Herbal plant like *Aloe vera* Linne or *Aloe barbadensis* Miller (family Liliaceae) is perennial resisting and succulent plant. It is one of those medicinal plants having immense importance. Genus *Aloe* contains 400 different species with its origin in African continent (Foster, 1999). The leaves have a high capacity of retaining water; therefore A. *vera* can survive very harsh circumstances (Agarry et al., 2005). Essentially, two products are obtained from *A. vera* leaves the clear gel and the yellow sap, which is very bitter (Bloomfield, 1985). The gel contains 99.3% water and the remaining 0.7% is solids (Heggers et al., 1996). One table spoon of *A. vera* gel contains 75 different chemicals which have biological activity. The clear gel is used to treat skin irritation/inflammation due to the presences of sterols while sap is used as a laxative (Grindlay and Reynolds, 1986.). *A. vera* gel may reduce symptoms and inflammation in patients with ulcerative colitis (Langmead et al., 2004). The injection of *A. vera* extracts to treat cancer has resulted in the deaths of several patients (Langmead, 1997). Wound healing capability of *A. vera* in both oral and topical form, due to the presence of saponin in gel it help in natural cleansing of skin (Davis, 1997). The gel enhances the immune system and help detoxification. The *A. vera* is used as a natural antibiotic and painkiller and gives mild laxative effect due to the presence of Anthroquinones. The cosmetic and alternative medicine industries regularly make claims regarding the soothing, moisturizing and healing properties of *A. vera*, especially through internet advertising (Boudreau and Beland, 2006).

A. vera is used as additive in shampoos and moisturizers it strengthens the hair and cares for the scalp, preventing hair break fall and splitting. Reports

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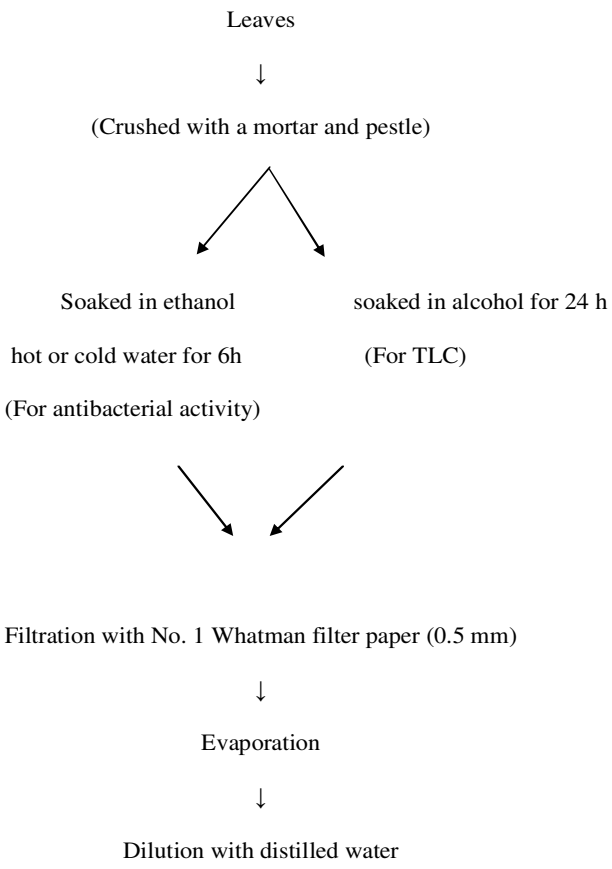

Figure 1. A schematic representation of *A. vera*

suggests that the beneficial effects of *A. vera* gel are due to its high molecular weight compounds such as polysaccharides, lectin like proteins (Grindlay and Reynadds 1986) and prostagladins (Azfal et al., 1991). *A. vera* promotes good metabolism, which improves the overall body function leading to higher energy level.

It is powerhouse of nutrition as it contains a variety vitamins like A, C, E, B1, B5 and B6 besides B12 which promote the formation of a net of fibers that trap the red corpuscles of the blood and minerals like iron, Magnesium, Manganese, Sodium, Phosphorus, Phosphorus, Calcium and Copper (Farooqi and Sreeramu, 2001). *A. vera* extracts can be used in formulation of new pharmaceutical products due to the medicinal importance and antibacterial property (Agarry et al., 2005). *A. vera* leaf gel can inhibit the growth of the two Gram-positive bacteria *Shigella flexneri* and *Streptococcus progenies* (Ferro et al., 2003).

Specific plant compounds such as anthraquinones (Garcia-Sosa et al., 2006) and dihhydroxyanthraquinones (Wu et al., 2006), as well as saponins (Reynolds and Dweck, 1999), have been proposed to have direct antimicrobial activity. *A. vera* extracts have antibacterial and antifungal activities (Sumbul et al., 2004). Acemannan, a polysaccharide component from whole plant material, has been proposed to have indirect antimicrobial activity through its ability to stimulate phagocytic leukocytes (Pugh et al 2001).

The present study was carried out to assess the flavonoid and alkaloid components of *A. vera* plant extract by the Thin Layer Chromatography (TLC) method and to find out the antimicrobial activity of this extract against different pathogenic strains.

MATERIALS AND METHODS

A. vera (barbadensis) plants were collected from the nursery of PCSIR Laboratories Complex, Lahore, Pakistan. The bacterial strains *Escherichia coli, Streptococcus sp., Staphylococcus aureus, Salmonella typhi, Bacillus subtilus* were acquired from Pakistan Type Culture Collection (PTCC), PCSIR laboratories complex Lahore. The identification of the organisms was confirmed using various morphological and biochemical tests, according to Barrow and Feltham (1993). Determination of antimicrobial activity of *A. vera* (barbadensis Miller) plant extracts was carried out by paper disc method. The active compounds of *A. vera* leaves were extracted according to the procedure described by Coats (1979) with minor changes for antibacterial activity as well as for TLC. A schematic representation is illustrated in Figure 1. The leaves were thoroughly washed with distilled water for surface sterilization and dried with the hot air oven at 60°C for 3 days. The amount of water

Table1. Effect of extraction solvent on antimicrobial activity of leaf extracts.

Organisms	Zone diameter of inhibition (mm)		
	Ethanol	Hot water	Cold water
S. aureus	9.33 ± 3.05	32.33 ± 2.88	-
B. subtilus	20.33 ± 1.52	33.66 ± 2.51	28.66 ± 1.15
Streptococcus sp.	10.0 ± 2.00	19.0 ± 2.51	-
S. typhi	26.33 ± 2.51	42.0 ± 4.00	37.33 ± 4.61
E.coli	-	42.0 + 4.00	-

loss was calculated by subtracting the weight of fresh leaves from the dry leaves. The dry leaves were pounded with the mortar and pestle and 2.5 g of each sample was placed into three separate 100 mL beakers. In the first beaker, 25 mL of hot water was poured, the second had 25 mL of ethanol while in the third beaker, 25 mL of cold distilled water was added. The beakers were then covered with aluminum foil and allowed to stand for 48 h with occasional stirring. The content was filtered by passing the suspension through sterile Whatman filter paper disc 1 (0.5 mm), the ethanol filterate was evaporated after filtration. Double dilution of the gel was prepared by diluting against distilled water.

For alkaloids TLC, 100 g of ground leaves were soaked in 400 mL alcohol for 48 h. Then 5% of aqueous solution of acetic acid was added to this solution. After 1 h, the extract was filtered and then 50 ml of dichloromethane was added to this filtrate in a separating funnel. Aqueous layer was separated and basified to pH 10 with 10% aqueous solution of sodium carbonate. Again 50 ml of dichloromethane was added to the filtrate in a separating funnel. Organic layer was separated and was evaporated to get the alkaloid residue (Surya and John, 2001).

For flavonoids TLC, 100 g of ground leaves were soaked in 400 ml alcohol for 48 h. After filtration, 30 ml of distilled water and 50 ml of ethyl acetate were added to this filtrate in a separating funnel. Allowed to settle down and separate the layers. Upper ethyl acetate layer was removed and residue was dissolved in the ethanol to make alcoholic extract (Ali, 1997).

RESULTS AND DISCUSSION

TLC of the extracts (alcoholic, hot water or cold water) revealed the presence of alkaloids in the organic layer of the extracts separately. Alkaloids were identified by the appearance of orange spots after spray of dragondorff's reagent on the chromatogram. In case of flavonoids, TLC chromatogram of aloe hot water extract sprayed with 10% $AlCl_3$ solution. The presence of flavonoids was confirmed by the fluorescence when observed under ultraviolet (UV) light, as stated by Egger (1969). The results obtained from the antimicrobial activity assay showed that *A. vera (barbadensis)* extract in various solvents has variable but significant antibacterial activity against the five clinical isolates used in this study. Results are shown in Table 1. It is shown by the results, recorded for antimicrobial activity using paper disc method, that *S. aureus, B. subtilus, Streptococcus sp. and S. typhi* were not susceptible to the ethanolic extracts of leaves, and zones of inhibition having diameter of 9.333 ± 3.05, 20.33 ± 1.52, 10.00 ± 2.0 and 26.33 ± 2.51 mm were recorded respectively whereas, ethanolic extract of *A. vera* had no antibacterial activity against *E. coli* (Table 1). Agarry et al. (2005) also observed that ethanolic extract of both *A. vera* gel as well as leaves inhibited the growth of *S. aureus.* The hot water extract produced zones of inhibition of 32.33 ± 2.88, 33.66 ± 2.51, 19.00 ± 2.51 and 42.00 ± 4.00 mm for *S. aureus, Bacillus sp, Streptococcus, S. typhi and E. coli* respectively (Table 1).

In case of distilled water extract, the inhibitory effect was relatively more pronounced on *Bacillus* and *Salmonella* as diameter of zones of inhibition recorded were 28.66 ± 1.15 and 37.33 ± 4.61 mm respectively. While other organisms tested were resistant (Table 1) as there is no zone of inhibition against *Streptococcus sp., S. aureus* and *E. coli*. These results are in accordance with who has reported that cold water extract did not inhibit the activity of *E. coli* (Esumeh et al., 2007).

Results from this study showed that hot water extract of the leaves of *A. vera* has more antibacterial activity followed by ethanolic and distilled water extracts on all the test organisms. This indicates that hot water and ethanolic extracts are more effective than cold water extract. It also showed that of all the organisms tested *S. typhi* and *B. subtilius* were susceptible to all kinds of extracts (ethanolic, hot water, cold water) with inhibitory zones ranging from 26.33 - 42.0 and 20.33 - 33.66 mm respectively (Table 1).

Results for the *in vitro* antibiotic susceptibility test are recorded in Table 2. These results indicate that the extract could be effectively used in the treatment of some infections or diseases caused by the various test organisms. For instance *E. coli* was resistant to various

Table 2. Antibiotic susceptibility test of the isolates.

	Zone diameter of inhibition (mm)				
Antibiotics (50 µg)	***S. aureus***	***B. subtilus.***	***Streptococcus sp.***	***S. typhi***	***E. coli***
D-Oxycycline hylate Ridox	47.66 ± 0.57735	37.66 ± 0.5773	37.66 ± 1.5275	35.33 ± 1.1547	-
Omeprazole USP OMOL	17.33 ± 1.154701	32.33 ± 1.5275	55.33 ± 1.1547	-	-
D- Oxy cycline Vibramicin	41.33 ± 2.081	24 ± 3.0550	41.33 ± 1.5275	42 ± 2.00	26.66 ± 1.1547
Ampicillin penbritin	31.33 ± 1.527	-	36.33 ± 1.5275	32.66 ± 1.1547	22.66 ± 1.1547
Omeprazole SOMEZOL	24.66 ± 1.1547	-	-	-	-
D-Oxy cycline USP (D-Oxysyclin)	36 ± 1.00	25.66 ± 1.5275	35.66 ± 1.5275	36.66 ± 1.1547	27 ± 1.00
Cephradine USP VERICEF	40.66 ± 1.1547	-	41 ± 1.00	55 ± 1.00	-
Cefadroxil NEOCEF	34.33 ± 1.5275	36 ±2.00	43.33 ± 1.1547	41 ± 1.00	-
Lincomycin HCl USP Lincocin	16 ± 2.00	27.66 ± 0.5773	-	50.66 ± 1.1547	-
Ampicillin+CloxacillinAmpiclox	25.66 ± 1.5275	-	-	45.33 ± 1.1547	-

antibiotics like D-Oxycycline hylate, Omeprazole USP, Omeprazole, Cephradine USP, Cefadroxil, Lincomycin HCl USP, Ampicillin + Cloxacillin but susceptible to hot water extract of *A. vera* leaves. The results were similar to those reported by Esumeh et al. (2007) who stated that due different healing properties of *A. vera*, it is one of the most promising plants for its use in modern medicine. Although, *in vitro* zone of inhibition observed with one drug cannot be compared with those obtained with another antimicrobial agent due to the difference in rate of diffusion through agar gel amongst other factors the zones observed for *A. vera* extract compares favorably with those of standard zone for known organisms (Barry and Thornsberry, 1993).

It can be concluded by comparing table 1 and 2 in the present study that *A. vera* gel could be a good substitute for certain antibiotics against different microbes. TLC of the extracts indicated the present extraction procedure was successful in extracting the alkaloids as well as flavonoids. The results of this study confirm why *A. vera* (*barbadensis)* gel is used as a common additive to shampoos, moisturizers and soaps as earlier reported by Schulz and Schynder (1993). It is believed that the antibacterial constituents of this plant leaf when extracted and purified could be a useful formulation in the treatment of some bacterial infections.

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