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Research Article

IN VITRO ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL SCREENING OF *ALOE VERA* LINN.

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ABSTRACT

The *in vitro* antimicrobial activity of aqueous and methanolic extracts of the roots of *Aloe vera* was investigated against *Bacillus cereus, Escherichia coli, Pseudomonas aerugenosa* and *Enterobacter aerogens* using the agar diffusion method. The methanol extract inhibited the growth of all the presently investigated bacteria with zone of inhibition between 7 - 20 mm at 100 µg/ml whereas, water extract exhibited zone of inhibition ranging from 2-4mm at the concentration of 100 µg/ml. The minimum inhibitory concentration (MIC) of the methanol extract was between 10 and 25 µg/ml while that of aqueous extract ranged from 25 to 60 µg/ml. The minimum bactericidal concentration (MBC) for methanol extract ranged between 20 and 50 µg/ml. The MBC of aqueous extract ranged from 50 to 120 µg/ml. Preliminary phytochemical analyses showed that the extracts contain flavonoids, terpenoids, tannins, saponins, reducing sugars and anthraquinones.

Keywords: *Aloe vera,* Antimicrobial activity, Phytochemical screening, Minimum inhibitory concentration (MIC), Minimum bactericidal concentration (MBC).

INTRODUCTION

The local use of natural plants as primary health remedies, due to their pharmacological properties, is quite common in Asia, Latin America and Africa (Bibitha *et al*., 2002). Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world's population, especially in the developing world (WHO, 2002). Infectious diseases continue to be the major concern for health institutions, pharmaceutical companies and governments all over the world (accounting for over 50, 000 deaths every day), especially with the current increasing trends of multidrug resistance among emerging and re-emerging bacterial pathogens to the available modern drugs or antibiotics (Franklin and Snow, 1989; Prescott *et al*., 2002). The search for newer sources of antibiotics is a global challenge preoccupying research institutions, pharmaceutical companies and academia, since many infectious agents are becoming resistant to synthetic drugs (Latha and Kannabiran, 2006). It is therefore very necessary that the search for newer antibiotic sources be a continued process. Plants are the cheapest and safer alternative sources of antimicrobials (Pretorious and Watt, 2001; Sharif and Banik, 2006; Doughari *et al*., 2007). In view of the importance of *A. vera* in ethnobothany as health remedy, in this paper, the antimicrobial property of crude extracts of the root of *Aloe vera* has been studied as part of the exploration for new and novel bio-active compounds.

Aloe vera a member of liliaceae family *is* an ornamental and medicinal plant. It is being used therapeutically, since Roman times and perhaps long before different properties being ascribed to the inner colorless leaf gel and to the exudates from the outer layers (Crosswhite and Crosswhite, 1984; Morton, 1961). It is a cactus like plant with green, dagger- shaped leaves that are fleshy, tapering, spiny, marginated and filled with a clear viscous gel (Yates, 2002). *Aloe vera* has been used to treat various skin conditions such as cuts, burns and eczema. It is alleged that sap from this plant eases pain and reduces inflammation.Evidence on the effects of *Aloe vera* sap on wound healing, however, is contradictory (Vogler *et al*., 1999). *Aloe vera* is as old as civilization and throughout history it has been used as a popular folk medicine. It is present in the arid regions of India and is believed to be effective in treating stomach ailments, gastrointestinal problems, skin diseases, constipation for radiation injury, for its anti-inflammatory effect, for wound healing and burns, as an anti-ulcer and diabetes. Currently the plant is widely used in skin care, cosmetics and as nutraceuticals (Klein *et al*., 1988).

MATERIALS AND METHODS

Plant materials and preparation of extract

Fresh roots of *Aloe vera* was collected from a local farm in Mysore city, Karnataka state, India in the month of July, 2009 and was identified and authenticated by the Botany Department University of Mysore. The fresh roots were washed with distilled water to remove dirt and shade dried to constant weight for 7 days. The dried roots were then blended using a household electrical blender. 20g of the pulverized root powder was extracted in methanol and distilled water separately. The extracts were then filtered using Whatman No. 1 filter paper. The methanol filtrate was concentrated to dryness *in vacuo* at 40º C using a rotary evaporator. The aqueous extract was lyophilized to obtain a dry powder (Akerele *et al*., 2008).

Test organisms

The bacterial cultures viz., *Bacillus cereus, Escherichia coli, Pseudomonas aerugenosa* and *Enterobacter aerogens* used for the present investigations were obtained from the Institute of Microbial Technology (IMTECH), Chandigarh, India. The bacterial cultures were maintained at 4^0C on nutrient agar.

Determination of phytochemical constituents

The freshly prepared extracts were subjected to standard phytochemical analyses for different constituents such as tannins, alkaloids, flavonoids, anthraquinones, glycosides, saponins, terpenoids and reducing sugars as described by Jigna *et al*. (2006) and Harbourne (1998).

Antimicrobial assay

Antibacterial activity of the aqueous and methanolic extracts were evaluated by the agar diffusion method (Aida et al., 2001) with slight modification. The bacterial cultures were inoculated onto Mueller Hinton agar plates. A sterile cork borer was used to make a well (6 mm in diameter) on the Muller Hinton Agar plates. 100 µl of both the aqueous and methonolic extracts at concentrations of 25, 50, 75 and 100 µg/ml, were applied separately in each of the wells in the culture plates previously seeded with the test organisms. The cultures were incubated at 37^oC for 24 h. Controls were set up in parallel using the solvents that were used to reconstitute the extract. The effects were compared with streptomycin at a concentration of 10 µg/ml. Antimicrobial activity was determined by measuring the zone of inhibition around each well (excluding the diameter of the well). For each extract, three replicate trials were conducted against each organism.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The estimation of MIC of the crude extracts was carried out using the method of Akinpelu and Kolawole (2004). The MIC was taken as the lowest concentration that prevented the growth of the test microorganism. To 0.5 ml of varying concentrations of the extracts (25, 50, 75 and100 µg/ml) in test tubes, Nutrient broth (2 ml) was

added and then a loopful of the test organism was introduced. The procedure was repeated on the test organisms using the standard antibiotic streptomycin. A tube containing Nutrient broth only was seeded with the test organisms as described above to serve as controls. The culture tubes were then incubated at 37oC for 24 h. After incubation the tubes were then examined for microbial growth by observing for turbidity. To determine the MBC, for each set of test tubes in the MIC determination, a loopful of broth was collected from those tubes that did not show any growth and inoculated onto sterile Nutrient agar by streaking. Nutrient agar plates only were also streaked with the respective test organisms to serve as controls. All the plates were then incubated at 37°C for 24 h. After incubation, the concentration at which no visible growth was seen was noted as the Minimum Bactericidal Concentration (MBC) Spencer and Spencer (2004).

RESULTS AND DISCUSSION

Table 1 shows the results of the preliminary phytochemical analyses of the root extracts of *A. vera.* The result revealed methanol extract possessed saponins, tannins, flavonoids, terpenoids, reducing sugars and anthraquinones, while the water extract contained terpenoids, tannins and reducing sugars. Phytoconstituents have been found to inhibit bacteria, fungi, viruses and pests. The presence of phytoconstituents in the root extracts may be responsible for the antibacterial activity of the plant (Marjorie, 1999).

Both the aqueous and methanolic extracts of the plant showed varying degree of antibacterial activities against the test bacterial species (Table 2). The antibacterial activities of the methanol extract compared favourably with standard antibiotic streptomycin. The methanol extract exerted highest activity on bacterial agents tested compared to the aqueous extract. The methanol extract at the concentration of 100 µg / ml showed 7 mm diameter zone of inhibition against *E.aerogens.* This was followed by 10, 17 and 20 mm zone of inhibition against *E. coli, B. cereus* and *P. aeruginosa* respectively (Table 2). Whereas at the same concentration the aqueous extract exerted highest activity against *B. cereus* with diameter zone of inhibition of 4 mm followed by 3, 2 and 2 mm zone of inhibition against *E. coli, P. aeruginosa* and *E.aerogens* respectively. The differences in the observed activities of the extracts may be due to varying degrees of solubility of the active constituents in the solvents used. The minimum inhibitory concentration (MIC) of the methanol extract for different organisms ranged between 10 and 25 µg/ml, while that of the aqueous extract ranged between 25 and 60 µg/ml (Table 3). The minimum bactericidal concentration (MBC) of the extract for different bacteria ranged between 20 and 50 µg / ml for the methanol extract and for the aqueous extract ranged between 50 and 120 µg / ml (Table 3).

The presence of bioactive substances have been reported to confer resistance to plants against bacteria, fungi and pests and therefore explains the demonstration of antibacterial activity by the plant extracts used in this study (Srinivasan *et al*., 2001). The results of this study showed that the methanolic extract was more effective than aqueous extract. This may be due to the better solubility of the active components in organic solvents (de Boer *et al*., 2005). The demonstration of activity against the test bacteria provides scientific bases for the local usage of this plant in the treatment of various ailments. The fact that the extracts were active against both gram-negative and gram-positive bacteria tested may indicate a broad spectrum of activity. This observation is very significant because of the possibility of developing therapeutic substances that will be active against multidrug-resistant organisms. The results of the study supports the traditional application of the plant and suggests that the plant extracts possess compounds with antibacterial properties that can be used as antibacterial agents in novel drugs for the treatment of bacterial diseases. Further pharmacological evaluations, toxicological studies and possible isolation of the therapeutic antibacterial from this plant are the future challenges.

Table 1: Showing phytochemical constituents of root extracts of *Aloe vera.*

S. No.	Test	Methanolic extract	Aqueous extract
1	Flavonoids	++	--
2	Terpenoids	++	++
3	Tannins	++	++
4	Saponins	++	--
5	Alkaloids	--	--
6	Reducing sugars	++	++
7	Anthraquinones	++	--
8	Cardiac glycosides	--	--

++ Presence of constituent; -- Absence of constituent

Table 2: Antibacterial efficacy of the root extracts of *Aloe vera* (Zone of inhibition measured in mm)

S. No.	Test organisms	Aqueous extract (µg/disc)				Methanolic extract (µg/disc)				Streptomycin
		25	50	75	100	25	50	75	100	(10 µg/disc)
1	*E.coli*	0	0	2±0.12	3±0.16	1±0.10	3±0.41	6±0.34	10±0.41	13 ± 0.76
2	*B.cereus*	1±0.21	2±0.11	3±0.44	4±0.31	6±0.12	10±0.21	15±0.31	17±0.42	12 ± 0.53
3	*P. aeruginosa*	0	0	1±0.08	2±0.23	5±0.24	15±0.42	19±0.65	20±0.90	18 ± 0.68
4	*E.aerogens*	0	0	1±0.11	2±0.21	1±0.11	2±0.13	4±0.34	7±0.42	5 ± 0.58

Values are shown in mean ± SE

Table 3: Antibacterial activity (MIC and MBC in µg/ml) of the root extracts of *Aloe vera*

S. No.	Test organisms	Aqueous extract		Methanolic extract	
		MIC	MBC	MIC	MBC
1	*E.coli*	40	80	25	50
2	*B.cereus*	30	60	20	40
3	*P. aeruginosa*	60	120	10	20
4	*E.aerogens*	25	50	15	30

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