

Original Research Article

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Antimicrobial Activity and Phytochemical Screening of *Aloe vera* (*Aloe barbadensis* Miller)

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ABSTRACT

Keywords

Antimicrobial activity, *Aloe vera*, *A. barbadensis* Miller, TLC Bioautography, Phytochemical analysis.

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The present study was conducted to assess the antimicrobial potential and phytochemical analysis of *Aloe vera* (*Aloe barbadensis* Miller) leaves extracts. The extracts were prepared by the sequential cold maceration method by using hexane, ethyl acetate, methanol and distilled water as a solvent. Antimicrobial activity of four extracts was performed by agar well diffusion method against different bacteria and fungi. Determination of Minimum Inhibitory Concentration (MIC) of different extracts, Thin Layer Chromatography (TLC), TLC bioautography and qualitative phytochemical analysis were also performed. The antimicrobial activity of *A. barbadensis* leaves extracts was found maximum against *S. marcescens* with a Zone of Inhibition (ZOI) of 13.67 ± 0.57 mm by hexane extract. The MIC of different extracts ranged between 6.25 and 50.00 mg/ml. Among all the fungi used in the study, all the three *Aspergillus* species were slightly inhibited by the specific extracts. The finding of TLC bioautography showed that compounds eluted at R_f 0.65 demonstrated strong antimicrobial activity whereas compounds eluted at R_f 0.41 and R_f 0.82 exhibited moderate antimicrobial activity against *S. marcescens*. Phytochemical analysis indicated the presence of phytochemicals present in various extracts. The results of the investigation clearly indicate that *A. barbadensis* leaves extract have a potential antimicrobial activity against various microorganisms due to the presence of various phytochemicals.

Introduction

The resistance of microorganisms against antimicrobial drugs is a major problem of recent times, which is increasing day by day (Cohen, 2000; Kumar *et al.*, 2013). As synthetic antimicrobials or antibiotics have considerable side effects over natural antimicrobial agents it is compulsory need to

search for drugs which are effective against a wide range of microorganisms with minimal or no side-effects (Shrikanth *et al.*, 2015). To tackle this problem, medicinal plants with ethnobotanical importance can be act as a source for the identification of the new drugs. Medicinal plants are considered as the

greatest pharmaceutical stores existing on the earth as they can produce eternal secondary phytochemicals having bioactive properties. These phytochemicals work efficiently to cure various diseases and illnesses since ancient times (Abdallah, 2011).

Aloe barbadensis Miller (*Aloe vera* L.) is an herb found all over the world. It is revealed that it has conspicuous pharmacological activities such as antibacterial (Subramanian *et al.*, 2006; Arunkumar and Muthuselvam, 2009; Saritha *et al.*, 2010; Fani and Kohanteb, 2012; Nejatizadeh-Barandozi, 2013), antifungal (Bajwa *et al.*, 2007; Rosca-Casian *et al.*, 2007; Khaing, 2011; Sitara *et al.*, 2011), antiviral (Zandi *et al.*, 2007), antioxidant (Baradaran *et al.*, 2013; Ray *et al.*, 2013; Kang *et al.*, 2014), cytotoxic (Jose *et al.*, 2014; Shalabi *et al.*, 2015), antidiabetic (Tanaka *et al.*, 2006; Choudhary *et al.*, 2014; Suleyman *et al.*, 2014), anti-inflammatory (Vijayalakshmi *et al.*, 2012; Bhattacharjee *et al.*, 2014), antitumor (El-Shemy *et al.*, 2010; Srihari *et al.*, 2015), nephroprotective (Iftikhar *et al.*, 2015; Virani *et al.*, 2016), antiulcer (Borra *et al.*, 2011) and anti-aging effects which can be used as a moisturizing agent to cure cardiovascular diseases as well as to enhance the immune system (Chatterjee *et al.*, 2013). It is used as an herbal medicine since long time which contains more than 100 bioactive constituents. Aloe plant is a rich source of many natural phytochemicals possessing health-promoting effects like, anthraquinones, vitamins, minerals, polysaccharides, sterols, amino acids, saponins, salicylic acids and may more (Surjushe *et al.*, 2008; Chatterjee *et al.*, 2013).

This might be the first report of the evaluation of antimicrobial activity of *A. barbadensis* leaves extracts against two bacteria *viz.*, *Serratia marcescens* and *Bacillus cereus* as well as four fungi used in the present study. Thus, the aim of the present investigation was

to evaluate the inhibitory effects of *A. barbadensis* leaves extracts against pathogenic bacteria and fungus in addition to elucidate the possible class of phytochemicals responsible for their antimicrobial activity.

Materials and Methods

Plant material used

Fresh leaves of *A. barbadensis* were collected from the botanical garden of G. J. Patel Institute of Ayurvedic Studies and Research, New Vallabh Vidhyanagar, Gujarat, India. The taxonomical identification was done by the taxonomist. The fresh leaves were washed with distilled water and air dried. After drying, leaves were powdered and stored at 4°C in airtight bottles for further study.

Preparation of plant extracts

Four solvents *viz.*, hexane, ethyl acetate, methanol and distilled water were used in the sequential cold maceration method (Dharajiya *et al.*, 2014) as described in flow chart given in Figure 1. At the end of extraction process four different extracts were prepared and further used for antimicrobial study. Test samples of 100 mg of extract/ml of dimethyl sulphoxide (DMSO) were prepared to perform antimicrobial assay.

Test microorganisms

All the microorganisms used in the present study were collected from the Department of Microbiology, ARIBAS, Gujarat, India. Total four bacteria were used in the study, of which three were Gram negative bacteria *viz.*, *Escherichia coli* (MTCC No. 448), *Pseudomonas aeruginosa* (MTCC No. 7436) and *Serratia marcescens* (MTCC No. 3124) while one was Gram positive bacterium namely, *Bacillus cereus* (MTCC No. 135). Total five fungal strains were used *viz.*,

Aspergillus niger, *Aspergillus flavus*, *Aspergillus oryzae*, *Penicillium chrysogenum* and *Trichoderma viridae*. The bacterial cultures were maintained on nutrient agar medium and the fungal strains were maintained on Potato Dextrose Agar (PDA) medium at 4°C.

Antimicrobial activity

The antibacterial and antifungal activities of the extracts were carried out by agar well diffusion method as described by Dharajiya *et al.*, 2014 and Dharajiya *et al.*, 2015a. The positive control wells were filled with Gentamicin (10 µg/ml) and Fluconazole (10 mcg/disc) against bacteria and fungi, respectively. The negative control wells were filled with DMSO.

Determination of Minimum Inhibitory Concentration (MIC)

The determination of MIC of different extracts with respect to different bacteria and fungi was determined by using the broth dilution method as explained by Dharajiya *et al.*, 2014.

Analytical Thin Layer Chromatography (TLC)

Analytical TLC was performed to identify an appropriate solvent system to generate the chromatogram. Various solvent systems were applied on the pre-coated TLC plates (Merck, silica gel 60 F254 plate, 0.25 mm) for the development of the chromatogram.

Among all the solvent systems, chloroform: methanol: distilled water (50:40:10) was found best and used for the TLC analysis as well as TLC bioautography analysis. The TLC plates were visualized under visible light for compounds separated followed by the calculation of R_f values.

TLC Bioautography

The hexane extract of *A. barbadensis* leaves was separated on TLC plate and the same plate was used for the TLC bioautography against *S. marcescens*. The TLC plate was developed using chloroform: methanol: distilled water (50:40:10) solvent, which separated components. The same TLC plate was dried at room temperature for the complete removal of solvents and placed in the petri plate followed by over laying of nutrient agar seeded with an overnight culture of *S. marcescens*. The petri plate was incubated at 37°C for 24 h. After incubation, an aqueous solution of 5 mg/ml of methylthiazoletetrazolium (Sigma-Aldrich) was sprayed on the plate. The clear zone of inhibition was observed against pink/purple background and their R_f values were compared with the reference TLC plate (Dharajiya *et al.*, 2016).

Qualitative phytochemical analysis

The extracts were tested for the presence of alkaloids, tannins, saponins, cardiac glycosides, steroids, phenols and flavonoids according to the standard protocols for detecting the presence of different phytochemicals in the plant extracts as described by Dharajiya *et al.*, 2012 and Dharajiya *et al.*, 2015b.

Results and Discussion

The problem of microbial resistance towards antimicrobial drugs is becoming a major problem for humankind as it leads to the death of millions of people (Cohen, 2000). Most of the world's population relies on plant derived traditional medicines for the need of their primary health care (Duraipandian *et al.*, 2006). Plants can be a very important source of newer drugs or antimicrobial compounds as they exhibit a vast range of

phytochemicals. Various *Aloe* species are found all over the world which are used in cosmetics, medicine/pharma and food industry (Park and Jo, 2006). *Aloe* leaves contain various chemicals from different classes which have antimicrobial activity (Arunkumar and Muthuselvam, 2009). Hence, the present study was carried out to evaluate the efficiency of different four extracts as an antimicrobial agent as well as to access the presence of phytochemicals in each extract.

Antimicrobial activity

Antimicrobial activity (in terms of the zone of inhibition) of the extracts was evaluated against selected pathogenic bacterial and fungal strains by agar well diffusion method. In the present investigation, total four extracts *viz.*, hexane, ethyl acetate, methanol and aqueous extracts of *A. barbadensis* leaves with a concentration of 100 mg/ml were tried. All the extracts except ethyl acetate showed antimicrobial activity against different test microorganisms. The maximum antibacterial effect of *A. barbadensis* leaves extracts was found against *S. marcescens* [Zone of inhibition (ZOI) = 13.67±0.57mm] by hexane extract followed by inhibition of *B. cereus* (ZOI = 12.33±0.57 mm) by the methanol extract. The methanol extract showed inhibitory effect against all the tested bacterial strains while ethyl acetate extract failed to inhibit the growth of any of the bacterial strains evaluated in the present study. In case of antifungal activity, the maximum inhibitory activity was found by aqueous extract against *A. niger* with 09.6±0.57mm zone of inhibition. Out of the four extracts tested, two extracts *viz.*, hexane and ethyl acetate failed to express antifungal activity against any of the fungal strains use in the study. The methanol extract exhibited slight inhibitory action against *A. oryzae*. Out of all the microorganisms, *P. chrysogenum* and *T. viridae* were found to be resistant to all the

four extracts of *A. barbadensis* leaves. The complete findings regarding antimicrobial activity are represented in Table 1.

The inhibitory activities of *A. barbadensis* or *Aloe vera* leaves against some bacteria *viz.*, *Aeromonas hydrophius*, *Aggregatibacter actinomycetemcomitans*, *Bacillus sphaericus*, *Bacteroides fragilis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, *Listeria monocytogenes*, *Micrococcus luteus*, *Morganella morganii*, *Mycobacterium smegmatis*, *Porphyromonas gingivalis*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Shigella boydii*, *Staphylococcus aureus*, *Streptococcus mutans*, *Streptococcus pyogenes* and *Vibrio parahaemolyticus* have been evaluated (Alemdar and Agaoglu, 2009; Arunkumar and Muthuselvam, 2009; Pandey and Mishra, 2010; Saritha *et al.*, 2010; Fani and Kohanteb, 2012; Nejatizadeh-Barandozi, 2013) in the recent past.

There are very few reports on antifungal activity of *Aloe* sp. which included the antifungal activity against some fungi *viz.*, *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Botrytis gladiolorum*, *Candida albicans*, *Colletotrichum coccodes*, *Cryptococcus neoformans*, *Drechslera hawaiiensis*, *Fusarium oxysporum*, *Heterosporium pruneti*, *Microsporium canis*, *Penicillium gladioli*, *Penicillium maneffei*, *Penicillium digitatum*, *Phythium sp.*, *Rhizoctonia solani*, *Trichophyton mentagraphytes* and *Trichophyton schoenleini* (Agarry and Olaleye, 2005; De Rodriguez *et al.*, 2005; Rosca-Casian *et al.*, 2007; Alemdar and Agaoglu, 2009; Khaing, 2011; Sitara *et al.*, 2011).

Hence, possibly it is the first study showing antimicrobial activity of *A. barbadensis* leaves extracts against two bacteria *viz.*, *S. marcescens* and *B. cereus* as well as four

fungus strains viz., *Aspergillus flavus*, *Aspergillus oryzae*, *Penicillium chrysogenum* and *Trichoderma viridae*.

Determination of MIC

The MIC values of various extracts with respect to specific microorganism were resolute using the broth dilution method as given in Table 2. All the extracts exhibiting antimicrobial activity in the agar well diffusion method were advanced to determine MIC values. As per the MIC results found in the present study, the range of MIC of various extracts was 6.25 to 50.00 mg/ml. In the present investigation, the lowest MIC value recorded was 6.25 mg/ml for the hexane extract against *S. marcescens* which indicated maximum power to inhibit the growth of the specific bacterial strain. The highest MIC value was 50 mg/ml for methanol and aqueous extracts against *A. oryzae* and *A. flavus*, respectively.

There are few reports of determination of MIC of various extracts of *Aloe sp.* against

different bacterial strains. One of the previous study indicated that the range of MIC of *A. vera* gel was 12.5-50.0 µg/ml against some periodontopathic and cariogenic bacterial isolates (Fani and Kohanteb, 2012). Another report revealed that the range of MIC of *A. barbadensis* extract against various pathogenic bacteria was 0.10-10.0 mg/ml (Pandey and Mishra, 2010). Ultimately, there are very few reports of MIC determination for *A. barbadensis* leaf extracts against the strains used in the present study. Hence, present study can be utilized as a base for the development of the antimicrobial drugs from *A. barbadensis* leaf against some bacteria.

TLC and TLC Bioautography

Total five components from hexane extract of *A. barbadensis* leaves were separated by TLC and their R_f values are given in Table 3. The same plate was used for the TLC bioautography against *S. marcescens*. It allowed determining the active components of the hexane extract having antimicrobial activity against *S. marcescens*.

Table.1 Antimicrobial activity (Zone of Inhibition) of *A. barbadensis* leaves extracts

Microorganisms		Name of extract (Concentration = 100 mg/ml)				Positive control		Negative control
		Hexane	Ethyl Acetate	Methanol	Aqueous	Gentamicin (10 µg/ml)	Fluconazole (10 mcg/disc)	DMSO
Bacteria	<i>S. marcescens</i>	13.67±0.57	-	11.00±1.00	11.67±1.15	19.00±1.00	NA	-
	<i>B. cereus</i>	-	-	12.33±0.57	10.83±0.76	15.17±0.76	NA	-
	<i>P. aeruginosa</i>	-	-	08.83±0.76	-	15.00±1.00	NA	-
	<i>E. coli</i>	-	-	10.33±0.57	09.5±0.50	14.67±1.04	NA	-
Fungi	<i>A. niger</i>	-	-	-	09.6±0.57	NA	16.16±1.04	-
	<i>A. flavus</i>	-	-	-	08.1±0.28	NA	21.33±1.15	-
	<i>A. oryzae</i>	-	-	08.6±0.57	-	NA	16.00±1.00	-
	<i>P. chrysogenum</i>	-	-	-	-	NA	18.66±1.52	-
	<i>T. viridae</i>	-	-	-	-	NA	22.33±0.57	-

(-): No zone of inhibition, NA: Not Assessed, DMSO: Dimethyl sulphoxide, The test was done in triplicate, Diameter of the zone of inhibitions is given here as mean±standard deviation

Table.2 Minimum Inhibitory Concentration (MIC) values of *A. barbadensis* leaves extracts

Microorganisms		Name of extract			
		Hexane	Ethyl Acetate	Methanol	Aqueous
Bacteria	<i>S. marcescens</i>	06.25	NA	12.50	12.50
	<i>B. cereus</i>	NA	NA	12.50	25.00
	<i>P. aeruginosa</i>	NA	NA	25.00	NA
	<i>E. coli</i>	NA	NA	25.00	25.00
Fungi	<i>A. niger</i>	NA	NA	NA	25.00
	<i>A. flavus</i>	NA	NA	NA	50.00
	<i>A. oryzae</i>	NA	NA	50.00	NA
	<i>P. chrysogenum</i>	NA	NA	NA	NA
	<i>T. viridae</i>	NA	NA	NA	NA

MIC: Minimum Inhibitory Concentration (mg/ml),
NA: Not Assessed

Table.3 Thin Layer Chromatography (TLC) of hexane extract of *A. barbadensis* leaves

No. of Compound	R _f value	Band colour in visible light
1	0.35	Dark brown
2	0.41	Brown
3	0.65	Light yellow
4	0.82	Brown
5	0.90	Brown

Table.4 Qualitative phytochemical analysis of *A. barbadensis* leaves extracts

Name of test	Name of extract			
	Hexane	Ethyl Acetate	Methanol	Aqueous
Alkaloids	+	-	+	+
Saponins	+	+	+	-
Tannins	-	-	-	+
Sterols	+	+	+	+
Cardiac glycoside	-	-	-	-
Flavanoids	-	-	+	+
Phenol	+	+	+	+

(+): Present, (-): Absence

Fig.1 Sequential cold maceration method for preparation of plant extracts

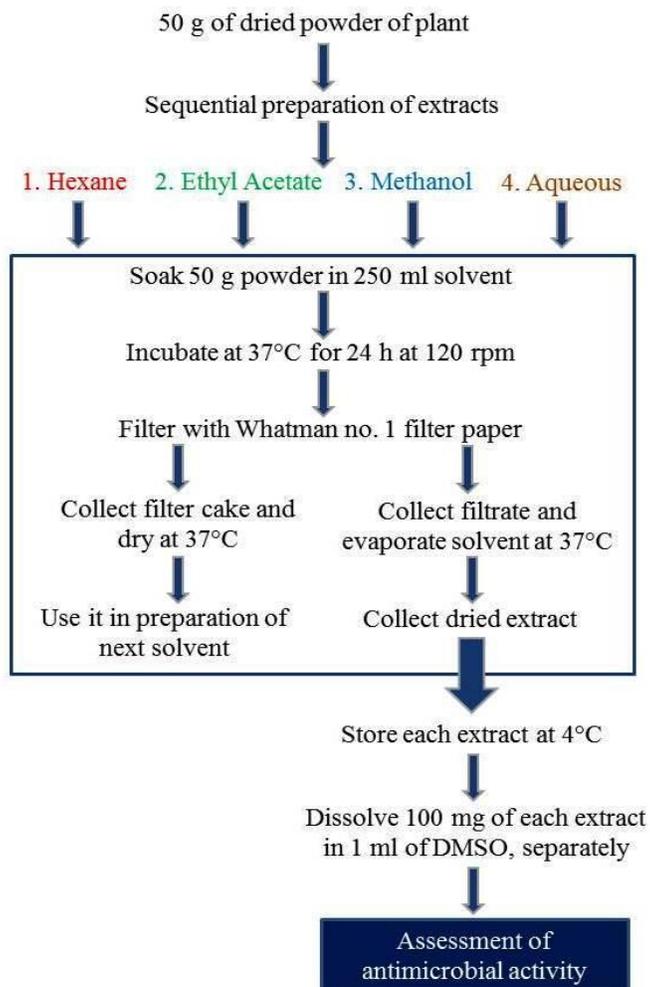


Fig.2 TLC bioautography of hexane extract of *A. barbadensis* leaves against *S. marcescens*



The result of TLC bioautography represented that components separated at R_f 0.65 possessed strong antimicrobial activity, whereas components with 0.41 and 0.82 R_f values exhibited moderate antimicrobial activity against *S. marcescens* which is represented as a clear zone of inhibition in Figure 2. Hence, the components with specific R_f values and having antimicrobial activity can be detected and purified for further specific analysis. The ethanol, acetone and methanol extracts of *A. vera* gel were used for the separation of the active components possessing antimicrobial activity (Lawrence *et al.*, 2009). Another study revealed that the component with 0.8 R_f value exhibited antimicrobial activity and identified as aloe-emodin (Nidiry *et al.*, 2011). In the present investigation, the component with 0.82 R_f value possessed antimicrobial activity which is indicative of the extraction of aloe-emodin in the hexane and other extracts showing antimicrobial activity.

Qualitative phytochemical analysis

The preliminary phytochemical analysis gives valuable information regarding the presence of important classes of phytochemicals present in the extracts. The outcomes of the qualitative phytochemical analysis of various extracts of *A. barbadensis* leaves are given in Table 4. The results point out to the presence of some phytochemicals in methanol, aqueous and hexane extracts as compared to ethyl acetate extract. It might be the reason behind no antimicrobial activity of ethyl acetate extract against the selected microorganisms. Similar investigations were carried out by other researchers for the determination of the class of phytochemicals present in various extracts of *Aloe* species (Arunkumar and Muthuselvam, 2009; Raphael, 2012).

In Conclusion, the current study revealed that the methanol extract of *A. barbadensis* leaves

possessed overall more antimicrobial activity followed by aqueous and hexane extracts, however hexane extract showed antimicrobial activity only against *S. marcescens* but with maximum zone of inhibition. Various phytochemicals may play role as antimicrobial agent which were extracted in different solvents. These phytochemicals having antimicrobial activity should be identified and purified from the crude extracts by various analytical techniques and can be implicated in the development of antimicrobial drugs against various pathogenic microorganisms.

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