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Aloe vera gel: Effective Therapeutic agent against Extended-Spectrum β-lactamase Producing *Escherichia coli* Isolated from Patients with Urinary Tract Infection in Tehran-Iran

Mehdi Goudarzi[1,2]*, Saeedeh Ghafari[1], Masoumeh Navidinia[3] and Hadi Azimi[4]

[1]Traditional Medicine and Materia Medical Research Center (TMRC),
Shahid Beheshti University of Medical Sciences, Iran.
[2]Department of Microbiology, School of Medicine,
Shahid Beheshti University of Medical Science, Tehran, Iran.
[3]Schools of Allied Medical Sciences,
Shahid Beheshti University of Medical Sciences, Tehran, IR Iran
[4]Division of English Language department at the School of Medicine,
Shahid Beheshti University of Medical Sciences, Tehran, Iran.

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***Aloe vera*, as a traditional folk medicine plant, is used for its curative and therapeutic properties. In the current study, attempts were made to evaluate *in vitro* antimicrobial activity of *A. vera* gel against *Escherichia coli* strains isolated from patients with urinary tract infection. A total of 150 *E. coli* isolates were recovered from urine samples between July 2015 and September 2015. Extended-spectrum ²-lactamase (ESBL) screening in *Escherichia coli* isolates was on the basis of double disc synergy tests and combined disk diffusion test. Epsilometer test was performed to determine susceptibilities of *E. coli* isolates to 13 antimicrobial agents. Metallo-beta-lactamase (MBL) production was detected by performing combined disc test. Also, the micro broth dilution method was used to determine the antimicrobial activity of *A. vera* gel against *E. coli* isolates.In the present study, out of 150 *E. coli* isolates, 110 (73.3%) were confirmed as ESBL. MBL screening, using phenotypic methods, indicated that 33 (22%) isolates were positive. The antibiogram revealed that 148 isolates (98.7%) were multi drug resistance *E. coli* strains. The coexistences of ESBL and MBL were found in 15 isolates (10%). All of ESBL and MBL *E. coli* strains were inhibited by ethanol extract of *A. vera* gel at minimum inhibitory concentration £200 μg/ml. More than half of the tested isolates (53.3%) were inhibited by concentrations that did not exceed 50 μg/ml for ethanol extract from *A. vera* gel.The results of the present study highlighted that *A. vera* gel, at various concentrations, could be used as an antibacterial agent for treatment and prevention of UTIs.**

Keywords: *Aloe vera* gel, *Escherichia coli*, Multidrug-resistant, ESBL, MBL, MIC.

Urinary Tract Infection (UTI) is one of the most frequent types of nosocomial infections and affects probably about one-half of all individuals during their lifetimes. The urinary tract is normally sterile and without bacteria. The entrance of bacteria into the urinary tract may cause asymptomatic or irritative symptoms such as frequency and urgency [1]. UTIs are usually caused as a consequence of bacterial invasion of the urinary tract, including the lower urinary tract (cystitis) and the upper urinary tract (pyelonephritis). The bacterial entry into the blood stream is associated with severe morbidity, including sepsis and death. UTI affects both men and women, but it is particularly common among the female population and increases directly with sexual activity and childbearing [1, 2]. It is estimated

* To whom all correspondence should be addressed.
Tel.: +98 (21) 23872556; Fax: +98 (21) 23872556;
E-mail: gudarzim@yahoo.com

that about 50–60% of women have experienced at least one UTI in their lifetime and one-third will have at least one symptomatic UTI necessitating antibiotic treatment by age. Various bacteria are responsible for UTI. Among the bacterial species, *Escherichia coli* is causing more than 80% of the UTI cases followed by *Staphylococcus, Klebsiella*, *Pseudomonas*, *Proteus* and *Enterococcus* species [3, 4].

Although antibiotic therapy is an important step of UTI treatment, during the past decades, the prevalence of resistance among E. coli strains has dramatically increased due to the improper and extensive use of antibiotics. In particular, extended-spectrum β-lactamase (ESBL) *E. coli* has emerged as a major public health concern [5, 6]. In the past few decades, an increase of resistance to antibiotics and the existence of Multi-Drug Resistance (MDR) in *E. coli* strains has become a public health problem. Rapid dissemination of MDR among *E. coli* strains has significantly limited the choice of available therapeutic options, and presents a particularly difficult challenge in these contexts [5]. The widespread MDR *E. coli* not only leads to increased economic burden, but it can also directly threaten the life of the patient. Although antibiotics are routinely used to prevent UTIs, and since there is the risk that bacteria will develop resistance to these agents, therapeutic strategies, with the emphasis on the use of extract and biologically active compounds isolated from herbal plants, should be revised [7].

The use of natural plants for treatment of many infectious diseases is a rich tradition in several cultures of many contemporary cultures, such as China, India, Japan, and Iran for millennia. Traditional medicine is quite common in Asia, Africa, and some areas of America where there is a belief in traditional medicine to maintain human health [8]. During the past several decades, many researchers have paid attention to antibacterial activities of medicinal plants. According to the estimates by World Health Organization, about 80% of the world's population, especially in the developing countries, still relies mainly on traditional medicine and the use of plant extracts as therapeutic choice [8, 9]. In the recent years, extracts or oils of medicinal plants with antimicrobial and anti-inflammatory effects have been used for treatment of many human infectious diseases. *Aloe vera* (*A. vera*) is one of these well-known medicinal plants [10].

A. vera belongs to the *Liliaceae* family of which there are over 360 known species. This plant is a cactus-like perennial, drought resistant, succulent plant with lance-shaped leaves containing clear gel in a central mucilaginous pulp. The gel consists of 99.3% water and the remaining 0.7% contains more than 75 active ingredients and a range of active compounds, including polysaccharides, vitamins, amino acids, phenolic compounds, and organic acids [11]. The gel of *A. vera* has been used for gastrointestinal disorders, healing wounds and burns, eczema, psoriasis, constipation, high blood pressure, ulcers, and diabetes since ancient times. Many studies have shown several biological activities of *A. vera* gel, such as cell growth stimulator activity, antioxidant, anti-inflammatory, immune modulating, as well as antibacterial, antiviral, and antifungal properties [12]. Although antimicrobial properties of *A. vera* gel are recognized primarily, there is still little information available on the uses of this gel and thus requires more evaluation. *E. coli* strains isolated from patients with UTI.

MATERIALS AND METHODS

Bacterial strains

The present descriptive study was conducted at the Shahid Beheshti University of Medical Sciences, Tehran, Iran. All the 150 *E. coli* strains tested in the present study were obtained from 245 urine specimens from hospitalized UTI patients during a period of three months from July 2015 and September 2015. The research was approved by the Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran (No IR.SBMU. REC.1394.6439). The inclusion criterion was existence of *E. coli* strains isolated from hospitalized patients. The exclusion criteria were community-acquired UTIs and repeated *E. coli* isolates. All the urine specimen were immediately transported to the laboratory. The identification of isolates was carried out based on standard microbiological procedures. The colony count semiquantitative method was performed according to the surface streak procedure using calibrated loops. The incubation was done for 24– 48 hours in aerobic conditions and at 37°C.

The growth of a single pathogen of $>10^5$ colony-forming units per milliliter (CFU/ml) from urine specimens was considered as positive UTI [13]. *E. coli* isolates were stored in Tryptic Soy Broth (TSB; Merck, Germany) containing 20% glycerol at -70°C for further investigations.

Antimicrobial Susceptibility Testing (AST)

Minimum Inhibitory Concentration (MIC) was determined for Ampicillin, Ceftriaxone, Cefazolin, Gentamicin, Ciprofloxacin, Amikacin, Imipenem, Meropenem, Nitrofurantoin, Piperacillin, Trimethoprim-sulfamethoxazole, Piperacillin/Tazobactam, and Nalidixic acid using E-test strips (Liofilchem; Italy) according to the manufacturer's protocol. Briefly, colonies from an overnight agar plate were suspended in broth to achieve a 0.5 McFarland standard and then inoculated on Mueller-Hinton agar (Oxoid, UK). After a brief drying period, E-test strips were located on the agar surface with the scale facing upwards. After overnight incubation at 35±2°C in the ambient atmosphere, the results were interpreted as susceptible and resistant according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [14]. MDR was defined as resistance to at least three or more unrelated antibiotics [5].

ESBL confirmatory test

Double Disc Synergy Test (DDST) and Combined Disk Diffusion Test (CDDT) were used to confirm ESBL phenotype based on CLSI criteria [14].

Double Disc Synergy Test (DDST)

To perform DDST Phenotypic method, Cefotaxime (30 µg) and Ceftazidime (30 µg), with and without Clavulanic acid (10 µg) discs, were placed on Mueller-Hinton agar (MHA, Oxoid, United Kingdom) with 25 mm apart from each other. The organism was regarded positive for the ESBL production when the zone of inhibition was equal or more than 5 mm for either antimicrobial agent tested with clavulanic acid versus its zone or when tested without clavulanic acid [15].

Combined Disc Diffusion Test (CDDT)

CDDT was done using both Ceftazidime (30 µg) disc alone and in combination with Clavulanic acid (30 µg/10 µg) discs placed 25 mm apart from each other on inoculated MHA. Enhancement of the inhibition zone diameter e"5 mm for antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was interpreted as ESBL-producing *E. coli* [16]. ATCC 25922 was used as quality control.

Phenotypic detection of Metallo-Beta-Lactamase (MBL)

Identification of MBL-producing isolates was performed using CDDT described by Galani et al [17]. In this test, Imipenem and Imipenem + EDTA discs were used. The diameter of inhibition zones of the Imipenem and Imipenem + EDTA discs were compared after 16-18 hours of incubation at 37°C. An increase in the inhibition zone of ≥7 mm around the Imipenem-EDTA disc, compared to that of the Imipenem disc alone, was considered as positive result, that indicated the presence of MBL in the tested isolate.

Preparation of *A. vera* gel

The leaves of *A. vera* plant were collected from a local farm in the south of Iran. The clean and fresh leaves of *A. vera* were dissected longitudinally into pieces. The thick straw-colored gel was collected in a sterile container. After the gel was homogenized with the blender, the ethanolic extract of the dry powdered gel was prepared by soaking 10 g of powdered gel in 60 ml of 95% ethanol for one day, shaking occasionally with a shaker. After one day, suspensions were filtered (Whatman no. 1 filter paper). Then, the ethanol was removed via evaporation using a rotary evaporator [10].

MIC of *Aloe vera* gel

Determination of MIC titer for ethanol gel extract of *A. vera* was carried out in 96-well cell culture plates. The MIC was defined as the lowest concentration of an antimicrobial agent that inhibits the visible growth of the tested isolate. In brief, bacterial suspension was adjusted equivalent to the 0.5 McFarland standard (approximately 1.0×10^8 CFU/mL) in nutrient broth medium (Merck, Germany). This suspension was further diluted 1:100 (10^6 CFU/mL) in the broth media. MIC values ranged between 25 µg/ml and 400 µg/ml. To each well, 100¼L of broth media was added. Then, 100µL of each concentration of extract was added to the wells. Wells with BHI medium (sterility control) and BHI and bacterial suspension (drug-free control) were also used as controls. The plates were wrapped loosely using cling film to ensure that the bacteria did not get dehydrated and were examined for determination of MIC after

incubation at 37°C overnight. To estimate MIC of *A. vera* gel extract, the absorbance of each well was measured at 595 nm. MIC level was defined as the lowest concentration at which no growth was observed [10].

RESULTS

During the seven-month period, 245 urine samples were collected from hospitalized patients with UTI and a total of 150 samples (61.2%) were confirmed as *E. coli* and thus entered the study. Patients' mean age was 44 years (median 43.2 years, ranging from 1 to 60 years). In the present investigation, 120 isolates (80%) were isolated from female and 30 isolates (20%) were isolated from male participants (M:F ratio was 0.25). The age distribution was 13.3% equal or less than 15 years, 56.7% from 16 to 35 years, 20% from 36 to 50 years, and 10% equal or greater than 51 years.

Table 1. The susceptibly pattern and MIC_{50} and MIC_{90} values of 150 *E. coli* clinical isolates

Agent	MIC (µg/ml)			No. (%) of isolates			MIC Interpretive Breakpoints[a]		
	Range	50%	90%	S	I	R	S	I	R
Aloe vera gel	25-800	50	200	-	-	-	-	-	-
Ampicillin	0.016-256	32	>32	5(3.3)	-	145(96.7)	≤8	16	≥32
Ceftriaxone	0.016-256	1	>4	28(18.7)	12(8)	110(73.3)	≤1	2	≥4
Cefazolin	0.016-256	2	>8	63(42)	-	87(58)	≤2	4	≥8
Piperacillin	0.016-256	16	128	41(27.3)	4(2.7)	105(70)	≤16	32-64	≥128
Imipenem	0.002-32	1	4	118(78.7)	-	32(21.3)	≤1	2	≥4
Meropenem	0.002-32	1	4	120(80)	-	30(20)	≤1	2	≥4
Gentamicin	0.016-256	16	>16	32(21.3)	-	118(78.7)	≤4	8	≥16
Amikacin	0.016-256	16	>64	88(58.7)	10(6.6)	52(34.7)	≤16	32	≥64
Ciprofloxacin	0.002-32	4	>4	30(20)	-	120(80)	≤1	2	≥4
Nitrofurantoin	0.032-512	32	128	43(28.7)	2(1.3)	105(70)	≤32	64	≥128
Nalidixic acid	0.016-256	16	32	40(26.7)	-	110(73.3)	≤16		≥32
Trimethoprim-sulfamethoxazole (1/19)	0.002-32	4/76	4/76	10(6.7)	-	140(93.3)	≤2/38		≥4/76
Piperacillin/ tazobactam (4 µg/mL)	0.016-256	16/4	128/4	97(64.7)	-	53(35.3)	≤16/4	32/4-64/4	≥128/4

Table 2. Resistance pattern and distribution of ESBL and MBL among tested isolates

Number of antibiotics	Resistance profile	ESBL No (%)	MBL No (%)	ESBL+ MBL No (%)	No (%)	Aloe vera gel MIC(µg/ml) 50%	90%
8	AMP, SXT, CIP, CN, NA, CRO, F, PIP	53(70.7)	5(6.7)	3(4)	75(50)	100	200
	AMP, SXT, CIP, PIP, TZP, AK, MRP, IMI	15(50)	2(6.7)	9(30)	30(20)	50	200
	AMP, SXT, CN, NA, CRO, TZP, F, AK	12(60)	4(20)	2(10)	20(13.4)	100	100
6	AMP, SXT, CIP, CN, NA, CRO	9(60)	3(20)	1(6.7)	15(10)	50	200
3	AMP, CN, F	5(100)	0(0)	0(0)	5(3.3)	50	100
	CN, F, TZP	0(0)	3(100)	0(0)	3(2)	50	50
	F, AK, IMI	1(50)	1(50)	0(0)	2(1.3)	100	100

AMP, ampicillin; CRO, ceftriaxone; KZ, cefazolin; CN, gentamicin; CIP, ciprofloxacin; AK, amikacin; IMI, imipenem; MRP, meropenem; F, nitrofurantoin; PIP, piperacillin; SXT, trimethoprim-sulfamethoxazole; TZP, piperacillin/tazobactam; NA, nalidixic acid.

Antimicrobial susceptibility

The results of the AST of 150 *E. coli* clinical isolates to 13 antibiotics tested and also MIC_{50} and MIC_{90} values are presented in Table 1. The results of AST showed that the majority of isolates were resistant to Ampicillin (96.7%), Trimethoprim-sulfamethoxazole (93.3%), Ciprofloxacin (80%), Gentamicin (78.7%), Ceftriaxone (73.3%), and Nalidixic acid (73.3%). The highest level of susceptibility was found to be related to Meropenem (80%) and Imipenem (78.7%). The resistance rates to other antimicrobial agents were: Piperacillin: 70%, Nitrofurantoin: 70%, Cefazolin: 58%, Piperacillin/Tazobactam: 35.3%, and Amikacin: 34.7%. A total of 148 isolates (98.7%) were characterized as MDR *E. coli* strains. AST showed seven different resistant profiles among the isolates where 125 isolates (83.3%) were resistant to eight antibiotics, 15 isolates (10%) were resistant to six antibiotics, and 10 isolates (6.7%) were resistant to three antibiotics.

ESBL screening using DDST and CDDT showed that 110 (73.3%) isolates were confirmed as ESBL belonging to female patients and age groups of 16-35 (56.7%) and 36-50 (16.7%) years old. MBL screening using phenotypic methods indicated that 33 (22%) isolates were positive for production of MBL and all belonged to female patients. The age of the patients with MBL positive isolates ranged from 1 to 35 years. Table 2 presents resistance profiles and distribution of ESBL and MBL among tested isolates. All the MBL producing isolates and ESBL producing isolates with the exception of two isolates were found to be MDR.

Antibacterial activity of *A. vera* gel

In the present study, antibacterial activity of *A. vera* gel was analyzed against *E. coli* strains. Three different concentrations (50, 100, and 200µg/mL) of gel extracts of *A. vera* showed the inhibitory effect on tested isolates. The gel extract showed good antibacterial activity against tested isolates. All of the ESBL and MBL *E. coli* strains were inhibited by ethanolic *A. vera* gel at MIC ≥200 µg/ml (Table 2). Out of 150 tested *E. coli* isolates, the growth of 80 (53.3%) isolates were inhibited by ethanolic *A. vera* gel extract at MIC ≥50 µg/ml, 12 (8%) isolates at MIC ≥100 µg/ml, and 58 (38.7%) isolates at MIC ≥200 µg/ml.

DISCUSSION

During the past decades, the extensive use of antibiotics for treatment of *E. coli* infections has led to dissemination of MDR, which is a serious threat increasing in the whole world [5]. Recently, increase of ESBL-producing *E. coli* isolates has been reported from different parts of the world, particularly Asia [6]. Based on literature, although overall trend of ESBL-producing *E. coli* is on the rise, distribution of ESBL-producing *E. coli* isolates and ESBL genotypes may vary greatly in different geographical areas and from institute to institute. In the current survey, the prevalence of ESBL-producing isolates was 73.3%, which was lower than that in Turkey (84%) [18], but higher than those in Portugal (67.9%) [19], India (66.7%) [20], United Arab Emirates (41%) [21], China (36.7%) [22], Kuwait (31.7%) [23], Saudi Arabia (30.6%) [24], Japan (20.4%) [25], Thailand (13.2%) [26], and Colombia (11.7%) [27]. These differences could be attributed to the factors such as type and volume of samples, design of the study, prescription pattern, and consumption of antibiotics in different geographical regions. Use of different phenotypic methods in various studies could also be listed as another reason.

The results of antibiotic susceptibility test revealed high resistance rate to ampicillin (96.7%), trimethoprim-sulfamethoxazole (93.3%), ciprofloxacin (80%), gentamicin (78.7%), ceftriaxone (73.3%) and nalidixic acid (73.3%), that is line with the previous data from Iran by Goudarzi et al [6] who reported full resistance to amoxicillin and penicillin and high resistance rates of tested isolates to co-trimoxazole (96%), ceftazidime (82%), cefotaxime (78%), cefoxitin (71%), ciprofloxacin (69%), gentamicin (65%), cephalexin (63%), nalidixic acid (61%), piperacillin (39%), and aztreonam (33%). Similar resistance patterns were reported by several investigators [22, 28, 29]. The present study indicated a high incidence of MBL-producing *E. coli* (22%) in patients with nosocomial UTI, which is higher than the reported rate from Nepal (18.98%) [30] and lower than that from earlier studies in Iran (31%) [29], India (27.59%) [31], and Iraq (45.2%) [32]. Numerous studies from other parts of Asia also demonstrated increasing incidence of MBL production in Enterobacteriaceae isolates [33, 34]

Overall, these findings exhibited a high frequency of resistance among *E. coli* isolates to the common antibiotics used routinely in the treatment of UTIs, especially in Iran. However, the application of antibiotics to eliminate the etiologic agents of UTIs, due to the risk of the bacteria developing MDR, is not recommended. This warrants a warning to the clinicians that prescription of these antibiotics must be changed.

According to the previous studies, it seems that many medicinal plants, as potential sources of novel antimicrobial compounds, could be used for treating some infections; among these medicina plants, *A. vera* is probably the most important one [10]. Previous studies have confirmed that the constituents of gel and leaf are distinct from each another, but it is clear that gel and leaf can complement one another in their medicinal capabilities. Several investigators believe that since *A. vera* gel is rich in a wide variety of pharmacologically active compounds, including anthraquinones, anthracene, anthranol, aloin, aloe mannan, aloetic acid, aloe-emodin, aloeride, chrysophanic acid, resistanol, and saponin, it can be more effective than the *A. vera* leaf [12].

The present survey showed all of MDR *E. coli* strains and also ESBL and MBL-producing strains were found to be sensitive to ethanolic *A. vera* gel extract at MIC ≥200 µg/ml; this indicates the good antibacterial activity of *A. vera* gel extract against our isolates.

There are differences in the antimicrobial effects of various extracts of *A. vera*. In a study conducted by Irshad *et al* to determine the antimicrobial activity of ethanol, methanol, and distilled water, it was shown that methanol extract of *A. vera* of leaves and gel has higher antibacterial activity compared with that of ethanol and distilled water extracts [35]. In another study carried out by Ibrahim *et al* in 2011 to evaluate and compare antimicrobial activity of *A. vera* gel against some human and plant pathogens, three different extracts forms of ethanol, acetone, and aqueous were compared with each other. They showed that among the three extracts, acetone extract *A. vera* gel was found to be more effective than ethanol and aqueous extracts [36]. Also, Pandey and Mishra compared the antimicrobial activities of aqueous extract of *A. vera* against Gram negative and Gram positive bacteria. They indicated that aqueous extract has a weak inhibitory effect on Gram positive bacteria while such effect on the Gram negative bacteria was not observed [37]. That various extracts of *A. vera* have different antimicrobial effects can be due to the difference in the solubility of different compounds found in *A. vera*, especially those solvents that have a particular antifungal or antimicrobial activity.

In the present survey, all the ESBL and MBL *E. coli* strains were inhibited by ethanolic *A. vera* gel extract at three MIC ≥50, 100, and 200 µg/ml, that represents strong antibacterial activity of *A. vera* gel. This finding is in conformity with the results of Fani et al who showed strong bactericidal activity of *A. vera* gel against some cariogenic and periodontopathic bacteria. They stated that *A. vera* gel with a MIC of 12.5 µg/ml was inhibited from growth of *Streptococcus mutans*, while the growth of *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Bacteroides fragilis* was inhibited at MIC 25-50 µg/ml [38].

Several studies have attempted to determine the antibacterial activity of *A. vera* gel, leaf, and juice against fungi and bacteria strains. Antonisamy *et al* investigated antibacterial anti-fungal activity of DMSO gel extracts of *A. vera* against five bacterial cultures *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, and three fungal cultures of *Aspergillus fumigatus*, *Candida albicans*, and *Penicillium sps*. They indicated good antibacterial and anti-fungal activity of DMSO gel extract of *A. vera* against tested microorganisms [35].

Agarry *et al* studied the antimicrobial activities of the gel and leaf of *A. vera* against *P. aeruginosa*, *S. aureus*, *Microsporum canis*, *Trichophyton schoenleinii*, *Trichophyton. mentagrophytes*, and *C. albicans*. The results clearly demonstrated that both the gel and the leaf had good antibacterial activities against *S. aureus*. The leaf possesses inhibitory effects on both *P. aeruginosa* and *C. albicans* while only the gel inhibited the growth of *T. mentagrophytes* [39].

In addition, in a study conducted by Kaithwas *et al* in 2008, antimicrobial activities of both *A. vera* gel and juice were studied. The results of the study showed that *A. vera* juice has higher antimicrobial activity than *A. vera* gel [40]. This difference in activity of the juice, as compared to gel activity, could be attributed to the presence of

greater amounts of the anthraquinones and phenolic antioxidants in the *A. vera* juice.

CONCLUSION

Based on the results of the present study, ethanolic *A. vera* gel extract proved to have good antibacterial activity against ESBL and MBL *E. coli* strains isolated from patients with nosocomial UTI. Yet, more studies should be carried out to determine bioactive components of *A. vera* gel and its effect on the wide range of bacteria and fungus including the pathogenic strains. Bearing in mind the results of the current study, we hope that *A. vera* gel will be used more as one of the most effective medicinal sources to treat infectious diseases.

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