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EFFECT OF HEAT TREATMENT (TEMPERATURE) ON STABILITY OF *ALOE VERA* L. (*IN VIVO* & *IN VITRO* REGENERATED) WHOLE LEAF AND INNER GEL EXTRACTS AGAINST SOME PATHOGENS OF HUMAN HEALTH SIGNIFICANCE

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ABSTRACT

The present study investigates the effect of heat treatment on the stability and antimicrobial activity of *Aloe vera* L. extracts obtained from both *in vivo* grown and *in vitro* regenerated plants. Whole leaf and inner gel extracts were prepared using ethyl acetate and subjected to different temperature treatments (25°C, 50°C, 75°C, and 100°C) for 30 minutes. The antimicrobial efficacy of treated and untreated extracts was evaluated against a wide range of human pathogenic microorganisms, including bacteria, fungi, and yeast, using the disc diffusion method. The results demonstrated that heat treatment up to 75°C did not significantly affect the antimicrobial activity of the extracts, indicating strong thermal stability. However, exposure to 100°C resulted in a significant reduction in inhibitory activity, although it did not completely eliminate the antimicrobial properties. Both *in vivo* and *in vitro* derived extracts exhibited broad-spectrum antimicrobial activity against tested pathogens such as *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus species*. Comparatively, inner gel extracts showed higher antimicrobial efficacy than whole leaf extracts. Statistical analysis using ANOVA confirmed that differences observed at higher temperatures were significant ($p < 0.05$). The findings suggest that *Aloe vera* L. extracts retain considerable bioactivity even after moderate heat exposure. This study highlights the potential application of *Aloe vera* L. extracts as natural antimicrobial agents in food preservation and pharmaceutical formulations. Their notable thermal stability enhances their suitability for industrial processing, contributing to extended shelf life and improved safety of products.

KEYWORDS: *Aloe vera* L.**,** Heat treatment**,** Antimicrobial activity**,** Thermal stability and Pathogenic microorganisms.

INTRODUCTION

In present investigation, number of pathogens of human health significance including bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Bacillus cereus)*, fungi (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Penicillium digitatum)* and yeast (*Candida albicans*, *Candida utilis*) were tested against the ethyl acetate extracts of *Aloe vera* L. (both *in vivo* grown and *in vitro* regenerated) whole leaf and inner gel at different temperatures to test the stability of *Aloe vera* L. extracts.

MATERIALS ANS METHODS

Preparation of crude extract

Leaves of the *Aloe vera* L. were collected from the already *in vitro* propagated and properly acclimatized 9-12 months old plants. *In vitro* propagation was the previous phase of our study to produce quality plant material to meet industrial requirement. Simultaneously leaves from 9-12 months old *in vivo* grown *Aloe vera* L. plants were also collected. Freshly collected *Aloe vera* L. leaves were washed with distilled water, followed by disinfecting with ethanol 70%. Later, in case of whole leaf crude extract preparation, leaves were chopped into

the small pieces and were exposed to 50ºC for 3 days to get dried. After complete drying, leaf parts were powdered using electric grinder, simultaneously in case of only gel crude extract preparation, upper green skin/rind of leaves was removed and latex was cut into small pieces and both types of leaf materials were homogenized separately. The homogenized materials were extracted with ethanol (95%). The ethanol from the extracted leaf materials was evaporated at 65^{o}C temperature in water bath. The solvent was completely removed and dried to get powder. All the powdered plant materials including whole leaf and only gel were used for the preparation of aqueous and solvent extracts.

Aqueous extract

Extracts were prepared using the modified method of Case.[1] 1:3 (w/v) ratios were used for the powdered leaf material and distilled water for extract preparation. The pulverized leaf material was used to prepare an infusion in hot (95ºC) distilled water. The infusion was left overnight under refrigeration (4ºC) to prevent any possible contamination. After 24 h the extracts were kept in rotary shaker at 100 rpm for 1 h and filtered with Whatman No.1 filter paper and subsequently subjected to lyophilization at – 47.5ºC. The frozen extract was then freeze dried to a powder, weighed, transferred into separate vial and preserved at 4ºC for future analysis.

Solvent extracts

As in case of aqueous extract here also 1:3 (w/v) ratios were used for the powdered leaf material and different solvents for extract preparation. The pulverized leaves material was mixed with sufficient quantity of solvent that is ethyl acetate. It was kept in rotary shaker at 100 rpm overnight and filtered with Whatman No.1 filter paper and subsequently subjected to lyophilization at – 47.5ºC. The dried extract thus obtained was weighed, transferred into vials and preserved at 4ºC for future analysis.

The ethyl acetate extracts of *Aloe vera* L. (both *in vivo* grown and *in vitro* regenerated) whole leaf and inner gel at a concentration of 100mg/10ml were incubated in a water bath for 30 min at 25, 50, 75 and 100°C respectively. The incubated extracts were then cooled and stored at− 80°C until use. Untreated extracts were used as controls. Antimicrobial activity of treated and control samples were determined by disc diffusion method. Experiments were done in triplicates.

Statistical analysis

All the analysis were carried out in triplicates and expressed as mean ± SD. Analysis of variance (ANOVA) were performed using the one-way analysis of variance. Significant differences between means were determined by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant.

RESULTS

All the outcomes of the effects of temperature on antimicrobial activity of ethyl acetate extract of *in vivo* and *in vitro* regenerated *Aloe vera* L. whole leaf and only gel are represented in Table 1 – 4. In present investigation the inhibitory actions of heat treated extract was not significantly different from that of untreated extracts, when the extracts were incubated at 25^{o}C, 50^{o}C and 75^{o}C for 30 minutes. While, boiling the extract at 100^{o}C for 30 minutes significantly reduced but did not eradicate their antimicrobial activities. Heat treatment of *Aloe vera* L. at 100^{o}C for 30 minutes significantly reduces their antimicrobial activity, but these extracts still maintained some of their inhibitory actions. The results of current investigations indicates that the *Aloe vera* L. extracts have significant thermal stability, which is regarded as an important property for compound to be used in food preservation or can extend the expiry of the product.

The thermal stability of plant extracts are critical aspects of their use in food processing applications as natural preservatives to control microbial growth.

Table 1: Effect of heat treatment on stability of ethyl acetate extract of *in vitro* regenerated *Aloe vera* L. whole leaf against pathogenic organisms.

S.No.	Microorganisms (Pathogens)	Control	Temperature ^{o}C			
			25	50	75	100
1	*Escherichia coli*	21.49 ± 1.87*	20.57 ± 0.63	21.73 ± 0.42	21.39 ± 0.57	14.80 ± 0.28
2	*Pseudomonas aeruginosa*	NT	NT	NT	NT	NT
3	*Proteus vulgaris*	16.20 ± 0.06	16.73 ± 0.36	16.11 ± 0.97	16.47 ± 0.35	11.19 ± 0.50
4	*Salmonella typhi*	17.54 ± 1.46	16.24 ± 0.19	17.14 ± 0.25	16.06 ± 0.63	10.36 ± 0.92
5	*Staphylococcus aureus*	15.62 ± 0.08	15.12 ± 0.60	15.92 ± 0.53	15.50 ± 0.15	10.18 ± 0.33
6	*Enterococcus faecalis*	16.02 ± 0.86	15.50 ± 0.12	16.38 ± 0.41	16.26 ± 0.73	12.72 ± 0.26
7	*Klebsiella pneumonia*	14.87 ± 0.16	13.28 ± 0.87	14.73 ± 0.24	13.49 ± 0.32	08.26 ± 0.82
8	*Bacillus subtilis*	21.34 ± 1.49	20.23 ± 1.41	21.29 ± 0.47	21.34 ± 0.46	12.11 ± 0.69
9	*Bacillus cereus*	17.54 ± 0.90	17.02 ± 0.24	17.23 ± 1.86	16.82 ± 1.39	10.98 ± 0.47
10	*Aspergillus niger*	14.23 ± 0.52	14.86 ± 0.62	14.30 ± 0.38	14.27 ± 0.57	08.40 ± 0.14
11	*Aspergillus flavus*	17.04 ± 0.62	16.75 ± 0.12	17.50 ± 0.09	17.71 ± 0.28	11.24 ± 1.73
12	*Aspergillus fumigatus*	14.48 ± 1.19	13.60 ± 0.23	14.56 ± 1.83	14.35 ± 0.50	08.48 ± 1.26
13	*Penicillium digitatum*	16.33 ± 0.36	16.80 ± 1.67	16.22 ± 0.31	15.83 ± 0.14	09.27 ± 0.21
14	*Candida albicans*	19.24 ± 0.02	19.81 ± 0.33	19.43 ± 0.26	19.33 ± 0.46	12.78 ± 0.63
15	*Candida utilis*	17.68 ± 0.42	16.76 ± 0.48	17.57 ± 0.72	17.19 ± 0.34	10.24 ± 0.15

The values are given as Mean ± SD of triplicates
The concentration of extracts used was 100 µg/disc
* Inhibitory zone in mm, including diameter of disc (5.0 mm)

Table 2: Effect of heat treatment on stability of ethyl acetate extract of *in vitro* regenerated *Aloe vera* L. only gel against pathogenic organisms.

S.No.	Microorganisms (Pathogens)	Control	Temperature ^{o}C			
			25	50	75	100
1	*Escherichia coli*	25.40 ± 1.54*	25.32 ± 0.28	25.58 ± 0.74	25.49 ± 1.26	17.26 ± 0.31
2	*Pseudomonas aeruginosa*	NT	NT	NT	NT	NT
3	*Proteus vulgaris*	19.36 ± 0.93	19.25 ± 0.62	19.50 ± 0.14	19.61 ± 1.39	13.67 ± 0.73
4	*Salmonella typhi*	21.43 ± 0.82	20.29 ± 0.57	21.45 ± 0.36	21.42 ± 0.40	15.10 ± 1.28
5	*Staphylococcus aureus*	19.33 ± 0.36	19.25 ± 1.84	19.36 ± 0.67	19.43 ± 0.16	12.47 ± 0.26
6	*Enterococcus faecalis*	20.25 ± 0.83	20.62 ± 0.33	20.21 ± 0.15	19.35 ± 1.42	13.60 ± 0.18
7	*Klebsiella pneumonia*	18.72 ± 1.20	18.38 ± 0.12	18.57 ± 0.12	18.24 ± 0.36	11.80 ± 0.92
8	*Bacillus subtilis*	25.32 ± 0.52	24.10 ± 1.24	25.52 ± 0.73	25.38 ± 0.55	14.32 ± 0.20
9	*Bacillus cereus*	22.25 ± 0.63	22.57 ± 0.86	22.83 ± 0.59	21.45 ± 1.17	12.49 ± 0.34
10	*Aspergillus niger*	19.57 ± 1.57	19.71 ± 0.20	19.42 ± 0.28	19.58 ± 0.32	11.21 ± 0.68
11	*Aspergillus flavus*	23.48 ± 0.83	22.29 ± 0.24	23.56 ± 1.36	23.07 ± 0.29	13.50 ± 0.76
12	*Aspergillus fumigatus*	18.33 ± 0.02	18.48 ± 0.57	18.35 ± 0.60	18.40 ± 0.52	10.39 ± 0.67
13	*Penicillium digitatum*	22.20 ± 0.12	22.55 ± 0.83	22.47 ± 0.39	21.83 ± 0.27	13.18 ± 0.52
14	*Candida albicans*	25.65 ± 0.18	25.43 ± 0.61	25.56 ± 0.38	25.36 ± 0.67	13.24 ± 1.91
15	*Candida utilis*	24.43 ± 0.10	23.20 ± 0.35	24.34 ± 0.42	24.67 ± 0.58	12.58 ± 0.13

The values are given as Mean ± SD of triplicates
The concentration of extracts used was 100 µg/disc
* Inhibitory zone in mm, including diameter of disc (5.0 mm)

Table 3: Effect of heat treatment on stability of ethyl acetate extract of *in vivo* grown *Aloe vera* L. whole leaf against pathogenic organisms.

S.No.	Microorganisms (Pathogens)	Control	Temperature ^{o}C			
			25	50	75	100
1	*Escherichia coli*	18.15 ± 0.05*	17.59 ± 0.37	18.06 ± 1.24	17.40 ± 0.87	10.27 ± 0.23
2	*Pseudomonas aeruginosa*	NT	NT	NT	NT	NT
3	*Proteus vulgaris*	13.24 ± 1.35	13.37 ± 0.82	13.28 ± 0.37	13.20 ± 0.18	08.16 ± 1.92
4	*Salmonella typhi*	15.62 ± 0.30	15.50 ± 1.46	15.33 ± 0.12	15.08 ± 0.14	09.29 ± 0.08
5	*Staphylococcus aureus*	12.31 ± 0.52	12.83 ± 0.17	12.43 ± 0.82	12.36 ± 0.79	08.43 ± 0.24
6	*Enterococcus faecalis*	12.57 ± 0.60	12.09 ± 0.68	12.27 ± 0.26	11.21 ± 0.33	08.61 ± 0.39
7	*Klebsiella pneumonia*	10.39 ± 0.56	10.74 ± 0.48	10.24 ± 0.72	10.56 ± 0.38	07.39 ± 0.57
8	*Bacillus subtilis*	18.62 ± 0.36	18.50 ± 0.63	18.20 ± 0.16	17.72 ± 0.42	11.35 ± 1.19
9	*Bacillus cereus*	14.54 ± 1.06	14.92 ± 0.45	14.18 ± 0.37	13.86 ± 0.72	08.28 ± 0.33
10	*Aspergillus niger*	11.86 ± 0.08	11.39 ± 0.94	11.37 ± 0.50	11.25 ± 0.46	07.76 ± 0.17
11	*Aspergillus flavus*	14.28 ± 0.20	13.26 ± 0.58	14.34 ± 1.02	14.19 ± 0.27	09.45 ± 0.25
12	*Aspergillus fumigatus*	12.50 ± 0.05	11.33 ± 1.87	12.83 ± 0.62	12.91 ± 0.18	07.51 ± 0.36
13	*Penicillium digitatum*	13.53 ± 0.84	13.73 ± 0.18	13.05 ± 0.49	12.43 ± 0.29	07.26 ± 1.24
14	*Candida albicans*	17.08 ± 0.47	17.12 ± 0.32	17.33 ± 0.57	17.58 ± 0.74	10.38 ± 1.39
15	*Candida utilis*	15.54 ± 0.05	14.37 ± 0.83	15.36 ± 0.40	15.84 ± 0.35	08.40 ± 0.46

The values are given as Mean ± SD of triplicates
The concentration of extracts used was 100 µg/disc
* Inhibitory zone in mm, including diameter of disc (5.0 mm)

Table 4: Effect of heat treatment on stability of ethyl acetate extract of *in vivo* grown *Aloe vera* L. only gel against pathogenic organisms.

S.No.	Microorganisms (Pathogens)	Control	Temperature ^{o}C			
			25	50	75	100
1	*Escherichia coli*	20.65 ± 0.28*	20.28 ± 0.72	20.16 ± 0.48	20.37 ± 0.50	12.08 ± 0.32
2	*Pseudomonas aeruginosa*	NT	NT	NT	NT	NT
3	*Proteus vulgaris*	14.36 ± 0.33	14.65 ± 0.40	14.54 ± 1.02	14.32 ± 0.36	09.39 ± 0.28
4	*Salmonella typhi*	16.55 ± 0.82	16.92 ± 0.57	16.62 ± 1.24	16.04 ± 0.21	10.26 ± 0.87
5	*Staphylococcus aureus*	14.62 ± 0.12	14.19 ± 0.25	14.09 ± 0.36	14.37 ± 1.83	09.52 ± 0.33
6	*Enterococcus faecalis*	13.43 ± 0.39	12.39 ± 0.42	13.86 ± 0.23	13.10 ± 0.09	08.92 ± 0.54
7	*Klebsiella pneumonia*	12.57 ± 0.52	12.75 ± 0.36	12.50 ± 0.82	12.17 ± 0.30	08.02 ± 0.46
8	*Bacillus subtilis*	20.33 ± 0.72	20.12 ± 0.65	20.47 ± 0.58	19.36 ± 1.18	11.24 ± 0.21
9	*Bacillus cereus*	15.85 ± 0.22	15.83 ± 1.26	15.38 ± 1.37	14.39 ± 0.40	09.35 ± 0.58
10	*Aspergillus niger*	12.26 ± 0.36	12.36 ± 0.14	12.70 ± 0.62	12.94 ± 0.34	07.88 ± 1.86
11	*Aspergillus flavus*	16.31 ± 0.70	15.48 ± 0.52	14.81 ± 1.24	14.28 ± 0.37	10.36 ± 0.18
12	*Aspergillus fumigatus*	13.57 ± 0.21	12.96 ± 0.71	13.26 ± 0.30	13.30 ± 0.12	07.43 ± 0.54
13	*Penicillium digitatum*	15.39 ± 0.15	15.28 ± 0.33	15.72 ± 0.56	14.25 ± 0.26	08.87 ± 0.60
14	*Candida albicans*	18.04 ± 0.42	18.35 ± 1.12	18.37 ± 0.04	18.72 ± 0.87	11.28 ± 0.39
15	*Candida utilis*	16.48 ± 0.62	16.18 ± 0.26	16.90 ± 0.67	16.34 ± 0.75	09.12 ± 0.24

The values are given as Mean ± SD of triplicates
The concentration of extracts used was 100 µg/disc
* Inhibitory zone in mm, including diameter of disc (5.0 mm)

DISCUSSION

The thermal stability of plant extracts is the critical aspects of their use in food processing applications as an important property for compound to be used in food preservation or can extend the expiry of the product. Consuming healthy, nutritious and contamination free food are the important necessary aspects in terms of human health.

So that in the current study the stability of the *Aloe vera* L. extracts under temperature conditions were assessed. Ethyl acetate extracts of *in vitro* and *in vivo* grown *Aloe vera* L. were assessed for its thermal stability. It was found that ethyl acetate extracts were showed the significant temperature stability.

Similar results were found by many other workers who tested the effects of temperature on different spices and herbs. Srinivasan *et al*. were examined the antibacterial activity and stability of garlic extract at different temperatures and found the similar results.[2]

Several other workers such as Bakhshayeshi *et al*., Mahmoud *et al*., Ruenroengklin *et al*., Jenshi roobha *et al*., Devi *et al*., Dehghan *et al*., Abu-Gharbia *et al*., Anees *et al*., Rakkimuthu *et al*., were also assessed the temperature stability of various herbal extracts for their antimicrobial activities against several pathogens of human health significance and found the similar results which strongly supports our current study.[3,4,5,6,7,8,9,10,11]

CONCLUSION

In current study the stability of ethyl acetate extract of the *Aloe vera* L. under various temperature conditions were assessed. It was found that ethyl acetate extracts were showed the significant stability against different temperatures or heat treatments. Therefore, in view of these results, the ability of the extracts to inhibit the growth of several bacterial and fungal species is an indication of the broad-spectrum antimicrobial potential of *Aloe vera* L. which makes the plant a candidate for bio-prospecting for antimicrobial drugs.

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