COMPARATIVE ANALYSIS OF PHYTOCHEMICAL POTENTIAL FROM THE *IN VITRO* EXPLANT REGENERATION PLANT *ALOE BARBADENSIS*

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ABSTRACT

Aloe vera is a succulent plant species of the genus Aloe. It is an ancient medicinal plant containing many medicinal properties for many diseases. It is an evergreen perennial, it originates from the Arabian Peninsula but grows wild in tropical climates round the arena and is cultivated for agricultural and medicinal uses. The species is also used for ornamental purposes and grows effectively indoors as a potted plant. There is a lack of production of Aloe leaf to meet the industry demand and so it is necessary to undertake large scale cultivation of Aloe. Large scale propagation can be standardized through Tissue culture where the yield is more and highly free from diseases, pests. In this study Aloe barbadensis were screened for important biochemical properties. As flavanoids are recognized to have potent biological activities their screening was performed along with various other test like chlorophyll test and carbohydrate test.

Keywords: Antioxidant, Ex vivo, In vitro, phytochemical, qualitative

I. INTRODUCTION

From earlier days plant extracts have been used as an effective tool for treating diseases. In India these phytochemicals lead a major role in industries like Ayurveda [1]. Nowadays the scope and productivity of phytochemicals have been relatively scaled down due to the advent of synthetic medicine industry. However the growing economy of these industries are on a hype,their very recent challenge holds up on control of drug resistant

bacterial species inhibition and providing efficient biosafety of the drug without any side effects [2]. This limitation of synthetic drugs leads to efficient drug. Aloe*vera* is a stemless or very short-stemmed plant growing to 60–100 cm tall, spreading by offsets. It is a unique plant which is a rich source of many chemical compounds and plays an important role in the international market [3]. Its leaves contain phytochemicals under study for possible bioactivity, such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones, and other anthraquinones, such as emodin and various lectins [4].

II. MATERIALS AND METHODS

i. Collection of samples

The healthy mature explants were collected from Genewin Biotech and maintained in green house.

ii. Preparation of the sample

The collected explants were brought to production of laboratory and washed thoroughly in running water for 10 minutes for the elimination of mud particles from the sample.

Fig1:Aloevera explants

iii. Sterilization of the sample

The leaves of Aloevera were carefully wiped with 70% ethanol with sterilized cotton for the removal of bacteria or other dust particles.

Fig 2: Explant sterilization

The new bud explants were soaked in antifungal, antibacterial, carbendazim and streptocycline(0.1%) for 15 minutes for the effective removal of all the microbes present in it. After this the explants were treated with a detergent, tween 20 for 20 minutes. For the complete removal of foam it was washed with sterile water and then soaked in 10% Sodium hypochlorite (40min), 10% NAOCl (40 min). The sterilization is further carried out inside laminar air flow chamber using surface sterilant and 2 sets ofexperiments were performed they are:

1. Mercuric chloride: leaves removed from this solution and wash with sterile water to eliminate toxic effects

2. Sodium hypochlorite: Bud break was recorded in each trial in 3 to 4 weeks.

iv. EXPLANTS INITIATION

It give rise to regeneration of new shoots from the selected explants in 15 days. The growth was monitored every week and inoculated in MS basal media treatment with 3% sucrose supplemented with various growth regulator concentration.

v. EXPLANTS MULTIPLICATION

The shoots arised from the explants was further transferred to multiplication stage for rapid propagation. Shoots were trimmed and inoculated in media consisting of basal MS media +3% sucrose. Four sub culturing was done every 3 to 4 weeks to increase multiplication ratio.

vi. PHYTOCHEMICAL AND QUANTITATIVE ANALYSIS

Qualitative and quantitative analysis of phytochemical test was done in invitro (For comparing the antioxidant such as: Alkaloids (wagner's test), Saponins and Carbohydrates (Molisch's test, Fehling's test, Benedict's test). The quantitative analysis include the determination of Carbohydrate content, protein content and Total chlorophyll content.

III. RESULTS

Comparing the exvivo and invitro studies it was found that the invitro explants possess elevative disease resistance at higher concentrations of antioxidants.

SURFACE STERLIZATION:

Majority of the explants after the treatment when exposed to sodium hypochlorite at 4% and the success rate was found as 63%. Other concentrations of Sodium hypochlorite (10% and 20%) showed success rate of bud break of about 42% and 21% and less percentage of explants also were non responsive.

i. SODIUM HYPOCHLORITE

Table 1:Treatment using sodium hypochlorate

TREATMENT OF SODIUM HYPOCHLORITE (%)	NO OF EXPLANTS TAKEN	MORTALITY RATE (%)	NO OF EXPLANTS NOT RESPONDED	BUD BREAK (%)
4	20	34±3.2	3	63±5.4
10		41±2.7	17	42±2.8
20		59±1.4	20	21±1.5

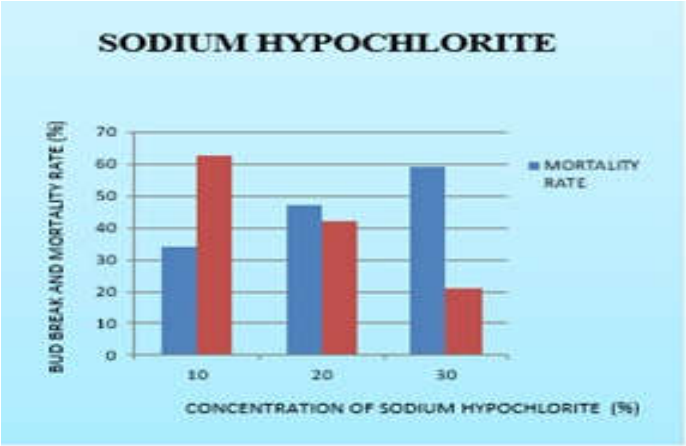

Fig3: Graph showing bud rate and mortality rate

ii. MERCURIC CHLORIDE

Table 2:Treatment using mercuric chloride

Treatment Of Mercuric Chloride (%)	NO Of Explants Taken	Mortality Rate (%)	No Of Explants Not Responded (Nos)	Bud Break (%)
0.1% $Hgcl_2$ – 3 Min	20	86±2.3	12	2±1.2
0.1% $Hgcl_2$ – 5 Min		20±3.5	66	14±0.3
0.1% $Hgcl_2$ – 7 Min		51±3.3	48	1±3.2
0.1% $Hgcl_2$ – 9 Min		47±4.1	53	0

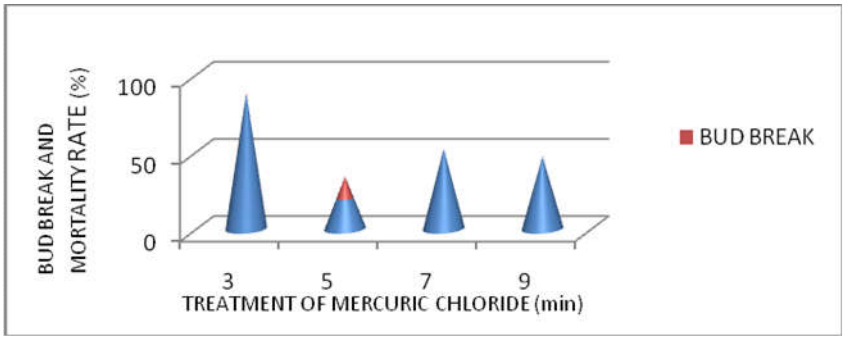

Fig 4: Graph showing bud rate and mortality rate

Majority of the explants survived after treatment when they were exposed to Sodium hypochlorite at 4% and the success rate was found as 63%. Other concentrations of Sodium hypochlorite (10% and 20%) showed success rate of bud break of about 42% and 21% and less percentage of explants also were non responsive.

iii. Initiation

Initiation of nodal response was found as 84% which trailed with the combination of 6BAP– 2 mg/l with mean height of 2.1 cm. other media trials with 6BAP of about 0.5,1,2 mg/l were found with lesser shoot regeneration and less shoot height and further transferred to multiplication stage.

Table 3: Initiation growth phase

TRIAL MEDIA 6BAP (mg/l)	NO.OF EXPLANTS TAKEN	NO.OF EXPLANTS RAISED SHOOTS	MEAN HEIGHT OF SHOOTS (cm)	MEAN % INITIATION RESPONSE	EFFECT OF BROWNING
0.5 + 0.1	20	9.3	0.8	27	+
1 + 0.1		10	0.92	31	+
1.5 + 0.1		5.1	1.03	37	-
2 + 0.1		16	2.10	84	++
2.5 + 0.1		4.2	0.51	51	++

+ = Slight Browning; - = No browning; ++ = Moderate Browning

iv. Multiplication

The inoculated explants were subjected to multiplication media and the *in vitro* propogated explants were experimented for various analysis.

Table 4: Multiplication of the explant

TRIAL MEDIA	MEAN SHOOT LENGTH (cm)	MEAN NUMBER OF SHOOTS (cm)
AVMM 1	0.51	1
AVMM 2	0.87	1
AVMM 3	1.13	1
AVMM 4	1.22	1
AVMM 5	1.28	2
AVMM 6	1.41	2
AVMM 7	1.58	2
AVMM 8	2.17	3

Fig.5: In vitropropogatedexplants of *Aloe barbedensis*

Phytochemical analysis was carried out using the selected plant parts such as leaves and gel using the water, acetone and ethanol solvents and the results were tabulated as below.

v. PHYTOCHEMICAL ANALYSIS

Phytochemical analysis was carried out using the selected parts such as leaves and gel by using the water, acetone and ethanol and the results were: Alkaloids were present in the ethanol and acetone extracts followed by flavonoids present in the ethanol and acetone extracts except water extracts. Negative results were recorded for the presence of carbohydrates, Tannins and saponins were observed to be nil in all the samples. Fats and oils were found only in water extract

Fig. 6: Phytochemical analysis

QUANTITATIVE ANALYSIS:

The quantitative analysis of invitro and exvivo was done for comparing the antioxidants such as carbohydrates, chlorophyll and Proteins.

vi. TEST FOR CARBOHYDRATES

Table 5: Ex vivo and In vitro analysis of carbohydrates

S.No	Samples	Ethanol Extract (mg/ml)
1.	Aloe leaf	38
2.	Aloe gel	53

S.No	Samples	Ethanol Extract (mg/ml)
1.	Aloe leaf	49.6
2.	Aloe gel	57

vii. TEST FOR TOTAL CHLOROPHYLL CONTENT

Table 6: Exvivo activity of chlorophyll

S.No	Samples	Leaf extract(%)
1.	Aloe(Ca)	1.07
2.	Aloe(Cb)	3.47
3.	Aloe(Cc)	4.53

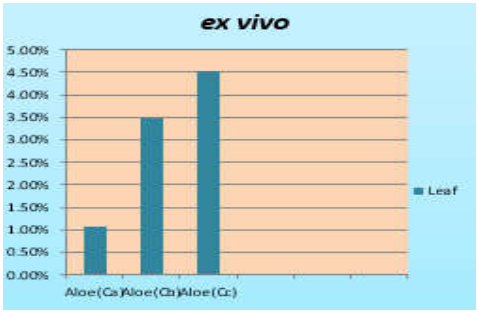

Fig7: Graph showing chlorophyll activity

Table 7: In vitro activity of chlorophyll

S.No	Samples	Leaf extract(%)
1.	Aloe(Ca)	9.62%
2.	Aloe(Cb)	6.22%
3.	Aloe(Cc)	8.11%

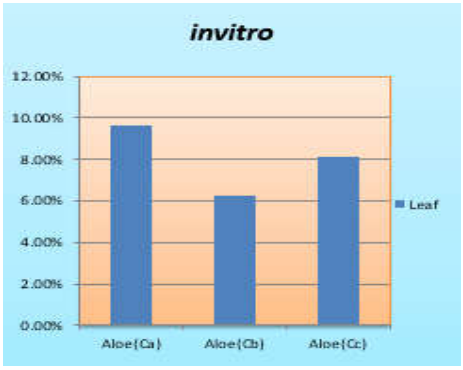

Fig8: Graph showing chlorophyll activity

viii. TEST FOR PROTEINS

Table 8: Table showing ex vivo activity of protein test

Ex vivo

S.No	Samples	Acetone extract (mg/ml)	Ethanol extract (mg/ml)	Water extract (mg/ml)
1.	Aloe leaf	6.4	6	5.4
2.	Aloe gel	6.5	6.6	6.3

Table 9: Table showing In vitro activity of protein test

In vitro

S.No	Samples	Acetone extract (mg/ml)	Ethanol extract (mg/ml)	Water extract (mg/ml)
1.	Aloe leaf	6.8	6	6.1
2.	Aloe gel	7.1	6.51	6.9

DISCUSSION

There is considerable use of *A. barbadensis*in folk medicine in the southern most United States, and some cosmetics and patent medicines generally found on the market are prepared from the gel in the leaves and from the juice. This demand for *Aloe vera* interferes with the fastest mode of propagation along with the large quantity. In this study, *Aloe* explants were selected and propagated through *in vitro* propagation. Majority of the explants survived after treatment when they were exposed to Sodium hypochlorite at 4% and the success rate was found as 63%. Other concentrations of Sodium hypochlorite (10% and 20%) showed success rate of bud break. Initiation response was found as 84% which trailed with the combination of 6BAP– 2 mg/l with mean height of 2.1 cm.

CONCLUSION

The in-vitro and ex vivo anti-oxidant properties of *Aloe barbadensis* were studied successfully. When the Quantitative analysis was studied, carbohydrates were measured higher in *in vitro* with 57 mg/ml and of *aloe* respectively. The chlorophyll content that was found to be higher in *in vitro aloe leaf* with 9.62% in Chlorophyll a, b and c than the *ex vivo* extracts. The total protein content that was found to be higher in aqueous extract of *aloe gel* in *in vitro* 6.9 mg/ml respectively.

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