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PRELIMINARY PHYTOCHEMICAL STUDIES ON *ALOE VERA* L. (*IN VIVO* & *IN VITRO* REGENERATED) WHOLE LEAF AND ONLY GEL EXTRACTS

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ABSTRACT

Present investigation was done to detect the preliminary phytochemical analysis of whole leaf and only gel from *in vivo* and *in vitro* regenerated *Aloe vera* L. after extraction with several solvents of different polarities (such as aqueous, methanol, ethyl acetate, chloroform and *n*-hexane) along with the detection of percent yield of extracted material. Among all the samples extracted including whole leaf and only gel samples from *in vivo* grown *Aloe vera* L. along with whole leaf and inner gel samples from *in vitro* regenerated *Aloe vera* L. the aqueous extracts of all the samples were showing the highest percent yield in comparison to other extracts. The phytochemical screening of yielded extracts showed the presence of flavonoids, alkaloids, saponins, carbohydrates, steroids, phenols, tannins, amino acids, proteins, anthraquinones and glycosides.

KEYWORDS: *Aloe vera* L., *in vitro*, *in vivo*, whole leaf, only gel, phytochemicals.

INTRODUCTION

Today, the *Aloe* industry has established high ethical standards for businesses and their products. There is a voluminous amount of anecdotal evidence showing that authentic, properly prepared *Aloe vera* has powerful healing properties in humans and animals. The virtues of *Aloe vera* have been recorded for thousands of years by many ancient civilizations, including Egypt, Persia, Greece, India and Africa.

The genus *Aloe* has about 400 species but only a few are medicinally important.[1] Among these, *Aloe vera* is the plant of greatest interest. Its leaves have been found to contain over 200 compounds, in which about 75 are biologically active constituents.[2,3]

The thick leaves of *Aloe vera* contain the water supply for the plant to survive long periods of drought.[4] Its leaves have a high capacity of retaining water also in very warm dry climates and therefore this plant can survive very harsh circumstances where most other vegetation disappears. An orange- yellow sap drip from the open end, after cutting the leaf, when the green skin of a leaf is removed a clear mucilaginous substance appears that contains fibres, water and the ingredient to retain the water in the leaf. This is called the gel. *Aloe vera* gel consists of 99.3% water. The remaining 0.7% is made up of solids with glucose and mannose constituting for a large part. These sugars together with the enzymes and amino acids in the gel give the special properties as a skin care product.[5] *Aloe vera* has medicinal and cosmetic properties.[6]

Aloe vera's mucopolysaccharides are long-chain sugars found in large amounts in the plant and properly prepared whole-leaf *Aloe* juice and juice concentrates. Researchers have just begun in the last few years to recognize the major role that mucopolysaccharides (MPS) play in human and animal health. *Aloe vera* plant, coupled with its direct anti-viral activity, explains why whole leaf *Aloe*, in addition to wound care, shows promise in a wide range of human and animal diseases including AIDS, cancer and ulcerative colitis. Other aspects of MPS are that they are found in every single cell in the body. MPS are as vital to a healthy body as bricks are to a brick house. The human body stops manufacturing MPS around puberty. After this, one must begin to receive the MPS from outside sources. One of the very best sources comes from whole leaf *Aloe vera.*[7]

Aloe vera Gel, like most natural juices, both fruit and vegetable, is an unstable product when extracted and is subject to discoloration and spoilage from contamination by microorganisms. The great success of *Aloe* as a commodity for use in nutritional foods and cosmetics is due to the proper stabilizing procedures that enable processors to store and ship the *Aloe* Gel without fear of spoilage throughout the market places of the world. Research conducted around the world leaves little doubt that certain biochemical properties of *Aloe* will be proven facts. Such attributes as moisturizing and penetrating properties are known, but the attributes such as its healing abilities and analgesic action to bacterial activity has not been clearly defined and documented through properly controlled scientific research and testing. The raw gel of

Aloe vera contains water soluble and fat-soluble vitamins, minerals, enzymes and polysaccharides. Phenolic compounds and organic acids.[8] It has been hypothesized that this heterogeneous composition of *Aloe vera* leaf gel may contribute to the diverse pharmacological and therapeutic activities which have been observed for *Aloe* gel.[9]

MATERIAL AND 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. plant 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.[10] 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 solvents viz., *n*-hexane, ethyl acetate, methanol and chloroform. 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 extracts thus obtained was weighed, transferred into separate vials and preserved at 4ºC for future analysis.

Preliminary phytochemical screening

The preliminary phytochemical screening of the sample was carried out as described by Nweze *et al*. and Senthilkumar and Reetha.[11,12] The samples were screened for flavonoids, alkaloids, saponins, carbohydrates, phytosterols and steroids, phenols, tannins, amino acids and proteins, anthraquinones and glycosides.

Test for flavonoids

a) To 2 ml of plant extract 1 ml of 1N aqueous NaOH solution was added and observed for the formation of yellow-orange colouration.

b) 2 ml of plant extract was treated with 4 drops of concentrated sulphuric acid and observed for the formation of orange colour.

Test for alkaloids

To 2 ml of plant extract, 2 ml of concentrated hydrochloric acid was added. Then 3 drops of Mayer's reagent were added. Presence of green colour or white precipitate indicates the presence of alkaloids.

Test for saponins

To 1 ml of plant extract, 5-10 ml of distilled water was added and shaken in a graduated cylinder for 15 min lengthwise. Formation of 1 cm layer of foam indicates the presence of saponin.

Test for carbohydrates

To 2 ml of plant extract, 1 ml of Molisch reagent and 4 drops of concentrated sulphuric acid were added. Formation of purple or reddish ring indicates the presence of carbohydrates.

Test for phytosterols and steroids

To 1 ml of plant extract, equal volume of chloroform and 3 drops of concentrated sulphuric acid were added. Formation of bluish green colour indicates the presence of phytosterols and formation of brown ring indicates the presence of steroids.

Test for phenols

To 1 ml of the extract, 2 ml of distilled water followed by 5 drops of 10% ferric chloride was added. Formation of blue or green colour indicates presence of phenols.

Test for tannins

To 1 ml of plant extract, 2 ml of 5% ferric chloride was added. Formation of dark blue or greenish black indicates the presence of tannins.

Test for amino acids and proteins

To 2 ml of plant extract, 4 drops of 0.2% Ninhydrin was added and heated to 100ºC. Formation of blue colour indicates the presence of proteins.

Test for anthraquinones

To 2 ml of plant extract is shaken with 10 ml of benzene and filtered. 5ml of 10% ammonia is added to the filtrate. The mixture is shaken and the presence of pink, red or violet colour indicates the presence of anthraquinones.

Test for glycosides

To 2 ml of plant extract, 1 ml of glacial acetic acid and 5% ferric chloride was added. To these 3 drops of concentrated sulphuric acid was added. Presence of greenish blue colour indicates the presence of glycosides.

RESULTS AND DISCUSSION

Percent yield of different solvent extracts

Gel extracts of *in vitro* regenerated *Aloe vera* L.

The percent yield of only gel extracts of *in vitro* regenerated *Aloe vera* L. with solvents of different polarity are 3.44, 3.46, 3.62, 3.62 and 3.64% for *n*-hexane, chloroform, ethyl acetate, methanol and aqueous respectively shown in Table 1.

Leaf extracts of *in vitro* regenerated *Aloe vera* L.

The percent yield of whole leaf extracts of *in vitro* regenerated *Aloe vera* L. with solvents of different polarity are 3.46, 3.56, 3.70, 3.74 and 3.80% for *n*-hexane, chloroform, ethyl acetate, methanol and aqueous respectively shown in Table 2.

Gel extracts of *in vivo* grown *Aloe vera* L.

The percent yield of only gel extracts of *in vivo* grown *Aloe vera* L. with solvents of different polarity are 3.42, 3.42, 3.56, 3.58 and 3.60% for *n*-hexane, chloroform, ethyl acetate, methanol and aqueous respectively shown in Table 3.

Leaf extracts from *in vivo* grown *Aloe vera* L.

The percent yield of whole leaf extracts of *in vivo* grown *Aloe vera* L. with solvents of different polarity are 3.42, 3.46, 3.58, 3.60 and 3.66% for *n*-hexane, chloroform, ethyl acetate, methanol and aqueous respectively shown in Table 4.

Preliminary phytochemical screening

The preliminary phytochemical screening of *in vitro* and *in vivo* grown *Aloe vera* L. whole leaf and inner gel extracts were showing the strong presence of various phytochemical substances such as flavonoids, alkaloids, saponins, carbohydrates, phytosterols & steroids, phenols, tannins, amino acids & proteins, anthraquinones and glycosides (Table 5).

Strong presence of flavonoid was there in methanol extracts of both whole leaf and only gel of all *in vitro* and *in vivo* grown *Aloe vera* L. whereas in ethyl acetate and chloroform extract it was in moderate amount and traces were found in aqueous and *n*-hexane extracts (Table 5). Alkaloid, saponins, carbohydrates, phytosterols & steroids, phenols were present in different amounts in different extracts of leaf and gel of *in vitro* and *in vivo* grown *Aloe vera* L. from strong to moderate, whereas in some extracts they were in trace or absent (Table 5).

Tannins and glycosides were found in moderate to trace amounts and in some extracts they were completely absent as well. Amino acids, proteins and anthraquinones were detected from moderate to trace amounts in different extracts (Table 5).

Grindly and Reynold[13] reported on a plant from west Bengal named as *Aloe barbadensis* showed quit different constituents. The principal component of the gel was a peptic substances containing mainly galacturonic acid and was accompanied by lesser amounts of a galactan, arabinan and non-acetylated glucomannon. *Aloe vera* plant from south India into four partially acetylated glucomannose, the whole having an average glucose/mannose ratio of 1:6, although the individual ratio varied from 1.5:1 to 1:19. The molecules were linear with 1-4 linkages between the sugar units. Trace of galacturonic acid, galactose, xylose and arabinose were also found.

Several workers found *Aloe vera* as an important antimicrobial and antioxidant components containing plant. Leaves of *Aloe vera* are the rich source of different useful phytochemicals. Phytoconstituents such as alkaloids, flavonoids, tannins, phenols, saponins and several other aromatic compounds in the plants serve as a defence mechanism against infection by many microorganisms.[14] While mechanism of action of these biochemical compounds is different. At the same time many important phytochemicals also found to work against the free radicals generated by oxidative stress thus protecting the system through their antioxidant properties. Several epidemiological studies suggest that the plant rich in antioxidants plays a protective role in health and against diseases and their consumption lowered risk of cancer, heart disease hypertension and stroke. The therapeutic effects of medicinal plants are due to the presence of different phytochemical constituents such as alkaloids, flavonoids, glycosides, phenols, saponins, sterols, etc.[15]

Table 1: Percent yield of gel extracts of *in vitro* regenerated *Aloe vera* L. with different solvents.

S.No.	Solvents	Weight of gel (g)	Weight of extract (g)	% yield of extract
1	*n*-Hexane	500	17.20	3.44
2	Chloroform	500	17.30	3.46
3	Ethyl acetate	500	18.10	3.62
4	Methanol	500	18.10	3.62
5	Aqueous	500	18.20	3.64

Table 2: Percent yield of whole leaf extracts of *in vitro* regenerated *Aloe vera* L. with different solvents.

S.No.	Solvents	Weight of gel (g)	Weight of extract (g)	% yield of extract
1	*n*-Hexane	500	17.30	3.46
2	Chloroform	500	17.80	3.56
3	Ethyl acetate	500	18.50	3.70
4	Methanol	500	18.70	3.74
5	Aqueous	500	19.00	3.80

Table 3: Percent yield of gel extracts of *in vivo* grown *Aloe vera* L. with different solvents.

S.No.	Solvents	Weight of gel (g)	Weight of extract (g)	% yield of extract
1	*n*-Hexane	500	17.10	3.42
2	Chloroform	500	17.10	3.42
3	Ethyl acetate	500	17.80	3.56
4	Methanol	500	17.90	3.58
5	Aqueous	500	18.00	3.60

Table 4: Percent yield of whole leaf extracts of *in vivo* grown *Aloe vera* L. with different solvents.

S.No.	Solvents	Weight of gel (g)	Weight of extract (g)	% yield of extract
1	*n*-Hexane	500	17.10	3.42
2	Chloroform	500	17.30	3.46
3	Ethyl acetate	500	17.90	3.58
4	Methanol	500	18.00	3.60
5	Aqueous	500	18.30	3.66

Table 5: Preliminary phytochemical screening of *in vitro* and *in vivo* regenerated *Aloe vera* L. (leaf and gel).

S.No.	Secondary Metabolites	*n*-Hexane				Chloroform				Ethyl acetate				Methanol				Aqueous			
		In vitro		*In vivo*		*In vitro*		*In vivo*		*In vitro*		*In vivo*		*In vitro*		*In vivo*		*In vitro*		*In vivo*	
		L	G	L	G	L	G	L	G	L	G	L	G	L	G	L	G	L	G	L	G
1	Flavonoid	+	+	+	+	++	++	++	++	++	++	++	++	+++	+++	+++	+++	+	+	+	+
2	Alkaloid	+	++	−	+	++	++	++	++	++	++	++	++	++	++	++	++	−	+	−	+
3	Saponines	+	+	+	+	+++	++	+++	++	++	++	++	++	+++	++	+++	++	++	+	++	+
4	Carbohydrates	−	++	−	++	++	++	++	++	+	++	+	++	++	+++	++	+++	+	++	+	++
5	Phytosterols & Steroids	−	+	−	+	+	++	+	++	++	++	++	++	++	+++	++	+++	+	+	+	+
6	Phenols	+	+	+	+	+	++	+	++	+	++	+	++	++	+++	++	+++	+	+	+	+
7	Tannins	−	+	−	+	+	++	+	++	+	++	+	++	+	++	+	++	−	+	−	+
8	Amino Acids & Proteins	+	+	+	+	++	++	++	++	+	++	+	++	++	++	++	++	+	+	+	+
9	Anthraquinones	+	+	+	+	++	++	++	++	+	++	+	++	++	++	++	++	+	+	+	+
10	Glycosides	−	+	−	+	+	++	+	++	+	++	+	++	+	++	++	+	−	+	−	+

L - Leaf extracts G - Gel extracts

+++ - Strongly positive ++ - Positive

+ - Trace − - Not detected

CONCLUSION

According to present study whole leaf and only gel extracts from *in vitro* and *in vivo* grown *Aloe vera* L. are showing good source of important phytochemical constituents of medicinal values including antioxidants and antimicrobials.

As in the present investigation we studied on the percent yield and preliminary phytochemicals present in *in vitro* and *in vivo* grown *Aloe vera* L. whole leaf and only gel, there is a need to study the effect of different climatic conditions, soil variations and seasons throughout the year on the composition of phytochemicals and medicinal properties present in *Aloe vera* L. whole leaf and inner gel.

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