

A Scientometric Assessment of Proximate Principles, Catalase Activity and Phytochemistry of *Aloe barbadensis* Miller.

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Abstract

Background: *Aloe vera*, a succulent perennial plant, belonging to *Aloaceae* family. The presence of bioactive compounds owns to its therapeutic properties and medicinal potential. *Aloevera* is effectual treatment for rejuvenation and recovery of body as it has anti-inflammatory, anti-bacterial, anti-tumor, anti-oxidant properties. Catalase, an enzyme present in *aloevera* protects the cell from oxidative damage by reactive oxygen species. Thus, it helps in maintaining the liver health, as a result optimum conditions to determine maximum catalase activity were studied. The total flavonoid and phenolic content were found to be 56 µg/mg & 41 µg/mg respectively. As *aloe vera* is a good source of vital bioactive and phytochemical compounds, it can be a potentially significant solution benefits for physical well-being by inclusion of *aloevera* into daily life.

Methods and Materials: *Aloe vera* leaves were collected from Bombay in October 2019. The traditional *Aloe vera* leaf processing method of Hand filleting was used. The juice thus obtained was filtered through muslin cloth and resulting filtrate was used in all the tests. The chemicals used in the duration of project were of analytical grade.

Result: The study measured high concentrations of carbohydrate, polysaccharide content and of protein content. Catalase was found to work optimally at physiological conditions. Methanol and water extract of *aloevera* were more efficient in comparison to chloroform extract to qualitatively determine the presence of phytochemicals. The total flavonoid and phenol contents were 56 ± 0.2 µg RE/ml and 41 ± 0.2 mg GAE/ml.

Conclusion: *Aloe vera* is a good source of vital bioactive and phytochemical compounds, it can be a potentially significant solution benefits for physical well-being by inclusion of *aloevera* into daily life.

Key Words: *Aloevera* Catalase Phytochemicals Flavonoid Phenol.

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I. Introduction

Aloe vera, a succulent perennial plant, belonging to *Aloaceae* family is widely cultivated for various purposes. It has been used since ages as the history suggests for its therapeutic properties and medicinal potential. Domesticated *Aloe vera* (*Aloe vera* (L.) Burm. F.) is botanically named as *Aloe barbadensis* Miller⁽¹⁰⁾. *Aloe barbadensis* Miller and *Aloe aborescens* Miller, are the only two species of *Aloe* grown today commercially⁽¹¹⁾. It contains 200 different bioactive compounds including vitamins, minerals, sugars, lignin, saponins, salicylic acids and amino acids⁽¹¹⁾. *Aloe vera* gel has broad spectrum of application. It shows antiseptic, antibacterial, antifungal action and is rich in anti-oxidants. The antimicrobial activity has been attributed to the plants natural anthraquinones⁽¹⁾. It clears acne and skin allergies, dark spots and skin blemishes, and makes the skin clearer⁽⁹⁾. The gel contains bradykinase, an anti-inflammatory compound. Nowadays large number of products in market contains *aloe vera* as one of the ingredients. Various preparation of *Aloe vera* has found its application in food, skincare, cosmetics and medical industry as active ingredients for extra therapeutic, rejuvenating, health enhances and hygienical effectives⁽⁶⁾. Despite large number of researches, safety of the *aloe gel* has not been scientifically proven. In order to achieve the above objective, identification and quantification of proximate principles and phytonutrient constituents of *aloe vera* was studied.

II. Material and Methods

Aloe vera leaves were collected from Bombay in October 2019. During the collection, healthy medium sized firm leaves samples were selected. The leaves were washed under tap water. The traditional *Aloe vera* leaf processing method of Hand filleting.

Study Design: Scientometric assessment study

Study Location: The study was carried out in Ramnarain Ruia Autonomous College at Matunga, Mumbai Maharashtra.

Study Duration: September 2019 January 2020.

Procedure Methodology:

Determination of Total Polysaccharide, Carbohydrate & Protein Content.

The phenol-sulphuric acid method was used to determine the total polysaccharide content. 5g% sample aliquots were treated with 5% phenol followed by addition of concentrated sulphuric acid. The additions were carried out in cold conditions. Absorbance was measured after 10 minutes of incubation at 488nm. The amount of carbohydrate was calculated using standard curve of glucose⁽⁴⁾.

The Anthrone method was used to determine total carbohydrate content. 1000 µl of 5 g% sample was treated with 4ml ice cold Anthrone reagent. It was vortexed and then heated in a boiling water bath for 90 seconds. The solution was immediately cooled in ice bath and absorbance was measured at 600 nm. The amount of carbohydrate was calculated using standard curve of glucose⁽⁵⁾.

Folin-Lowry method was used to determine total protein content. 1ml of 5 g% sample was prepared in phosphate buffer (pH 7) and added to 5 ml of freshly prepared alkaline copper sulphate. The reaction mixture was mixed well and allowed to stand for 10 minutes. followed by addition of 0.5ml phenol reagent (Folin-Ciocalteu reagent). After 30 minutes of incubation the absorbance was read at 620 nm. Amount of protein was estimated using standard curve of bovine serum albumin (BSA)⁽⁷⁾.

Effect OF pH, Temperature on Catalase.

The optimal pH for catalase was determined in potassium phosphate buffer system (pH 6.4-7.4). Hydrogen peroxide was used as substrate for these studies. 1000 µl of 5% enzyme extract was added to 1000 µl of 5% substrate in presence of phosphate buffer of varying pH range from (6.4-7.4) and freshly prepared dichromate acetic acid solution. The mixture was incubated at 37⁰C for 10 min. Absorbance was read at 620nm. The optimum pH of the enzyme was calculated by plotting a graph of pH vs absorbance.

The optimal temperature for catalase was determined in range of (0-100⁰C). 1000 µl 5 gm% of enzyme extract was added to 1000 µl of 5% substrate in presence of phosphate buffer of pH 7.0 and freshly prepared dichromate acetic acid. The test tubes were incubated at various temperature and absorbance was read at 620 nm. The optimum temperature of the enzyme was calculated by plotting a graph of temperature vs absorbance.

Phytochemical Qualitative Analysis:

The chloroform, methanolic, and aqueous extract solutions were assessed for the existence of the phytochemicals by using the defined tests.

Tests	Protocol
Molisch's Test for Carbohydrate	2ml plant extract + 1 ml Molisch's reagent + few drops of concentrated sulphuric acid.
Test for Tannin's	1 ml plant extract + 2 ml 5% ferric chloride.
Foam Test for Saponins	2ml of plant extract + 2 ml of distilled water. Shake in a graduated cylinder for 15 minutes lengthwise.
Test for Flavonoids	2ml of plant extract + 1 ml of 2N sodium hydroxide.
Mayer's Test for Alkaloids	2ml of plant extract + 2ml of concentrated hydrochloric acid + few drops of Mayer's reagent.
Test for Quinones	1ml plant extract + 1ml of concentrated sulphuric acid.
Salkowski's Test for Terpenoids	2ml of plant extract + 2ml of concentrated hydrochloric acid + few drops of Mayer's reagent.
Ferric chloride Test for Phenols	1 ml of extract + 2ml distilled water + few drops of 10% ferric chloride.
Salkowski's Test for Steroids and Phytosteroids	1ml of extract + equal volume of chloroform + few drops of concentrated sulphuric acid from the sides of the test tube.

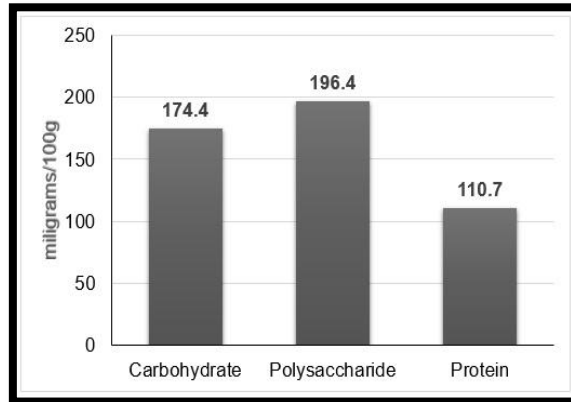
Determination of Total Flavonoid and Phenol Content.

The total flavonoid content was evaluated according to the aluminium chloride colorimetric method. The plant extract (2 mL) was added to 2% aluminium chloride solution (2 mL). The mixture was allowed to react at room temperature for 30 minutes and the absorbance was read at 420 nm. All determinations were performed in triplicate and results were expressed as µg rutin equivalent (RE)/mg crude extract⁽⁸⁾.

The total phenolic content was evaluated by modified Folin-Ciocalteu assay described by⁽²⁾. The plant extract (0.50ml) was added to a test tube containing a tenfold diluted Folin-Ciocalteu reagent solution (2.50ml) and sodium carbonate (2.0ml 7.5%). The mixture was allowed to react for 30 minutes at room temperature. The total phenolic content was then spectrophotometrically determined at 760 nm. All determinations were done in triplicate and results obtained were expressed as µg gallic acid equivalent (GAE)/mg crude extract.

III. Results

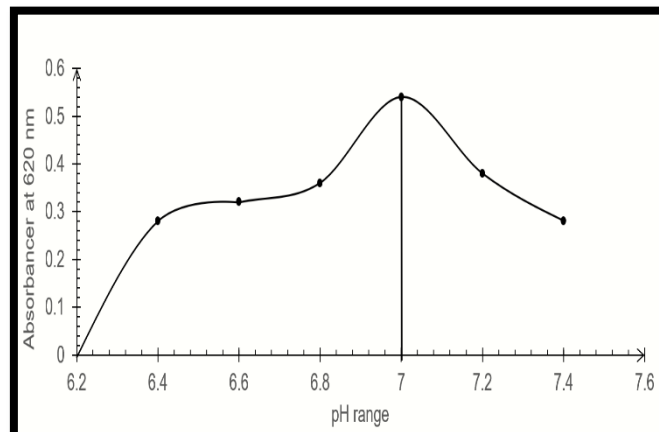
Biochemical Analysis of Proximate Principles.



Graph: Proximate Profile of Whole aloe vera leaf.

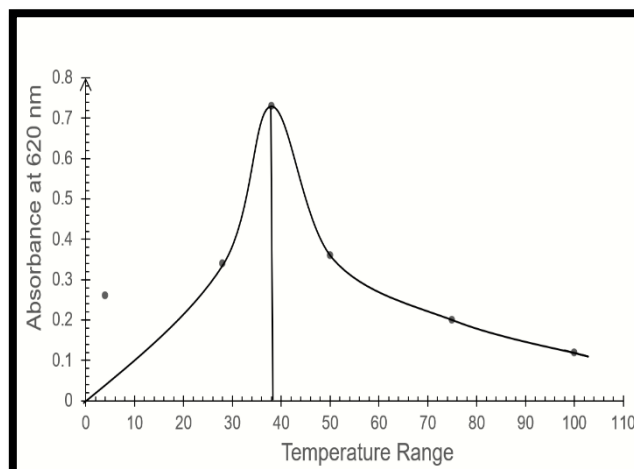
The Total Carbohydrate content, Total Polysaccharide content and Total Protein content of Whole leaf was found to be 174.4 mg %, 196.4 mg% and 110.7 mg% respectively.

Enzyme Assay.



Graph: Optimum pH of Catalase

The optimum pH profile of catalase was observed at pH 7. Catalase was active in the pH range of 6.4-7.4. There was a decline in activity at pH lower than 7.0.



Graph: Optimum Temperature of Catalase

The optimum activity occurred at 37°C when the enzyme was incubated with hydrogen peroxide at different temperatures.

Quantification of Phenol and Flavonoid.

Assays		Aloe vera
Total phenolic content		41 ± 0.2 µg ^a
Total flavonoid content		56 ± 0.2 µg ^b

a µg gallic acid equivalent (GAE)/mg, *b* µg rutin equivalent (RE)/mg.

The total flavonoid content was 56 ± 0.2 µg rutin equivalent (RE)/ml crude extract by reference to standard curve. The total phenol content is reported as gallic acid equivalent by reference to standard curve and was found to be 41 ± 0.2 mg GAE/g of extract.

Phytochemical Qualitative Analysis

Tests	Chloroform Extract	Methanol Extract	Water Extract
Carbohydrate	+	+	+
Tannins	-	+	+
Saponins	+	-	-
Flavonoids	+	+	+
Alkaloids	+	-	+
Quinones	-	+	+
Terpenoids	-	+	+
Phenols	-	+	+
Steroids	-	+	+

Key: + = Presence, - = Absence.

IV. Discussion

In the present study, unprocessed aloe vera gel was evaluated for biochemical analysis of proximate principles, enzymatic profile of catalase, screening of phytochemicals and quantification of phenol and flavonoid. The present study measured 174.4 mg% of carbohydrate, 196.4 mg% of polysaccharide content and 110.7 mg% of protein content. Catalase was found to work optimally at physiological conditions. Methanol and water extract of aloe vera were more efficient in comparison to chloroform extract to qualitatively determine the presence of phytochemicals. The total flavonoid and phenol contents were 56 ± 0.2 µg Rutin equivalent/ml and 41 ± 0.2 mg Gallic acid equivalent/ml. Disparities in the achieved results can be explained based on the fact of different experimental conditions. Both flavonoid and phenol exhibit antioxidant and anti-allergic properties. Enzyme catalase that functions by protecting the cell from oxidative damage, displayed optimum activity at physiological conditions during the study. In today's lifestyle poor diet situates the body under constant attack from oxidative stress. Oxidative stress poses serious harm to the body and Flavonoid, Phenol and catalase present in Aloe vera can aid fighting it.

V. Conclusion

Due to the versatile functions, properties and presence of significant bio-active compounds Aloe vera can be included in one's diet in appropriate amount to reap the benefits. It is also anticipated that data gained from the present study will open new avenues for the development of potential products that can be used for the citizenry.

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