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# ASSESSING THE POTENTIAL OF ALOE VERA FOR ITS APPLICATION AS A NATURAL PRESERVATIVE

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## INTRODUCTION:

Food preservation is an important criterion in maintaining the quality of a food product over a period of time by increasing its shelf life. This can be achieved by the addition of certain additives (either natural or chemical). Modern trends in food science contributed to the growth of widely researched additives like ascorbic acid, tocopherols and vinegar among others. These preservatives act by suppressing the growth of microorganisms and preventing the production of chemicals that might cause food spoilage. Preservatives also work by scavenging the Reactive Oxygen Species (ROS) that are produced due to prolonged storage and increased microbial growth. With emerging trends, a new range of chemical preservatives came to the front like nitrates, sorbates, benzoates and sulfites. Even though these are effective as preservatives, they also pose serious health threats to human on ingestion. For example: Sodium nitrite which is prevalent in the preservation of meats and hot dogs' results in the formation of N-nitrosamines when cooked at high temperature. N-nitrosamine is a known carcinogenic and it will also impede the supply of oxygen to cells in general (Sangeeta Dwivedi et al). As the health effects of some chemical preservatives exceeds its benefits there is quite a search for the natural preservatives both by researchers and industrialists in general. This shift is paradigm is strengthened by the shift in consumer preference for organic products. In this paper the use of different varieties of aloe vera extract as a viable option for natural preservative is investigated.

Key words: Aloe vera, natural preservatives, ROS, nitrates, carcinogenic

## 1. INTRODUCTION

According to FSSAI, a preservative **means "a substance which when added to food, is capable of inhibiting, retarding, or arresting the process of fermentation, acidification, or other**

**decomposition of food."** The latest technological development in food science and technology contributed to the development of various techniques that can arrest the growth of microorganisms completely like thermal processing, irradiation and freezing. Some Ready-to-eat food can be stored in retort packages effectively for a relatively long period of time. Even though they are effective, the high cost of these techniques make it difficult for it be adopted by small scale industries. At such case preservatives are the safe and effective option for food preservation.

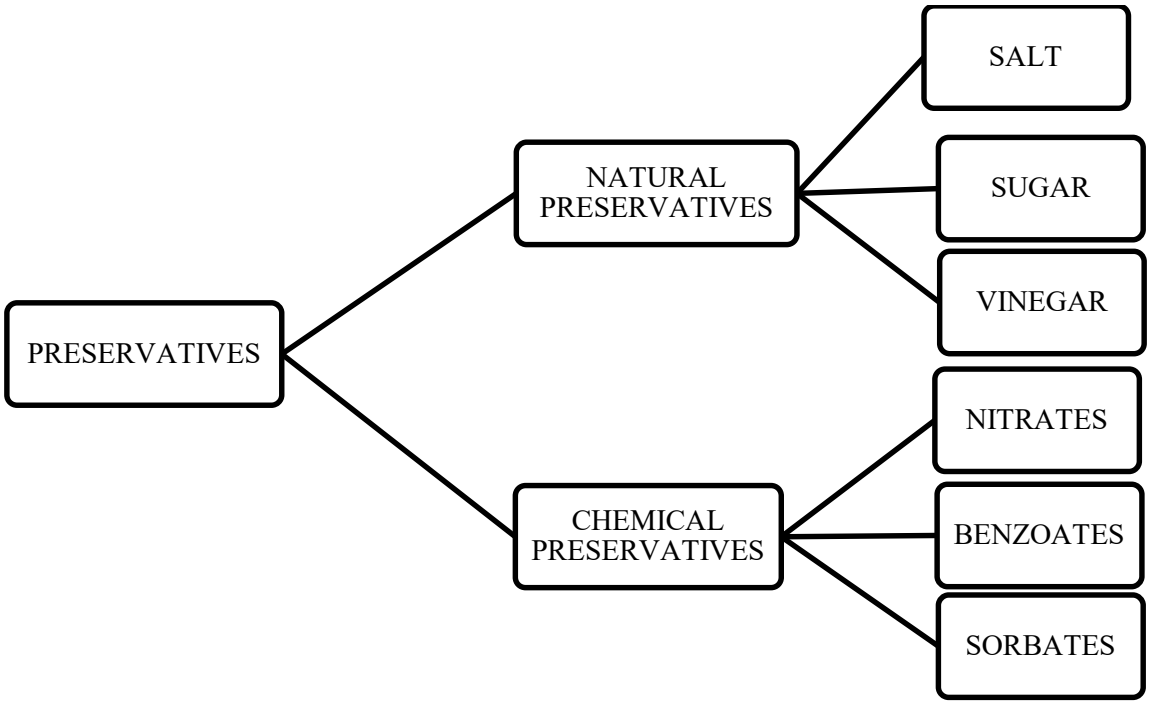
Preservatives can be classified into natural and chemical preservative based on the source.

## 1.1 NATURAL PRESERVATIVES:

Natural preservatives are substance derived from plants rich in bioactive molecules. These substances have inherent antimicrobial and antioxidant properties which make them an ideal choice for organic preservatives. Some of the compounds that belong to this class are salt, vinegar, organic acids, sugar and spices. These are commonly called as Class I preservation and their addition is not restricted.

## 1.2 CHEMICAL PRESERVATIVES:

Chemical preservatives can either be organic or inorganic. Examples of this Class II preservatives include nitrites of Sodium or Potassium, nisin, Calcium propionate etc., Addition of these preservatives are restricted by regulating agencies like FDA, Codex Alimentarius and FSSAI. These chemical preservatives act by disrupting the cell membrane of microorganisms and preventing the enzyme activity.



### 1.3 ALOE VERA:

Aloe vera is a medicinal semi-tropical plant widely cultivated across India. In India, it is found in Rajasthan, Andhra Pradesh, Gujarat, Maharashtra and Tamil Nadu. It has a wide range of bioactive compounds like Aloetic acid, aloin, anthraquinone, salicyclic acid, cinnamic acid and isobarbaloin. This plant has proven antidiabetic, wound healing and anticancerous properties.

## 2. MATERIALS AND METHODS:

### 2.1 COLLECTION AND PREPARATION OF ALOE VERA:

Fresh samples of Aloe vera is brought from the market and surface sterilized using Sodium Hypochlorite. Then the outer part is peeled off and the gel portion is collected. The gel is made into a homogenous substance and filtered. In order to decrease the bitterness of the aloe gel the concentration is maintained at 40% for palatability.

### 2.2 ANALYSIS METHOD:

### 2.2.1 QUALITATIVE PHYTOCHEMICAL ANALYSIS:

Phytochemical analysis of plant extracts indicates the presence of phytoconstituents like alkaloids, tannins, flavonoids, glycosides, phenols, saponins and terpenoids which are responsible for antimicrobial activity of the extract. Analysis was carried out on the ethanol, methanol, chloroform, Dichloromethane and water extract using standard procedures to identify the phytochemicals based on methods mentioned by Gul, R et al (2017).

Table 2.1: Qualitative Phytochemical analysis test procedure

| S.NO | TEST | PROCEDURE | INFERENCE |
|---|---|---|---|
| 1. | Test for Alkaloids (Mayer's test) | To 1ml of the extract 1ml of dilute Hydrochloric acid and a few drops of Mayer's reagent were added. | Formation of white precipitate confirms the presence of Alkaloids. |
| 2. | Test for Flavonoids | To a drop of the extract, 1ml of dilute sodium hydroxide was added. | An intense yellow color appears which became colorless on the addition of a few drops of dilute acid which indicates the presence of flavonoids. |

| | | | |
|---|---|---|---|
| 3. | Test for Phenols | To 1ml of the extract, equal volume of 1% Ferric chloride was added. | Formation of yellow or green color indicates the presence of phenols. |
| 4. | Test for Steroids (Liberman Burchard test) | To 1ml of extract, 1ml of chloroform and 3ml of acetic anhydride were added. Finally one or two drops of concentrated Sulphuric acid were added. | Appearance of dark green color confirms the presence of steroids. |
| 5. | Test for Tannins (Lead acetate test) | To 1ml of the extract 2ml of 10% lead acetate solution was added. | A white precipitate that was partially soluble in 1ml of 10% acetic acid indicates the presence of tannins. |
| 6. | Test for Saponins | 1ml of the extract was taken in a test tube and 5ml of distilled water is added to it followed by a vigorous shaking for 30 seconds. | The persistent froth indicates the presence of saponins. |
| 7. 7 | Test for Terpenoids (Salkowski test) | Few drops of concentrated Sulphuric acid were added to 1ml of the extract and shaken well. | After some time the lower layer turns golden yellow color indicating the presence of terpenoids. |
| 8. | Test for Carbohydrates (Molisch tests) | The extract was treated with 2ml of Molisch's reagent and few drops of concentrated Sulphuric acid were added along the side of test tube. | A reddish violet ring shows the presence of carbohydrates. |
| 9. | Test for Glycosides (Keller-Killani test) | The extract was dissolved in glacial acetic acid and two drops of ferric chloride solution was added to it. The contents were transferred to a test tube containing 2ml of concentrated Sulphuric acid. | A reddish brown color ring at the junction of two layers indicates the presence of glycosides. |
| 10. | Test for Proteins (Biuret test) | When the protein solution was made alkaline with sodium | A pink/violet color denotes the presence of proteins. |

| | | | |
|---|---|---|---|
| | | hydroxide and a drop of dilute copper sulphate solution was added to it. | |

### 2.2.2 DETERMINATION OF TOTAL PHENOLIC CONTENT

The total phenolic content was determined spectrophotometrically by the Folin-Ciocalteu method as mentioned in Katalinic, V., et al (2006) with minor modifications. Gallic acid was used as a standard. 1 ml of sample was mixed with 1 ml of Folin-Ciocalteu reagent (FCR). Then 10 ml of 7% $Na_2CO_3$ solution was added to the mixture after 10 minutes followed by the addition of 3ml of distilled water and the solution was mixed thoroughly. The mixture was kept in the dark at room temperature for 60 min, after which the absorbance was read at 750 nm. TPC was done for both the ethanol extract and methanol extract.

The TPC was determined from extrapolation of calibration curve which was made by preparing Gallic acid solution at different concentrations ranging from 50mg/ml to 1000mg/ml. The estimation of the phenolic compounds was carried out in triplicate. The TPC was expressed as milligrams of Gallic acid equivalents (GAE) per ml of sample.

### 2.2.3 DETERMINATION OF TOTAL FLAVONOID CONTENT

Total flavonoid content (TFC) was determined using colorimetric Aluminum chloride ($AlCl_3$) method as mentioned by Wanyo, P et al., (2014) with slight modification. To 1mL of the extract 3mL of distilled water in a test tube followed by addition of 1mL of 5% Sodium nitrite solution. After 7 min, 0.5 mL of 10% Aluminum chloride solution was added. After another 5 min 1.5 mL of 1 M Sodium Hydroxide was added. The absorbance was measured immediately after 20 min of incubation at 510 nm using spectrophotometer. TFC was done for both the ethanol and methanol extracts.

The TFC was determined from extrapolation of calibration curve which was made by preparing quercetin solution at different concentrations ranging from 50mg/ml to 1000mg/ml. The estimation of the phenolic compounds was carried out in triplicate. The TFC was expressed as milligrams of quercetin equivalents (QE) per g of sample. Results were expressed as mg quercetin equivalents in 1 ml of sample (mg QE/ml).

### 2.2.4 ANTIMICROBIAL ACTIVITY OF THE EXTRACT

### AGAR WELL-DIFFUSION METHOD

Agar well-diffusion method was followed according to in order to determine the antimicrobial activity. The organisms taken for studying the effect of Aloe vera are *Bacillus subtilis, Staphylococcus aureus* and *Bacillus ceres*. Nutrient agar (NA) plates were swabbed (sterile cotton swabs) with 8 h old -broth culture of respective bacteria. Wells (10 mm diameter) were made in each of these plates using sterile cork borer. Stock solution of plant extract was prepared at a

concentration of 1 mg/ml in plant extracts viz., Ethanol, about 100 µl of different concentrations of plant solvent extracts were added sterile syringe into the wells and allowed to diffuse at room temperature for 2 h. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. The agar well diffusion method was carried out in triplicates for each organism and the results are tabulated. (Faden, A. A. et al., 2018)

### 2.2.5ANTIOXIDANT ACTIVITY OF THE *ALOE VERA* EXTRACT

### DPPH SCAVENGING ACTIVITY:

The DPPH scavenging activity assay was done according to the method followed by *Sridhar, K., & Charles, A. L. (2019).* 0.1ml of standard and sample extracts were taken at varying concentrations (2 µg, 4 µg, 6 µg, 8 µg, 10 µg, 12 µg, 14 µg, 16 µg, 18 µg and 20 µg) and 0.1ml of 0.100 µM Methanolic DPPH was added. The reaction mixture was incubated in dark at room temperature for 10 minutes. Finally, the absorbance was read at 517nm. A graph was plotted comparing the scavenging activity of both the standard (Ascorbic acid) and the sample extract.

$$\textbf{SCAVENGING ACTIVITY}(\%) = \left( \frac{\textbf{Absorbance}_{\textbf{Control}} - \textbf{Absorbance}_{\textbf{Sample}}}{\textbf{Absorbance}_{\textbf{Control}}} \times \mathbf{100} \right)$$

### PHOSPHOMOLYBDATE ASSAY:

The Phosphomolybdate assay was done according to the method followed by Sahreen, S et al.,*)*. 0.1ml of standard and sample extracts were taken at varying concentrations ( 2 µg, 4 µg, 6 µg, 8 µg,10 µg, 12 µg, 14 µg, 16 µg, 18 µg and 20 µg ) and 0.1ml of reagent solution ( 0.6M Sulphuric acid, 28mM Sodium Phosphate and 4mM ammonium molybdate) was added. The test tubes were capped with silver foil and the reaction mixture was incubated in a water bath at 95ºC for 90 minutes. Cool the sample to room temperature. Finally, the absorbance was read at 765nm. A graph was plotted comparing the scavenging activity of both the standard (Ascorbic acid) and the sample extract.

## 3. RESULTS AND DISCUSSION

The subjective phytochemical analysis of *Aloe vera* for alkaloids, anthraquinones, carbohydrates, cardiac glycosides, coumarins, flavonoids, glycosides, phenols, phlobatannins, phytosteroids, saponins, steroids, tannins, terpenoids and triterpenoids Table 3.1.

Among the extracts tested Ethanol extract test positive for most of the phytochemicals followed by Methanol, Chloroform, Water and Dichloromethane.

| S.no | Phytochemicals | Ethanol extract | Methanol extract | Chloroform extract | DCM extract | Aqueous extract |
|---|---|---|---|---|---|---|
| 1 | Carbohydrates | - | - | - | - | - |
| 2 | Protein | + | + | - | + | - |
| 3 | Alkaloid | + | + | + | + | + |
| 4 | Triterpenoid | + | - | - | - | - |
| 5 | Flavonoids | + | + | + | - | + |
| 6 | Phenols | + | + | + | + | + |
| 7 | Phytosterols | + | _ | _ | - | - |
| 8 | Tannins | + | + | _ | + | - |
| 9 | Saponins | + | + | + | + | + |
| 10 | Glycosides | + | + | - | - | - |

Table 3.1: Phytochemical analysis of *Aloe vera* extract using different solvents

### 3.2 TOTAL PHENOLIC CONTENT

Phenolic compounds are important plant constituents with redox properties responsible for antioxidant activity. The hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging. As a basis, phenolic content was measured using the Folin-Ciocalteu reagent in each extract.

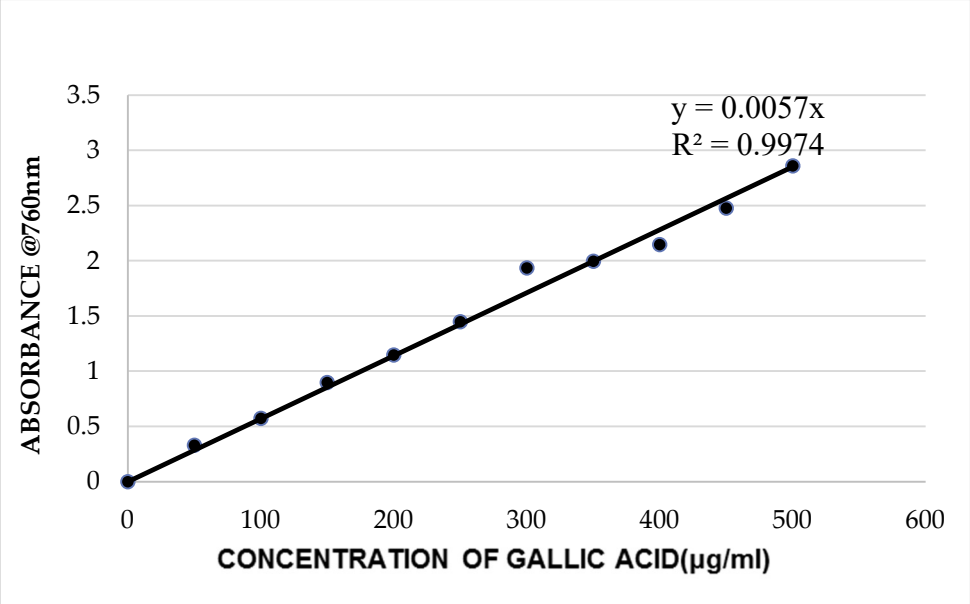


Fig 3.1: Total phenolic content estimation by Folin-Ciocalteu method- calibration curve using Gallic acid as standard

The results were derived from a calibration curve (y = 0.0057x, $R^2$ = 0.990) of Gallic acid (0-1000 µg/mL) and expressed in Gallic acid equivalents (GAE) per gram dry extract weight using the formula, C = cV/M; where C = total content of phenolic compounds in mg/g GAE, c = the concentration of Gallic acid established from the calibration curve, V = volume of extract and M = the weight of the extract.

The content of phenolic compounds in ethanol extract is 334.46±2.39mg GAE/ml, and the content of phenolic compounds in methanol extract is 297.46±4.78mg GAE/ml.

## 3.3 TOTAL FLAVONOID CONTENT

Flavonoids are secondary metabolites with antioxidant activity, the potency of which depends on the number and position of free OH groups. As a basis of quantitative determination, flavonoid contents in selected plant extracts were determined using aluminum chloride in a colorimetric method.

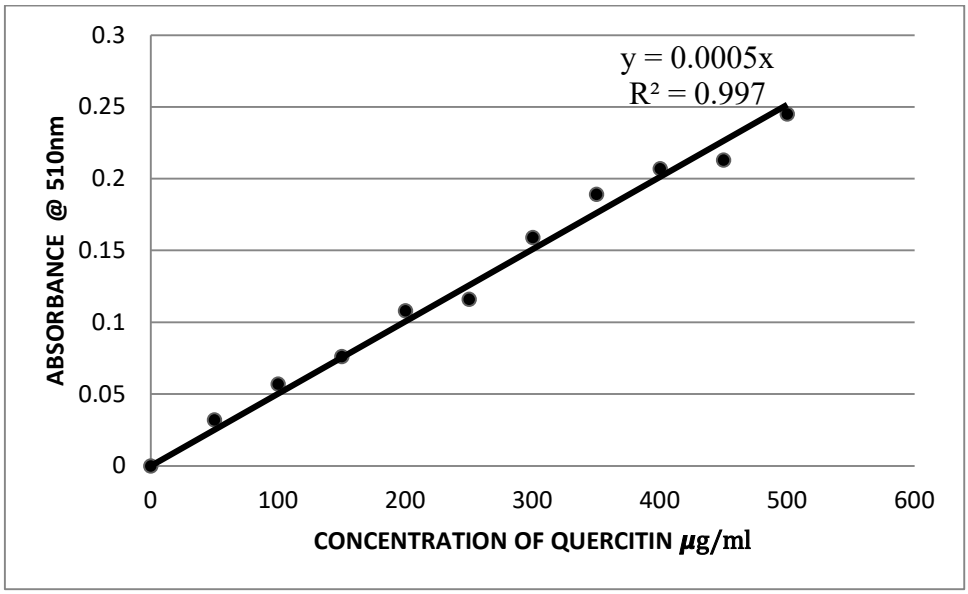



Fig 3.2: Total flavonoids content estimation by $Alcl_3$ method- calibration curve using Gallic acid as standard

The results were derived from the calibration curve (y = 0.0005, $R^2$ = 0.988) of quercetin (0–1000 µg/mL) and expressed in quercetin equivalents (QE) per gram dry extract weight using the formula, C= cV/M; where C= total content of phenolic compounds in mg/g QE, c= the concentration of quercetin established from the calibration curve, V= volume of extract and M = the weight of the extract. The flavonoid content in ethanol extract is 188±9.9 mg QE/ml, whereas the flavonoid content in methanol extract is 162±4.68 mg QE/ml.

## 3.4 ANTIMICROBIAL ACTIVITY

Inhibitory activity was measured based on the clear zone surrounding the well. If there is no clear

zone, it is assumed that there is no inhibition.

The results showed that the Aloe vera gel was effective against *Bacillus subtilis, Bacillus ceres* and *Staphylococcus aureus*. As the concentration of the extract increased, the zone of inhibition also increased significantly.

Table 3.2: Antimicrobial activity- Zone of inhibition (mm) for various microorganisms using Aloe vera extract.

| | **Concentration (mg/ml)** | **Gram (+ve) bacteria Zone of inhibition (mm)** | | |
|---|---|---|---|---|
| | | ***Bacillus subtilis*** | ***Bacillus ceres*** | ***Staphylococcus aureus*** |
| **Amoxycylin** | 100µg/ml | 15.33±0.57 | 16.33±0.57 | 15.33±0.41 |
| **Ethanolic extract of *Aloe vera*** | 50µg/ml | 12.3±0.58 | 10.33±0.58 | 13.2±0.20 |
| | 100µg/ml | 13.1±0.28 | 11.66±0.58 | 14.23±0.30 |
| | 150µg/ml | 13.93±0.11 | 12.67±1.15 | 15.13±0.37 |
| | 200µg/ml | 15.3±0.3 | 13.00±.00 | 15.36±0.25 |
| | 250µg/ml | 16.3±0.36 | 13.3±0.58 | 16.33±0.15 |
| | 500µg/ml | 18.13±0.30 | 14.67±0.58 | 16.33±0.57 |
| | 750µg/ml | 18.93±0.30 | 16.00 | 16.83±0.05 |
| | 1000µg/ml | 19±0.2 | 17.67± 0.58 | 18.43±0.15 |

## CONCLUSION:

The above analysis shows that aloe vera has a wide range of phenolics and other bioactive compounds which make them the ideal choice for their use as an organic preservative.

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