



Evaluation Of Antioxidant & Nutritional Activity Of Amla Wine & Fermented Amla Leaves

Author: Rohit Darade , Krupa shah

(Department of food technology, faculty of Applied Sciences, Parul university Vadodara-391760 Gujrat state, India.)

ABSTRACT: A Comprehensive Study This study aimed to assess the antioxidant and nutritional properties of Amla wine and fermented Amla leaves through proximate analysis, sensory analysis, physico-chemical analysis, and microbiological analysis. Fermented Amla products, including wine, hold significant potential in meeting human nutritional needs and are recognized for their therapeutic properties, including anti-cholesterolemic and anti-carcinogenic effects. Amla wine, fermented through anaerobic fermentation, is particularly valued for its enhanced antioxidant and nutritional content. Fresh Amla fruit and leaves were sourced from local markets and farms, while freeze-dried *Saccharomyces cerevisiae* cultures from reputable institutions were employed for fermentation. Proximate analysis of the raw materials, including pH, acidity, ethanol, sugar, antioxidant, and phytochemicals contents such as polyphenols and flavonoids, was conducted. Amla wine formulations included 10% and 20% Amla fruit wine, as well as 10% Amla leaves wine. The study's findings, encompassing the results of various analyses, provide valuable insights into the antioxidant and nutritional properties of Amla wine and fermented Amla leaves, contributing to a deeper understanding of their potential health benefits and applications in human nutrition and well-being.

1.1. INTRODUCTION

India's super food amla is amazing. It's referred to as Gooseberry or Indian Gooseberry everywhere. This plant blooms and produces little, greenish-yellow fruits. It's the fruit tree with long-standing Ayurvedic benefits. It has been used to treat hunger and medical conditions. The amla plant can withstand extreme cold well, but it can't withstand extreme heat as well. The fruits have a tart flavor and are high in nutrients.

Commonly referred to as amla or Indian gooseberry, *Phyllanthus emblica* L. is a transient

tree in the Euphorbeaceae family. Amla fruits are mostly found in India, Pakistan, China, Iran, and Southeast Asia.. In Indian traditional medicine, amla is used to treat a variety of ailments, including anxiety, burning sensations in the skin and eyes, anemia, male reproductive system and reproduction, digestion issues, liver health, and cardiovascular system tonicity. One of the most widely used botanicals, the fruit of *P. Emblica* L. has numerous applications in the culinary, cosmetic, and medical fields. It is a fantastic dietary supplement with a number of health advantages. Because of the high concentration of phenolic compounds, emblic fruit may be used as a plant source of nutraceuticals,

medicinal ingredients, and natural antioxidants. Customers enjoy the distinct flavor and lovely aroma of Emblic fruit.

In various animal and human investigations, amla has been proven to have anti-hyperglycemic, hypoglycemic, anti-inflammatory, anti hyperlipidemic, and antioxidant activities. Because amla is high in antioxidants including gallic acid, ascorbic acid, and phenolic compounds, it supports the immune and digestive systems of the body. Owing to the growing interest in and potential for *P. emblica* L., this paper attempts to give a general overview of the nutritional makeup, phytochemistry, and possible health advantages of consuming the naturally occurring phytochemicals in amla. In India, it is considered a sacred tree. The fruit has a lot of nutrients. Fresh and dried fruits can be eaten for health reasons. Because it offers treatments for a wide range of illnesses, it is frequently utilized in Ayurvedic medicine. In addition to having a high vitamin C content, gooseberries also include calcium, phosphorus, iron, beta-carotene, and vitamin B complex. An additional strong antioxidant is amla, or Indian gooseberry.

1.2. Nutritional Profile Of Amla

The nutritional profile of 100 grams of raw amla is as follows:

- Vitamin A
- Vitamin K
- Vitamin B-complex (including B1, B2, B3, and B5)
- Calcium
- Phosphorus
- Iron
- Potassium
- Magnesium
- Copper
- Manganese

Calories	44 kcal
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Carbohydrates	10.18 grams
Dietary Fiber	4.3 grams
Sugars	4.4 grams
Protein	0.88 grams
Fat	0.58 grams
Vitamin C	600-700 milligrams per 100 grams

Fig.1.3.1 nutritional components of amla.

1.3. MATERIALS REQUIRED & METHOD

The present research work was investigation on "Evaluation of Antioxidant & Nutritional Activity of Amla Wine & Fermented Amla Leaves."

- ▶ **Requirements:** 250ml Flask, Test-Tubes, Test-Tube Stand, Calorimeter, Incubator, Centrifuge, Magnetic Stirrer, Etc.
- ▶ **Chemicals :** DNSA reagent, CAN reagent, Gallic acid, Na_2CO_3 , Ethanol, Methanol, AlCl_3 , NaOH, F.C reagent, Ascorbic acid, Potassium per sulphate, ABTS.

Phase I: - Collection Of Materials:

fresh & mature amla fruits were procured from the local store in market, where as the fresh amla leaves were collected from amla tree planted in Bhilarewadi Village Farm Katraj, Pune.

The freeze dried culture used for preparation of wine using fermentation is *Saccharomyces cerevisiae*. Which is obtained from Sinhgad College Of Science, Savitribai Phule Pune University (SPPU), Pune. Jaggery juice was procured from the grocery store in local market.

Phase II: - Raw Material Preparation & Sample Preparation

Crushed Amla Fruit Sample:

1. Fully mature fruit were selected, washed to remove dirt and dust.
2. Seed was removed by using knife and then cutting amla into small pieces to grind into mortar & pestle till pulp is formed.

3. The amla fruit i.e. Cut into small pieces are weighed to make 10 % and 20% conc. wine.
4. Then the amla is crushed in mortar pestle making it fine paste.
5. The cut pieces of crushed amla are transferred to sterile conical flask for further fermentation process

Dried Amla Leaves Sample:

1. Keep the amla leaves at room temp 37°C for 24 hrs drying in a Petri-plate evenly.
2. Prepare a wine from the leaves of amla tree.
3. Take the leaves placed for drying after 24 hrs at room temperature.
4. The amla leaves are grinded into fine powder and weighed to make 10 %conc. of leaves, 20%conc. of jaggery and 70% distilled water.
5. Prepare this wine sample of amla leaves for total 200 ml volume.

Yeast Culture Preparation: The activated dried culture of *Saccharomyces cerevisiae*. Obtained from Sinhgad College Of Science, Savitribai Phule Pune University (SPPU), Pune

1. The yeast culture was maintained in sterilized sachet at room temperature.
2. *Saccharomyces cerevisiae* granules are weighed around 2.5 gm & mixed well in 50 ml of distilled water in sterile beaker.
3. Take O.D in calorimeter and make sure the O.D must be around 0.4 to 0.8. If not dilute it with distilled water & *Saccharomyces cerevisiae*.
4. After the O.D place the *Saccharomyces cerevisiae* (yeast) in refrigerator at refrigeration temperature of 5-7°C for 10-15 minutes.

Phase III: - Development Of Wine From Amla & Its Leaves By Fermentation By Using Yeast *Saccharomyces cerevisiae*.

Preparation Of Amla Wine:

1. 1. The crushed amla fruit sample prepared at **Phase II** i.e. 10% & 20% weighed crushed amla are transferred to sterile conical flask separately for further fermentation process.
2. After addition of crushed amla the add 20% jaggery juice to conical flask.
3. Now make up the total volume of wine 100% by addition of 70% distilled water.
10 % amla fruit +20% Jaggery juice +70. % D/W = 100 %.
4. Prepared the amla fruit wine of total volume 200 ml.
5. **For 10% amla fruit wine:** As per the given concentration percentage amla fruit pieces were weighed 20gm and 40 ml of jaggery juice. Then 140ml of distilled water is added to make final volume of 200 ml.
6. **For 20% amla fruit wine:** As per the given concentration percentage amla fruit pieces were weighed 40gm and 40 ml of jaggery juice. Then 120ml of distilled water is added to make final volume of 200 ml.
7. **For 10% amla leaves wine:** As per the given concentration percentage grinded powder of amla leaves was weighed 20gm and 40 ml of jaggery juice. Then 140ml of distilled water is added to make final volume of 200 ml.
8. After the initial preparation process add *Saccharomyces cerevisiae* solution prepared 1ml/100 ml of wine prepared i.e. (2 ml *Saccharomyces cerevisiae* for 200 ml of wine.

9. After the addition of yeast (*Saccharomyces cerevisiae*) close the lid of the conical flask with single hole rubber cork & make sure to attach "U shaped tube" into rubber cork that is tightly attached. The "U-shaped tube" is used for the anaerobic fermentation process.

Anaerobic fermentation: It is a metabolic process that converts carbohydrates (sugar) to organic acids, gases or alcohols under anaerobic conditions. The microorganisms break down biodegradable material in the absence of oxygen.

10. Cover the flask head, rubber cork, & U-shaped tube with parafilm tape properly so that no oxygen will pass through it. Take a beaker with water in it and dip the U-shaped tube tip in water so that the CO_2 produced in flask will get released in water.

Phase IV: - Sensory Evaluation Of The Product.

When the quality of a food product is assessed by means of human organs, the evaluation is said to be sensory or subjective or organoleptic. Every time food is eaten a judgment is made. Sensory quality is a combination of different senses of perception coming into play in choosing and eating a food. The effective characteristic is not the property of the food but the subject's reaction to the sensory qualities of foods.

Phase V: - Determine Physicochemical Constituents Of The Product.

1. pH (AOAC 2000) :-

The pH content was determined by (M. tronico digital-255) pH meter

2. Titratable acidity (FSSAI MANUAL,2015):

- ▶ Prepare 100 ml of NaOH solution of 1 N , Fill the burette with NaOH solution.
- ▶ Take a conical flask, Add 1 ml of wine sample and 9 ml distilled water in it.
- ▶ Add 2-3 drops of phenolphthalein indicator, Mix it well.
- ▶ Do the titration by adding 0.1 ml of NaOH solution slowly drop by drop.
- ▶ Add the NaOH solution till the color of the sample in flask changes to light pink .
- ▶ Note down the burette reading (ml) by which color has changed.

Calculation:

$$\text{Titratable acidity \%} = \frac{\text{No of ml of 0.1 N NaOH required for titration} \times 0.009 \times 100}{\text{Weight of sample}}$$

Weight of sample

(weight of sample = Vol of milk X specific gravity)

3. Sugar Content By DNSA Reagent :-

The color of the reagent changes from yellow to orange or red, depending upon the concentration of reducing sugar present. The DNSA test can detect concentrations of glucose between 0.5 mM (0.09% glucose w/v) and 40 mM (0.72% glucose w/v).

DNSA is more sensitive and easier to use than Benedict's reagent. Used with a colorimeter, it is ideal for measuring the action of enzymes such as

invertase, cellulase and amylase where reducing sugars are produced. Thus it helps to meet two of the important practical requirements of the current (English) biology specifications

Procedure:

- ▶ Take Gallic acid as a standard for checking sugar content in wine samples of Amla. Make (0.2, 0.4, 0.6, 0.8, 1.0,,1.8 (Gallic Conc. in mg) of Gallic acid concentration.
- ▶ Prepare DNSA reagent.
- ▶ Take 1ml standard solution sample add 9 ml distilled water in it + 1ml DNSA reagent.
- ▶ Keep it in boiling bath for 5 minutes till the color changes to brown red.
- ▶ Take 1 ml of amla wine sample add 9ml distilled water + 1 ml of DNSA reagent.
- ▶ After cooling the test tubes, take the absorption at [540](#) nm for standard solution and wine samples.
- ▶ Note down the readings and plot the graph.

4. Ethanol content by CAN reagent:-

Ethanol is biologically produced through the fermentation of sugars by various types of microbes or chemically produced via chemical reaction or petro-chemical processes.

Ceric ammonium nitrate test Take 1 ml solution of organic compound dissolved in a suitable solvent. Add a few drops of ceric ammonium nitrate solution. Appearance of red color shows the presence of alcoholic – OH group.

Procedure:

- ▶ Take ethanol as a standard for checking ethanol content in wine samples of amla .
- ▶ Make (10%, 20%, 30%, 40%, 50% Ethanol conc.) i.e. for 10 % of ethanol 1ml ethanol and 9 ml distilled water.

- ▶ Take 1ml ethanol standard solution add 1ml CAN reagent.
- ▶ Do same for samples
- ▶ Take 1 ml sample (amla fruit) (1:10 dilution).
- ▶ Now take 1ml wine sample, add 1ml CAN reagent.
- ▶ Take 1 ml sample (amla fruit) (1:10 dilution) add 1 ml CAN reagent.
- ▶ Take absorption of both standard and wine samples at [540](#) nm.

5. Phytochemical Analysis :-

Total Polyphenolic & Flavonoids content by using biochemical Assay.

- a. **Polyphenolic Content :** Total phenolic The total phenolic content of the extract was determined by Folin-Ciocalteu reagent in an alkaline atmosphere. Phenolic content is determined by measuring the absorbance of the sample solution at 765 nm and comparing with a calibration curve using gallic acid as a standard. polyphenols have antioxidant and anti-inflammatory properties that could have preventive and/or therapeutic effects.

Procedure:

- ▶ Take gallic acid as a standard for checking Polyphenolic content in wine samples of amla.
- ▶ Prepare 1 N Gallic acid as a standard solution ,Take 0.01 gm Gallic acid in 100 ml distilled water.
- ▶ Make 10%,20%,30%,40%,50% Gallic acid concentration for standard readings.

- ▶ Prepare F.C (Follin's Ciocaltaue) reagent in 1:1 dilution , and prepare 1.75 gm Na₂CO₃ solution in 25 ml of distilled water.
- ▶ Take 1ml standard solution add 9 ml distilled water in it (1:10 dilution).
- ▶ Add 1ml Na₂CO₃ in standard solution and keep it for 10 min dark incubation at RT.
- ▶ Add 1ml F.C reagent & again keep it for 60 min dark incubation at RT.
- ▶ After incubation take the absorbance at 760nm in calorimeter and note the O.D values.

b. Flavonoids Content :

Flavonoids are a diverse group of phytonutrients (plant chemicals) found in various fruits, vegetables, herbs, and beverages.

Flavonoids are potent antioxidants, meaning they can neutralize free radicals in the body, thereby reducing oxidative stress and inflammation. Scientific studies on flavonoids continue to explore their potential health effects and mechanisms of action. However, more research is needed to fully understand their role in human health and disease prevention.

Procedure:

- ▶ Take quercetin as a standard for checking flavonoids content in wine samples of amla.
- ▶ Take 20mg of Quercetin powder and dissolve with 20ml of Ethanol.
- ▶ Take 0.5gm AlCl₃ chemical and dissolve it in 25ml of Methanol.
- ▶ Make 10%,20%,30%,40% & 50% Quercetin conc. solution concentration by addition of 1ml,2ml,.....,5ml of Quercetin-Ethanol Solution into 5 test tube with 9ml,8ml,.....,5ml Distilled Water Respectively.

- ▶ Pipette out only 1 ml solution from all 5 tubes into another 5 tubes and mark them as Standard Solutions.
- ▶ Add 0.1ml of sample and 0.9ml of Distilled Water into another tube, Now all tubes would have 1ml of solution in them.
- ▶ Add 1ml of AlCl₃-Methanol solution in all of the tubes and now the total volume in all tube will be 2ml.
- ▶ Incubation in Dark for 10min at Room Temperature.
- ▶ After Incubation dilute all the tubes with 2ml of distilled water.
- ▶ Take the readings at 368nm on Colorimeter.
- ▶ Take 1ml of quercetin-ethanol solution and 1ml of AlCl₃-methanol solution in a tube and use it as BLANK.

Calculation:

$$\text{Flavonoids content (\% w/w)} = \frac{\text{RE} \times \text{V} \times \text{D} \times 10}{\text{W}} \times 100$$

RE =Rutin equivalent (µg/ml),

V =Total volume of sample (ml),

D =Dilution factor, W - sample weight (g).

6. Antioxidant Content By ABTS Radical Scavenging Assay :

ABTS Assay:- The ABTS assay measures the relative ability of antioxidants to scavenge the ABTS generated in aqueous phase, as compared with a Trolox (water soluble vitamin E) standard. The ABTS is generated by reacting with a strong oxidizing agent (Eg. potassium permanganate or potassium per sulfate) with the ABTS salt.

Procedure:

- ▶ Take ABTS stock solution as a standard for checking antioxidant content in wine samples of amla.

- ▶ Take 0.0247 gm ABTS powder in 50 ml D/W in conical flask (7mM/ litre).
- ▶ Add 0.0324 gm of phosphate persulfate (2.4 mmol/1 litre) in 50 ml distilled water in another flask.
- ▶ Mix both the solution make 100 ml ABTS stock solution.
- ▶ Keep it in dark incubation for 16 hrs.
- ▶ Take vitamin C (Ascorbic acid) as a standard.
- ▶ Add 0.017613 mg Ascorbic acid 100 ml (1 mg/ml).
- ▶ Make 10%, 20%, 30%, 40%, 50% standard solution of vitamin c (1 N).
- ▶ Take 10 µg i.e. 0.1 ml of vitamin c. For 10 %.
- ▶ Take 20 µg i.e. 0.2 ml of vitamin c. For 20 %.
- ▶ Take 30 µg i.e. 0.3 ml of vitamin c. For 30 %.
- ▶ Take 40 µg i.e. 0.4 ml of vitamin c. For 40 %.
- ▶ Take 50 µg i.e. 0.5 ml of vitamin c. For 50 %.
- ▶ After the 16 hours incubation of ABTS stock solution.
- ▶ Take 0.1 mg of standard solution + 0.9 mg of d/w, Add 1 ml of ABTS Solution.
- ▶ Do the same procedure for all samples.
- ▶ Take 0.1 ml of amla wine sample + 0.9 ml of distilled water
- ▶ Take 0.1 ml diluted sample, Add 1 ml of ABTS Solution Place it for 30 minutes dark incubation.

- ▶ Take absorption of samples at 724 nm in calorimeter.

7. Antimicrobial Activity:

- ▶ Petri plates & nutrient agar media were autoclaved.
- ▶ Agar-agar Media is poured in petri plates and kept it for solidify
- ▶ 4 different bacteria suspensions were prepared for spreading on agar plate.
 1. E-coli.
 2. Pseudomonas.
 3. S. aureus.
 4. Bacillus subtilis.
- ▶ After solidifying 0.1ml of each bacteria poured by using micropipette and spreaded over plate.
- ▶ With the help of sterile cork borer 5 wells were made in agar plate.
- ▶ Add samples in wells and add sterile distilled water in middle well as a control and label wells according to sample added.
- ▶ Place the petri plates for incubation at 37°C for 24 to 48 hrs.
- ▶ Check the inhibition of growth around wells.

RESULTS & DISCUSSION

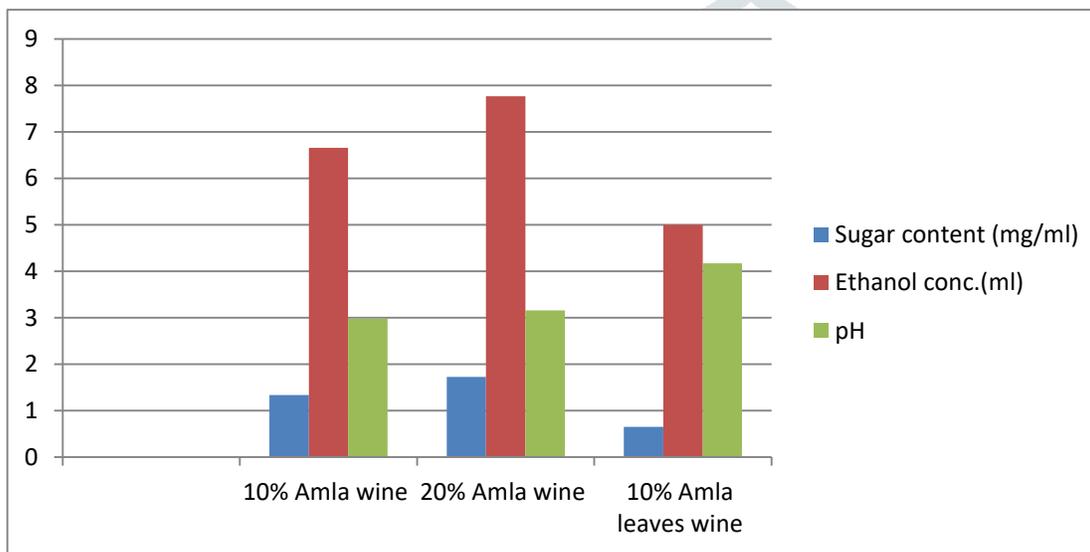
- ▶ The current study was designed to “Evaluation of Antioxidant & Nutritional Activity of Amla Wine & Fermented Amla Leaves” for proximate analysis of raw

materials, sensory analysis, physico-chemical analysis, microbiological analysis.

- ▶ Fermented Amla wine occupy a place in satisfying nutritional requirements of human consumption. Amla fermented and non fermented products have been well recognized to have therapeutic, anti-cholesterolemic, anti-carcinogenic properties. Amla wine is well known product for its enhanced antioxidant & nutritional content requirements because of fermentation by anaerobic fermentation. Amla wine is a sour, bitter, and astringent.,
- ▶ Physicochemical and microbiological analysis
- ▶ Fresh amla fruit and its leaves were procured from the local store in market , where as the fresh amla leaves were collected from amla tree planted in Bhilarewadi Village Farm Katraj, Pune .
- ▶ The freeze dried culture used for preparation of wine using fermentation is *Saccharomyces cerevisiae*. Which is obtained from Sinhgad College Of Science , Savitribai Phule Pune University (SPPU), Pune.
- ▶ Fresh amla and yeast was used for in making of amla wine was analyzed for proximate analysis compositions like pH, acidity, Ethanol, sugar content, antioxidant content, phytochemicals contents such as polyphenols & flavonoids.
- ▶ The amla wine is prepared for 10% amla fruit wine, 20% amla fruit wine, & 10% amla leaves wine.
- ▶ The result obtained from the present investigation has been summarized under following heads:

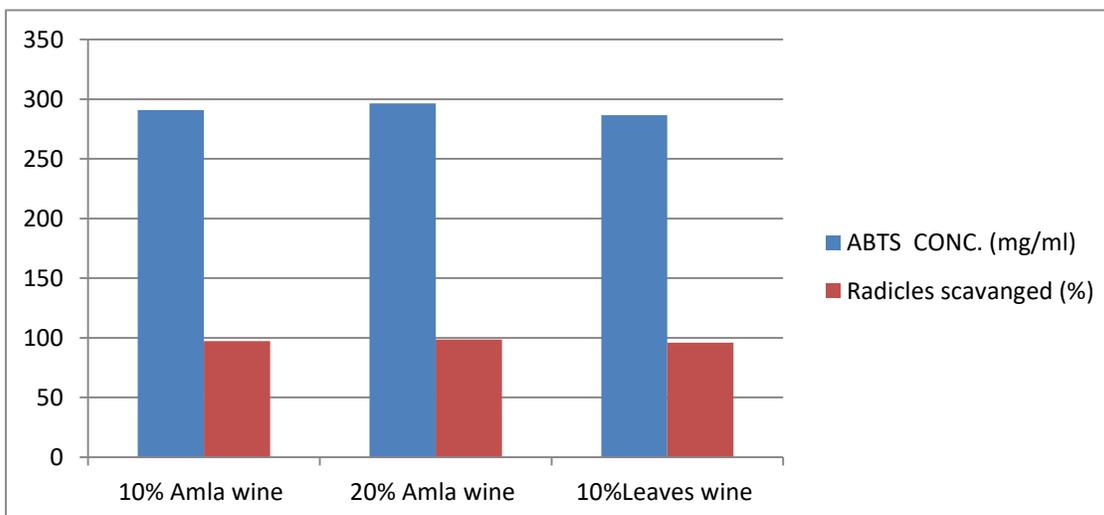
◎ **Chemical Analysis :**

Amla samples	Sugar content (mg/ml)	Ethanol conc. (ml)	pH	Total acidity (N)
10% Amla wine	1.34	6.66	2.98	0.007
20% Amla wine	1.73	7.77	3.16	0.009
10% Amla leaves wine	0.65	5.0	4.17	0.036



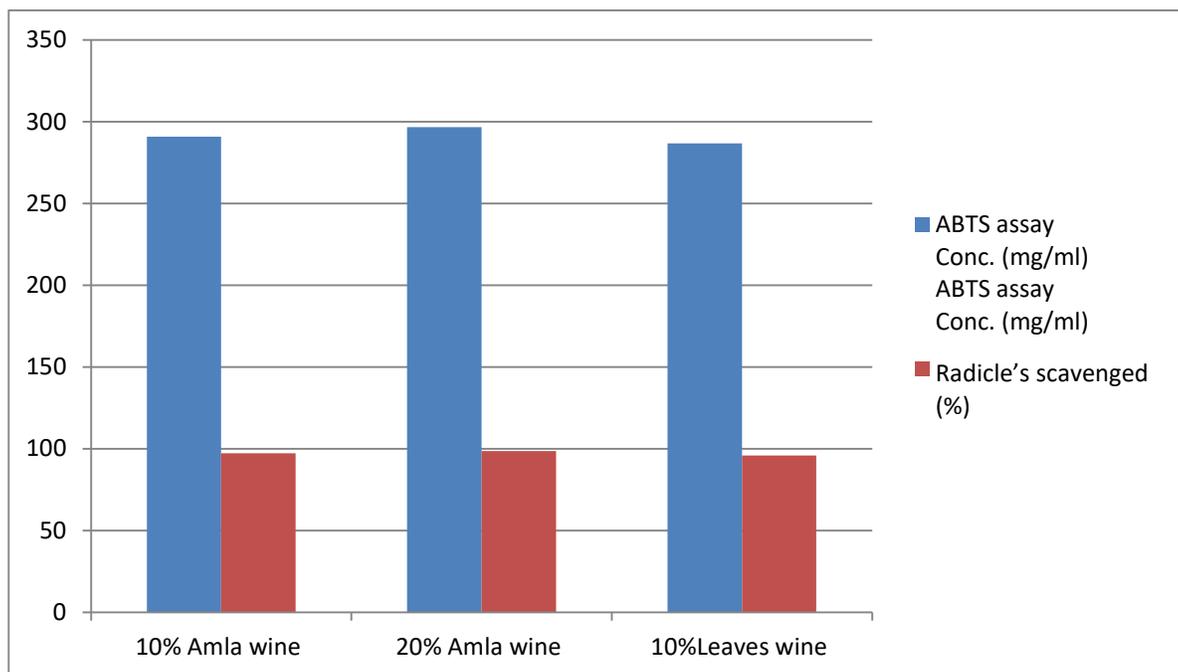
◎ **Antioxidant content :**

Amla samples	ABTS assay Conc. (mg/ml)	Radicle’s scavenged (%)
10% Amla wine	290.83	97.22
20% Amla wine	296.57	98.61
10%Leaves wine	286.70	95.8



◎ Polyphenolic & flavonoids :

Amla samples	Polyphenolic content (mg/ml)	Flavonoids (mg/ml)
10% Amla wine	70	0.33
20% Amla wine	85	0.41
10%Amla leaves wine	77.5	0.52



most

alkaline amla wine is 10% amla leaves wine having ph of 4.17.

SUMMARY & CONCLUSION

1. Maximum sugar content is observed in 20% amla wine 1.73mg/ml
2. Maximum ethanol content is observed in 20% amla wine 7.7%.
3. Most acidic amla wine is 10% amla fruit wine having ph of 2.98 and

4. Wine with highest Polyphenolic content is 20% amla fruit wine having value of 85mg/ml.
5. Wine with highest flavonoids content is 10% amla leaves having value of 0.52mg/ml.
6. Wine with highest antioxidant activity is 10% amla fruit wine having value of 295.83µg/ml.
7. Wine with highest antioxidant radicals scavenged is 10% amla fruit wine having value of 98.6%

8. All samples of wine were showing antimicrobial activity with clear zone of inhibitions.
9. Diameter of zone of inhibition for each wine sample are :
 - a. For 10% amla fruit wine sample: 1.8 cm.
 - b. For 20% amla fruit wine sample: 1.9 cm.
 - c. For 10% amla leaves wine sample: 2.0 cm.
 - d. For solid fermented leaves sample: 1.7cm.

Conclusion:

- ▶ We have got enhanced Antioxidant Activity after fermentation of amla wine.
- ▶ After fermentation sugar content is decreased in amla wine .
- ▶ Anti microbial activity of amla wine is observed only in Bacillus subtilis bacteria.
- ▶ Most alkaline amla wine is 10% amla leaves wine having ph of 4.17 which is best for consumption.

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