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DEVELOP AND FORMULATE POMEGRANATE (Punicagranatum L) JUICE ALONG WITH UTILIZATION OFALOE VERA (Aloe Barbadensis Miller) GEL

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ABSTRACT

Pomegranate with Aloe Vera is a cooling tonic to exhausted the summer heat for Pitta. Pomegranate juice is a polyphenol-rich fruit juice with great antioxidant capacity. In limited studies in human and murine models, pomegranate juice has been revealed to exert significant antiatherogenic, antioxidant, antihypertensive, and anti inflammatory effects. Pomegranate juice significantly decrease datherosclerotic lesionareas in immune-deficient mice and intima media thickness in cardiac patients on medications. It also decreased lipid peroxidation in patients with type 2 diabetes, and systolic blood pressure and serum angiotensin converting enzyme movement in hypertensive patients. Thus, the potential cardioprotective benefits of pomegranate juice deserve further clinical investigation, and evidence to date suggests it may be prudent to include this fruit juice in a heart-healthy diet. The Aloe vera plant has been recognized and used for centuries for its health, beauty, medicinal and skin care properties. Aloe vera gel has been used for medicinal purposes in numerous cultures for millennia: Greece, Egypt, India, Mexico, Japan and China. Aloe vera gel contains 75 potentially active constituents: vitamins, enzymes, minerals, sugars, lignin, saponins, salicylic acids and amino acids. Aloe vera gel is beneficial in the treatment of gastrointestinal problems, such as indigestion, yeast, heartburn and irritable bowel syndrome. Aloe vera extract increased cell tolerance to glucose in healthy and diabetic rats, and the usage of aloe vera juice (4 - 14 weeks) had a significant hypoglycemic effect.

KEYWORDS: *Pomegranate, Aloe Vera, Antiatherogenic, Active Constituents, Hypoglycemic Effect.*

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INTRODUCTION

The foremost supplement included in diet of people from time being are the fruits, as fruits are considered significant in body functioning. Fruits are rich in essential elements, such as water, vitamins, minerals (Ca, Mg, Zn, Fe, K etc.), fibers and other organic components, which renders fruit, through nutritional quality and importance. The bioactive components and antioxidants are typically found in significant amounts in fruits, namely apple, berries, cherries, grapes, pomegranate, citrus fruits The fruit-based foods like juices are considered a key source of nutrition and hence consumed globally, in fresh, packaged, ready to serve forms. Moreover, the juice acquired from citrus fruits cover aromatic volatiles, such as alcohols, esters, aldehydes, ketones and non-volatile compounds such as, sugars and organic compounds. The foremost component of fruit juice is water, followed by carbohydrate content (sucrose, fructose, glucose, and sorbitol), which is found in 0.44 kcal/mL to 0.64 kcal/mL . This can be associated to the human milk that contain 7 g percent of carbohydrate content. The functional foods are developed and standardized from fruits due to its valuable nutraceutical attributes, along

with rising research and studies that help understand functional and healthiness promoting components of fruits. Fruits and fruit juices are brilliant source of phytonutrients or phytochemicals, that help in preventing diseases, builds immunity and defends intra-cellular and extra-cellular oxidative damages caused due to free radicals.

A literature study of rise in antioxidant activity of serum or reduction in oxidative harms of biomolecules, drew the comparison between fruit juice, green tea and black tea. The study exposed that pomegranate juice possesses cardioprotective characteristics, decreased or reversed the progression of ischemic lesion regions, and positively affected intimal media thickness and systolic blood pressure. The studies have also stated that pomegranate juice has positive effects in patients on multiple medications associated to heart diseases, thus confirming to be the good source of add on to the phytotherapy in patients. The **pomegranate** or pomegranate juice contains major content of ellagitannins and ellagic acid, which are otherwise obtained by hydrolysis of tannins, whereas, Gallic Acid (EA) is found pomegranate in distinct quantities. However, ellagitannins when metabolized by intestinal microflora forms EA and the compound is also analogous to urolithins. The urolithins are reported to be systematically bioavailable where they accumulate in organs such as colon, prostate and intestine. **Stevia** is a high intensity non-nutritive sweetener and has wide range of application, while extensively used as sweetener, nowadays in food industry. This white, crystalline and odorless powder, extracted from leaves of *Stevia rebaudiana Bertoni*, a plant native to north eastern Paraguay, termed as Stevia, is approximately 300 times sweeter than sucrose \. *Stevia rebaudiana* plant species contains most potential sweetening agents compared to other Stevia plant species. The sweetening agent extracted from this plant species is Diterpene glycosides, a natural sweetener. The leaves of wild Stevia plant contain 0.3 percent dulcoside, 0.6 percent rebaudioside C, 3.8 percent rebaudioside A and 9.1 percent stevioside. **Aloe vera** (*Aloe barbadensis Miller*) belongs to Lileacea family (Volger& Ernest 1999), fresh leaves of which are utilized for extraction of bitter yellow latex from aloe vera sap (peripheral bundle sheath) and a mucilaginous gel, which has increased utility in health care and cosmetics industries. The major component of aloe vera leaf is water (95%) and contains nutrients like vitamins, amino acids and minerals, bioactive compounds, enzymes, all contribute to its functional characteristics. The gel obtained from aloe vera has a bitter taste, but its palatability could be improved with addition of fruit juices. Aloe vera has been used by Homoeopathic, Allopathic and Ayurvedic practitioners around the globe attributing to its properties such as emollient, anti-inflammatory, purgative, anti-microbial, aphrodisiac, antifungal, antioxidant characteristics and other reasons like cosmetic qualities.

The food manufacturing industries, namely juice sector which focusses on preservation of juice products without decreasing the organoleptic properties, by the pasteurization method. Thermal treatment is one of the pasteurization methods for preservation of juices, used in juice production industries. But thermal processing leads to degradation of heat-liable antioxidants and essential compounds in the juice. Additional pasteurization technique, microwave heating, generates volumetric heating within the food. This results in microwave heating for short period and produce high quality food products that are self- stable. Therefore, this electrical method, microwave heating preserves organoleptic characteristics of food during processing. The method disables enzymes and a microorganism in less time compare to other conventional heating methods and also prevents the loss of significant compounds from food.

MATERIALS AND METHODS

Pomegranate

Pomegranate of variety „Bhagwa' was bought from a local market in Hadapsar, Pune, and stevia leaf powder was taken from medical store as the experimental material for this study. The fresh fruits and aloe Vera were collected for the experiment.

plate 1 – *Pomegranate of variety 'Bhagwa'.*

Figure 1

Aloe Vera Leaves

Aloe Vera leaves of variety „Barbadensis Miller' was taken from a local market in Hadapsar, Pune, and stevia leaf powder was bought from medical store as the experimental material for this study. The fresh aloe Vera were collected for the experiment.

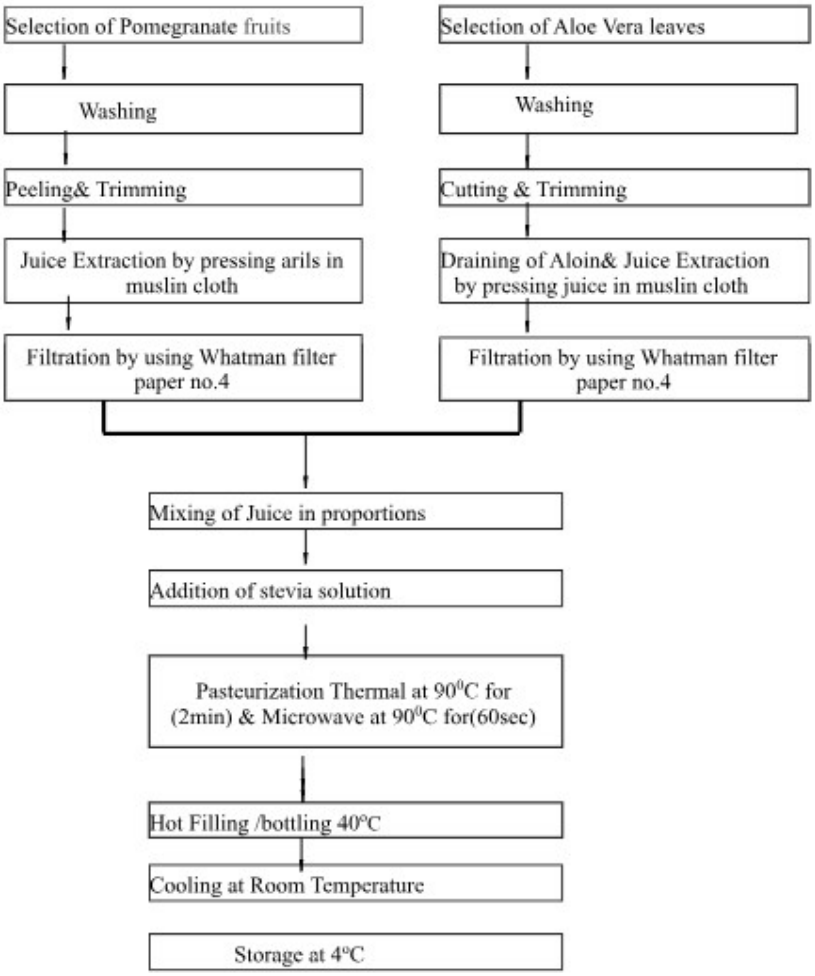
Plate 2. - *Aloe vera* leaves of variety *„Barbadensis Miller'*

Figure 2

Stevia Powder

Stevia leaf powder was bought from medical store as the experimental material for this study.

Flow Sheet for Preparation of Pomegranate and Aloe Vera Mix Juice



Fruit Selection and Juice Extraction

Pomegranate of variety Bhagwa' and Aloe Vera leaves of variety Barbadensis Miller' was bought from a local market in Hadapsar, Pune, and stevia leaf powder was taken from medical store as the experimental material for this study. The fresh fruits and aloe Vera were collected for the experiment. The Pomegranates and aloe vera leaves were thoroughly cleaned. The skin of the pomegranate was peeled and clipped to separate the arils from the skin. Aloin was drained appropriately from aloe vera leaves, then peeled and clipped to extract aloe vera gel from the skin.

Filtration of Juice

A triple layer of muslin cloth was employed to stop contamination and big particles from the juice of the pomegranate and aloe vera that had been extracted. The juices were chilled separately for a full night at 4°C. After 24 hours, Whatman filter paper no. 4 was used to filter each juice in order to produce clarified juice.

Figure 3

Mixing of Juice

The clarified juice of aloe vera and pomegranate juices and stevia were mixed in proportions as per the below table.1 and the treatments are labeled as S0, S1, S2, respectively with an increasing proportion of stevia solution by 0.5% in each trial.

Table 1: Treatment of Formulation of Juice for Thermal and Microwave Pasteurization

Treatments	Pomegranate Juice	Aloe vera Juice	Stevia
S0	80%	20%	1.5%
S1	80%	20%	2%
S2	80%	20%	2.5%

Pasteurization of Juices

Thermal pasteurization of all juice samples (pomegranate, aloevera with juice addition) remained performed at 90 ^{0}C for 2 minutes. And the Microwave Pasteurization at 90 ^{0}C for 60 seconds

Table 2: Pasteurization Time and Temperature for Juice

Thermal Pasteurization		Microwave pasteurization	
Time	Temperature	Time	Temperature
4 min	70	120 sec	70
3 min	80	80 sec	80
2 min	90	60 sec	90

Bottling

The juice was pasteurized using thermal and microwave pasteurization techniques. It was formerly chilled at 40 °C, poured into clean, pre-sterilized dry food-grade PET bottles with a capacity of 50 ml, left with 2.0 cm of headspace, and then sealed airtight using a screw type lid.

Storage

Both the experimental sets of (Thermal and Microwave pasteurized) were stored at refrigeration temperature (4+10C).

RESULTS AND DISCUSSION

Analysis of Physicochemical Properties of Raw Materials at 0 Days

The physicochemical properties such as color, pH, TSS, acidity, antioxidant (DPPH) % Inhibition, antioxidant (FRAP) mg/ml AAE) and phenolic content (mg/ml GAE) of raw materials (pomegranate fruit and aloe vera) were determined and recorded in table 4 The color of pomegranate fruit and aloe vera leaf was observed that reddish- violet and light green, respectively. Whereas, the pH value was found to be 3.9±0.0 for pomegranate juice and 4.6±0.0 for aloe vera juice. The Total Soluble Solids (TSS) of pomegranate juice and aloe vera was expected to be 13.8±0.0 and 3.7± 0.01, respectively, while it was also determined that the acidity of pomegranate juice (4.8±0.70) is greater than aloe vera gel/juice (1.27±0.64).

The antioxidant content was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and Ferric Reducing Ability of Plasma (FRAP). The study exposed that antioxidant content evaluated by DPPH (89.8± 0.1% inhibition) was more than FRAP method (58.4 ± 0.78mg/ml AAE). The similar results were obtained for antioxidant content approximation of aloe vera juice, wherein DPPH results presented antioxidant content of 58.3± 0.1% inhibition and FRAP method showed antioxidant content of 24.7± 0.64mg/ml AAE. The nonsignificant difference was observed in the projected results of phenolic content of aloe vera and pomegranate juice, as 1.31±0.1mg/ml GAE and 1.48±0.1mg/ml GAE, respectively. The total phenolic content results were in accordance to the literature Keskin-Šašić *et al.,* 2012. The results showed that the phenolic content of pomegranate juice was greater compared to that of aloe vera.

Table 3: Physicochemical Analysis of Raw Materials at 0 Days

Parameters	Pomegranate	Aloe Vera
Color	Reddish-Violet	Light Green
pH	3.9± 0.0	4.6± 0.0
TSS	13.8± 0.0	3.7± 0.01
Acidity	4.8±0.70	1.27±0.64
Antioxidant (DPPH)% Inhibition	89.8± 0.1	58.3± 0.1
Antioxidant (FRAP) mg/ml AAE	58.4 ± 0.78	24.7± 0.64
Phenolic Content (mg/ml GAE)	1.48± 0.1	1.31± 0.1

*Each Value is Average of Three Determination ± SD

Formulation and Standardization of Pomegranate Aloe Vera Mix Juice

The pomegranate aloe vera mix juice formulation was standardized by using ingredients namely, pomegranate juice, aloe vera juice and stevia.

Table 4: Formulation of Pomegranate Aloe Vera Mix Juice with Addition of Stevia

Sample	Pomegranate Juice (ml/100ml)	Aloe Vera Juice (ml/100ml)	Stevia (ml)
S0	80	20	0
S1	80	20	1.5
S2	80	20	2
S3	80	20	2.5

The ratio of pomegranate juice to aloe juice was kept continuous as 80:20 and the quantity of stevia was modified. Thus, four formulations of pomegranate and aloe vera juice mix were prepared, namely S0, S1, S2 and S3, as shown in table 4. The proportion of pomegranate juice: aloe vera juice: stevia in S0, S1, S2 and S3 was 80:20:0, 80:20:1.5,

80:20:2 and 80:20:2.5, respectively. S0 was viewed as control formulation, without stevia content. All the four formulations were evaluated for sensory characteristics.

Sensory Evaluation of Pomegranate Aloe Vera Mix Juice

All three formulations of pomegranate aloe vera mix juice with addition of stevia were prepared and evaluated for sensory characteristics such as color, appearance, mouthfeel, flavor and overall acceptability, by a semi-trained panel, using a 9-point hedonic scale. The data taken from the sensory evaluation of S0, S1, S2 and S3 formulations, exposed that formulation S2 obtained a maximum score (overall acceptability) of 8, with scores for color (8), appearance (8), mouthfeel (8.1), flavor (8), taste (8) overall acceptability as shown Thus, formulation S2 was selected for further analysis and study.

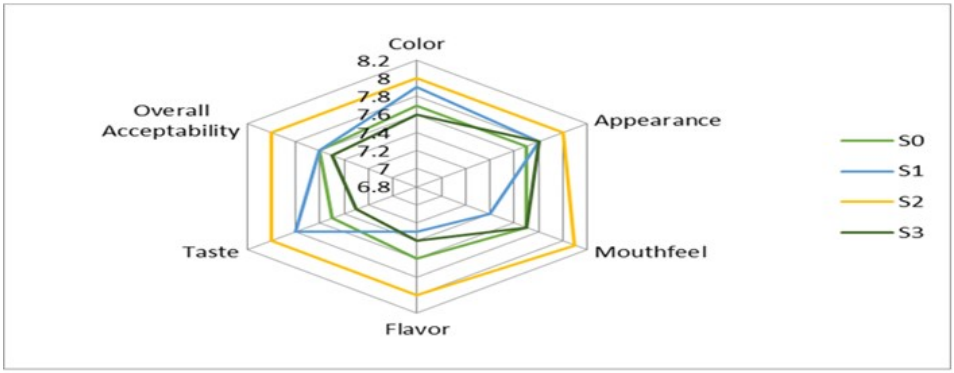


Figure 4

Analysis of Physicochemical Properties of Pomegranate Aloe Vera Mix Juice

The physicochemical attributes of the developed and standardized formulation were determined and noted in table 7. The color of developed pomegranate aloe vera mix juice was found to be reddish, whereas the pH and TSS was expected to be 3.9±0.0 and 13.2± 0.0, respectively. The acidity of developed juice was found to be 3.3±0.68 and phenolic content was estimated as 1.36± 0.1mg/ml GAE. The antioxidant content was determined by DPPH and FRAP assay and the results indicated the content to be 84.8± 0.1% inhibition and 42.6± 0.76mg/ml AAE, respectively

Physicochemical Properties of Pomegranate Aloe Vera Mix Juice at 0 Days

Table

Parameters	Values
Color	Red
pH	3.9± 0.0
TSS	13.2± 0.0
Acidity	3.3±0.68
Antioxidant (DPPH) % Inhibition	88.8± 0.1
Antioxidant (FRAP) (mg/ml AAE)	42.6± 0.76
Phenolic Content (mg/ml GAE)	1.36± 0.1

*Values are mean of triplicate readings

The values of antioxidant activity (DPPH & FRAP) were reduced compared to the fresh pomegranate juice. This could be attributed to the addition of aloe vera juice. The total phenolic content of aloe vera extract was valued to be 2.7 mg of GAE/100 g, in the study of antibacterial activities and antioxidant capacity of aloe vera, by Nejat Zadeh, 2013. The antioxidant content of aloe vera juice was estimated to be little compared to the pomegranate juice.

Microbial Analysis of Pomegranate Aloe Vera Mix Juice

The analysis of microbial load was achieved by determining Total Plate Count of two variants of developed pomegranate aloe vera mix juice for 42 days. The two alternatives were thermal pasteurized pomegranate aloe vera mix juice and microwave pasteurized pomegranate aloe vera mix juice, which were analyzed with the interval of 7 days, as recorded in table .

Furthermore, the temperature of thermal pasteurization and microwave pasteurization was determined by conducting microbial analysis, i.e., Total Plate Count of control (neither thermal nor microwave treatment) and developed product treated with thermal pasteurization at 70 ^{o}C, 80 ^{o}C and 90 ^{o}C for time period of 2 minutes. Similarly, the Total Plate Count of microwave pasteurized developed product was determined for temperatures 70 ^{o}C, 80 ^{o}C and 90 ^{o}C for time period of 60 seconds. The results exposed the presence of several bacterial colonies at 70 ^{o}C and 80 ^{o}C of thermal treatment, whereas at 90 ^{o}C the bacterial colonies were absent. The presence of bacterial colonies at 70 ^{o}C and 80 ^{o}C of microwave treatment was detected, while the total plate count was found to be zero at 90 ^{o}C. Therefore, the temperature of thermal and microwave pasteurization was standardized as 90 ^{o}C.

The results of microbial analysis discovered that the negligible bacterial count was observed for 35 days in the thermal pasteurized (90 ^{o}C for 2 minutes) sample, whereas the sample developed 1.8 CFU per ml of bacterial count. Additionally, no development of bacterial colonies was observed for the developed juice treated with microwave pasteurization (90 ^{o}C for 60 seconds).

Table 5: Microbial Analysis of Pomegranate Aloe Vera Mix Juice

Treatments	Days						
	0	7	14	21	28	35	42
Thermal (CFU/ml)	ND	ND	ND	ND	ND	ND	1.8
Microwave (CFU/ml)	ND	ND	ND	ND	ND	ND	ND

*Values are mean of triplicate readings

Storage Studies of Pomegranate Aloe Vera Mix Juice

The study on the effect of storage period on the functional or bioactive properties of the developed pomegranate aloe mix juice cured with thermal pasteurization and microwave pasteurization was conducted. The selected formulation was stored at refrigeration temperature at 4±1 ^{o}C. for 42 days and the bioactive properties were examined with the time interval of 7 days, starting from 0 days.

SUMMARY AND CONCLUSION

Consuming a variability of fruits or drinking fruit juices is the best approach to gain all of the vitamins, minerals, micronutrients, and antioxidants we need. Among all fruits, pomegranate have relatively great antioxidants and phenolic compounds and aloe vera has been proven for its medicinal and nutritional properties and hence, pomegranate and aloe vera were selected for further studies and formulation of the juice. The pomegranate fruit is well-known not only for its medicinal and health benefits, but also because it is high in antioxidants, dietary fibers, vitamins, and minerals. Aloe vera has been used for medicinal purposes for thousands of years. It is used in the pharmaceutical, food, and cosmetic industries all around the world due to its adaptability. Pomegranate juice and aloe vera juice were mixed in 80:20 proportion and stevia in various proportions, and the important physicochemical properties like total phenol content, the antioxidant activity of these mixed juices were analyzed. The thermal and microwave pasteurization effect and storage effect (42 days

at 4°C) on bioactive properties was also ascertained in this study. The pH of developed juice was reduced overtime during storage same as TSS of the juice which was also decreased overtime during the storage period but the increased trend in the acidity of the juice was recorded during storage time.

The consequences from sensory study exhibited Pomegranate: aloe vera, (80:20:2) have recorded the utmost acceptability in terms of color, flavor, mouthfeel, taste, overall acceptability. The microwave pasteurization of the juice was carried out at 90°C for 60 sec resulted in a significant loss in the nutrient content as well as antioxidant activity of juice which was may be due to heat intolerance and reduction of heat sensible limitations of the juice Whereas in thermal pasteurized juice was carried out at 90 ^{0}C for 2min resulted extra loss as compared to microwave pasteurization. The juice samples when stored at 4 ^{0}C statically non- significant reduction in the bioactive compounds was noted in both the juices until 42 days. The antioxidant activity (DPPH and FRAP) of the mixed juice showed a decreasing trend during the storage period of 42 days as antioxidant activity (DPPH) reduced by 4% and the antioxidant activity (FRAP) decreased up to 2%consistently. The Total Phenol Content were also found to be reduced during a storage period of 42 days that is 11% respectively. This is because anthocyanins are heat and pH sensible compounds which displays different characteristics on different pH levels.

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