

Synopsis Physicochemical Stability, Antioxidant Activity, and Acceptance of Bael Incorporated RTS

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Submitted on,

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CERTIFICATE

This is to certify that **Anshid V** (Registration No. **11710516**) has personally completed M.Sc. Dissertation-I entitled, "**Physicochemical Stability, Antioxidant Activity, and Acceptance of Bael incorporated RTS**" under my guidance and supervision. To the best of my knowledge, the present work is the result of her original investigation and study. No part of pre-dissertation has ever been submitted for any other purpose at any University.

The project report is appropriate for the submission and the partial fulfillment of the conditions for the evaluation leading to the award of Master of Food Technology.

Date: ____- - 2018

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DECLARATION

I hereby declare that the work presented in the dissertation 1 entitled "Physicochemical Stability, Antioxidant Activity, and Acceptance of Bael incorporated RTS" is my own and original. Work will be carried out by me at School of Agriculture, Lovely Professional University, Phagwara, Punjab, India under the guidance of **Dr. Anil Panghal** (20785), Research & Consultancy Coordinator & Associate Professor, School of Agriculture, Lovely Professional University, Phagwara, Punjab, India, for the award of the degree of Master of Science in Food Technology.

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I certified that the above statement made by the student is correct to the best of my knowledge and belief.

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INTRODUCTION

Aegle mermelos (L.) corr. (Bael) is known as a primitive plant of India having several medicinal properties and is from the family Rutaceae. Bael tree is pre-historically well-known inside India and regarded as a sacred tree, Yajurveda and Ramayana mentioned about bael in it, because of this bael has great methodological significance. Importance 11of bael increases with its curative properties, each part belongs to the plant (root; trunk; bark; leaf; flower; fruit and seed) are used in formulation of ayurvedic medicines. Purification of bael produce several biologically active compounds, can act against diseases like cancer and cardiac vascular diseases. Characteristics of ripe bael fruit include sweet, aromatic, nutritious and highly palatable. Bael fruit cannot be eaten out of hand because of several reasons includes hardness of shell, mucilaginous texture and number of seeds and content of fibre. Pulp of bael fruit contains carotenoids; phenolics; terpenoids; coumarins; flavonoids and alkaloids these are functionally bioactive in nature. By using bael fruit various by products can be developed. Products like candy, toffy, RTS, etc. Details incorporated further in this paper deals with the bael fruit processed product development studies, its medicinal properties and related composition of particular bael fruit.

The fruit of bael plant (Aegle marmelos) is richly available India (Family- Rotaceae) (Jindal et al, 2012). Commonly known as bael in Hindi and in the Ayurvedic Pharmacopoeia of India is a official fruit (Rana et al, 2012). Fruit composed of important compounds like carotenoids; phenolics; alkaloid; terpenoids; coumarins; flavonoids and pectins and these are bio-active in nature (Rana et al, 2012). The amount of total sugar present is low also contains slight quantity of non-reducing sugar; tannins and reducing sugar (Rana et al, 2012). Amount of pectin is high in bael fruit (Rana et al, 2012). In unripe bael fruit each seed is enveloped by gum. The gum present around each seed helps in showing adhesive, water proofing also oil emulsion coating properties (Jindal et al, 2012). As the bael fruit is edible its recommended use actions include anti-amoebic, anti-diabetic and anti-histaminic (Rana et al, 2012). Considering different indigenous fruits of India bael fruit occupies an imperative place. Jain literature and Buddhist literature mentioned about bael product and also about the same was mentioned in Vedas and Ramayana (Singh et al, 2014). The marmelosin ($C_{13}H_{12}O_3$) and it is known as a remedy for all stomach based problems and is found in the bael fruit (Singh et al, 2014). Bael's high nutritional and medicinal properties is because of its good source of nutrients and vitamins which are most valuable with their properties (Singh et al, 2014). The unripe fruit of bael prescribed for diarrhoea and dysentery treatment and unripe fruits are astringent; digestive; stomachic (Singh et al, 2014). Bael tree is spiny and richly grown under sub-tropical and tropical climatic conditions of India as well as other African and Asian countries (Patra et al, 2015). It's used as a ethno medicine as a solution for various human ailments and this property of bael is present all the stages of maturity of the fruit (Patra et al, 2015). The bael fruit consist of shell very hard in nature and the pulp (edible) inside the fruit seen in yellowish or orange in colour which is sweet slightly and having a unique flowery odour and pleasant flavour (Patra et al, 2015). The composition of mash is the reason behind the protective property against several chronic diseases (Patra et al, 2015). The soluble and in-soluble mucilaginous dietary fibre and pectin are together called total dietary fibre present in bael fruit (Patra et al, 2015). In addition to this fibres bael fruit composed of many minerals and vitamins such as thiamine; riboflavin; vitamin C; vitamin A; calcium; niacin and phosphorus (Patra et al, 2015). Because of the richness of amino acids; vitamins and minerals bael can give important contribution to maintain nutrient requirement and to prevent various deficiency disorders (Patra et al, 2015). Bael is used in production of herbal medicines. The matured and ripened bael fruit is consumed freshly and also used in production of nectar; squash; jam; marmalade and cream (Patra et al, 2015). Bael fruit contains ant nutritional factors that help in controlling blood sugar (Asadullah et al, 2015). In Thailand bael fruit is dried and packed in tea bags and also preserved in syrup for the further use as desserts and ingredient for cake (Asadullah et al, 2015). The bael leaves have been claimed to be traditionally utilized for the amelioration of numerous diseases or disorders mainly to treat inflammation asthma; hypoglycaemia; febrifuge; hepatitis and is also used as analgesic (Asadullah et al, 2015). Typical bael fruit image Fig 1.



Fig 1: Typical bael fruit image (Source: https://www.google.com/)

In Thailand, the immature leaves and shoots are consumed as vegetables and young leaves and shoots are consumed as seasonal food in Indonesia. Inside Pakistan, no information available on nutritional profiles of parts bael grown (Asadullah et al, 2015). Usually processed products from bael includes preserves; refreshing beverages powder; leather; squash; nectar; jam; syrup. For bael based product development pulp is the pre-requisite (Singh et al, 2014).

Bael plant take harmful poisonous gas from surrounding atmosphere and make them inert or neutral to remove their poisonous property. In plant species group bael plant is called as "climate purifier". As compared other plants bael can release higher amount of O_2 in presence of sunlight (Bansal et al, 2007). Bael is included in a category of 'Fragrant' species, which can neutralize the bad odour of the air by deodorization process.

PROBLEM BACKGROUND

Bael having amazing medical advantages, restorative advantages, custom utilize and home remedy. Commendable flavour and activity as resuscitating refreshment of bael incorporated beverages increases its importance. Big scale production of bael used products is not yet happened only unorganized sectors are there in case of bael related product processing. Bael used product development and its business sides as value added products also impressive. To find the unexplored facts based on bael and bael incorporated products efficiently engaged research is required. It is a best home cure which battles with different everyday life medical issues like obstruction; peptic ulcer; acid reflux; respiratory issues; the runs; loose bowels; heaps; diabetes; sexual dysfunctions thus numerous. Wood apple gives insusceptibility to battle different contaminations like bacterial; viral: parasitic every day utilization of the bael helps in regarding normal stomach related issues, for example, looseness of the bowels; blockage; cholera; haemorrhoids; and numerous more because of its accessibility of tannin. Bael content high nutritive esteem which is exceptionally utilize full for the wellbeing point view.

REVIEW OF LITRATURE

Bael Fruit

Fruit is the most prized part of the plant. The bael product, fruit is a pyriform, may fluctuate from oval to round and measure differs from 10 to 20 cm in width. The fruit is utilized to prepare juice; to stick: squash; jam toffee and different items. The mash of bael fruit contains water; sugars; protein; fibre; protein; fat: calcium; phosphorous; potassium; iron; minerals and vitamins (Chakraborty et al, 2012). Data related to physical parameters of the fruit is delineated in **Table 1**.

Plant Profile

| Scientific classification. (Sood and Katoch, 2014) | <i>Vernacular names.</i> (Chaurasiya et al, 2015) |
|---|--|
| Kingdom: Plantae. | Hindi: Bel, Beli, Belgiri. |
| Sub-kingdom: Tracheobionta. | Sanskrit: Bilva, Shiva druma, Shiva phala, |
| Super division: Spermatophyta | Vilva. |
| Division: Magnoliophyta | English: Bael, Bengal quince, Golden apple. |
| Class: Magnoliopsida | Urdu: Bel, Bel kham. |
| Sub-class: Rosidae | Assamese and Marathi: Bel. |
| Order: Spanidales | Malayalam: Marredy. |
| Family: Rutacae | Oriya: Belo. |
| Genus: Aegle | Tamil: Vilvama, Vilva marum. |
| Species: marmelos | Telugu: Bilva, Bilava pandu. |
| | 1 |

| PARAMETERS | VALUE |
|--------------------------|-----------------|
| External colour | Brownish yellow |
| Pulp colour | Bright yellow |
| Weight (g) | 1120 |
| Polar diameter (cm) | 12.96 |
| Transverse diameter (cm) | 13.35 |
| Specific gravity (g/cc) | 1.11 |
| Volume (I) | 1.01 |
| Peel (%) | 24 |
| Pulp (%) | 68 |
| Seed (%) | 1.3 |
| Shape (%) | Roundish-oblong |

Table 1 physical parameters of the fruit is delineated (Amarjeet kaur and manoranjan kalia 2017).

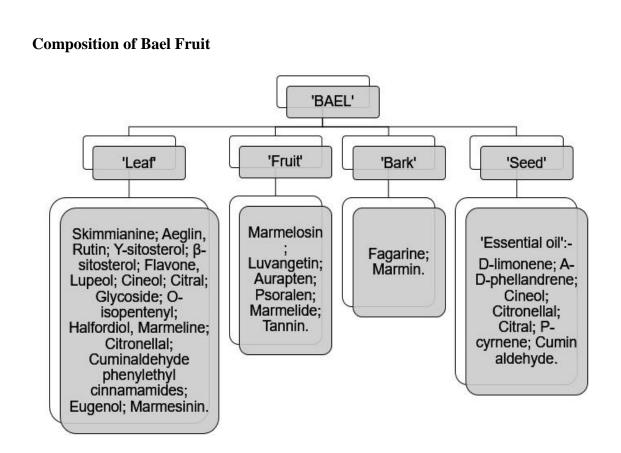


Fig 3 Different alkaloids; different Carotenoids; different Coumarins also Flavonoids Terpenes present in bael.

Presence of vital bioactive compound in pulp of bael fruit is proven with th help of different bael based researches. Bio active elements in bael includes terpenoids; coumarins; carotenoids; phenolics; flavonoids; alkaloids; tannins and pectins. Other than these some volatile compounds such as β -caryophyllene; isoamyl acetate; hexadecanoic acid; β -ionone; Hexanal; acetoin; carvone; 3,5octadiene-2-one; p-cymene; dihydro-β-ionone; (E)- 2-octenal; humulene oxide; αhumulene; α -cubebene; caryophyllene oxide; limonene; (E,E)- 2,4 heptadienal; citronellal; β cubebene; dehydro-p-cymene; citronella; cineole; verbenone; p-cymene; pulegone; β-phellandrene; hexadecane; 3,5-octadiene-2-one; verbenone; trans-p-mentha-2,8-dienol; (E)- 6,10-dimethyl-5,9undecadien-2-one; linalool; carvyl acetate; cuminaldehyde; citral (Suvimol and Pranee, 2008). They additionally contain marmelosin and coumarins like o-isopentenyl halfordinol; marmelin; alloimperatorin; furocoumarins; aegeline; o-mtheyl halfordinol; aegelenine; psoralen. Likewise they also contain linoleic acid; phlobatannins; anthocyanins; tannins; tartaric acid; flavonoid glycosides; leucoanthocyanins and flavon-3-ols (Maity et al., 2009). Different phytochemicals present different parts of the bael plant is delineated in **Fig 2**. Data related to chemical constituents of bael fruit; mineral constituents of bael (mg/ 100 g); sugar content in bael (percent); anti-nutritional content in bael; ascorbic acid content in bael (mg/100 g) of bael pulp; seed and pericarp delineated Table 2. Different alkaloids; different Carotenoids; different Coumarins also Flavonoids Terpenes present in bael is delineated in Fig 3.

| | <u>(</u> | CHEMI | CAL CONSTITU | <u>ENTS OF BAI</u> | EL FRU | <u>JIT</u> | | | | |
|----------------------------|----------|-------------|----------------------|--------------------|----------------|---------------|---------|------|--|--|
| Parameter | Moisture | pН | Acidity Crude As | | Ash | Crude | Crude | TSS | | |
| | (%) | (%) | (% citric acid) | protein (%) (%) | | fibre (%) | fat (%) | brix | | |
| Pulp | 61.06 | 4.95 | 0.30 | 3.64 | 2.85 | 4.80 | 0.43 | 36 | | |
| Seed | 31.8 | 5.49 | 0.06 | 1.01 | 4.02 | - | 1.08 | - | | |
| Pericarp | 38.92 | 5.28 | 0.29 | 1.31 | 3.18 | 30.65 | 0.06 | - | | |
| | <u>M</u> | INERA | <u>L CONSTITUEN</u> | TS OF BAEL | <u>(mg/ 10</u> | <u>)0 g)</u> | | | | |
| Mineral Cu Zi Ca K P Mg Fe | | | | | | | | | | |
| Pulp | 0.19 | 0.28 | 78.00 | 603 | | 51.60 | 4.00 | 0.55 | | |
| Seed | 0.01 | 0.03 | - | 108 | | 3.30 | 0.82 | 0.08 | | |
| Pericarp | - | 0.02 | 6.00 | 210 | | 2.80 | 0.91 | 0.02 | | |
| | | <u>SUGA</u> | <u>R CONTENT IN</u> | BAEL (PER C | CENET | <u>)</u> | | | | |
| Sugar | Reducing | sugar | Non - reducing Sugar | | Total sugar | | | | | |
| - | | | sugar | _ | | | | | | |
| Pulp | 4.42 | · | 9.93 | - | | | 14.35 | | | |
| Seed | - | | - | - | | | - | | | |
| Pericarp | 0.92 | r | 0.91 | - | | | 1.83 | | | |
| | | ANTI | -NUTRITIONAL | CONTENT IN | N BAEL | <u>_</u> | | | | |
| Constituent | Tannic a | cid (Gal | llo-tannic acid) | | Oxala | ates (g/100 g | g) | | | |
| Pulp | | 0.2 | 2 | | | 0.96 | | | | |
| Seed | | - | | | | 0.20 | | | | |
| Pericarp | | 1.0 | 03 | | | 0.30 | | | | |
| | AS | SCORB | IC ACID CONTE | <u>ENT IN BAEL</u> | <u>(mg/10</u> | <u>0 g)</u> | | | | |
| Pulp | | | | 22.5 | | | | | | |
| Seed | | | | 2.80 | | | | | | |

Table 2 Chemical constituents of bael fruit; mineral constituents of bael (mg/ 100 g); sugar content in bael (per cenet); anti-nutritional content in bael; ascorbic acid content in bael (mg/100 g) of bael pulp; seed and pericarp (Amarjeet kaur and manoranjan kalia 2017).

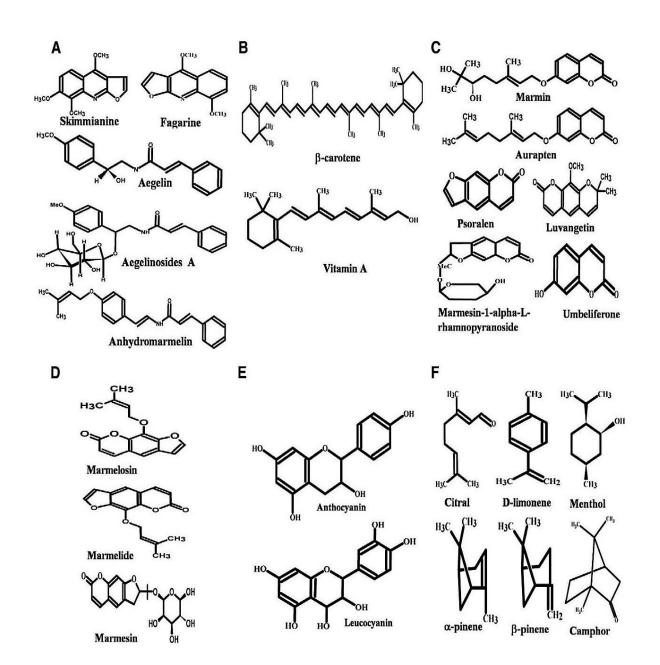


Fig 3.Different alkaloids; different Carotenoids; different Coumarins also Flavonoids Terpenes present in bael is delineated.

Remedial Importance of Bael

• Diarrhea and dysentery

Half ripe or full ripe bael fruit can act as a medicine against chronic diarrhea and dysentery without fever. Consumption of bael helps in making the faecal matter excrete in solid form and prevented the loss of blood through fecal matter (Sharma et al., 2006; Patel et al., 2012)

• Antinuclear action

Bael having ability to show antinuclear activity, bael shows gastro protective activity. In gastro intestinal tract gastroduodenal ulcer is a disease generally seen. Because of the presence of a compound called in Luvangetin bael fruit it can deliver a hindrance to absolute ethanol induced gastric mucosal harming. Gastric ulcer is induced mostly because of the oxidative stress this can also be prevented by the compound Luvangetin (Maity et al., 2009).

• Anti-diabetic action

Bael extract consumption with a dose accordingly 250 milligram per kilogram of body mass acts against diabetics. Bael extract can give better results than glycenamide is an anti-diabetic drug. These characteristic anti-diabetic action of bael fruit is due to the presence of particular compound called coumarins, inducing the activity of beta cells of islet of Langerhans producing insulin, helps in controlling diabetics (Kamalakkannan and prince 2003).

• Anti-hyperlipidemic activity

Hydrolysis of triglycerides mobilize deposited fat helps in reduction of lipid in level present blood. Oral intake of watery concentrate of bael fruit and the seed and related components separately at a measured dose of 250 milligram per kilogram of the body helps to regulate the triglycerides hydrolysis (Kamalakkannan and prince 2003).

• Acting against oxidation

Bael extract consumption with a dose accordingly 250 milligram per kilogram of body mass maintain the action of reactive oxygen species scavengers (ROS). ROS scavengers includes Glutathione reductase; glutathione peroxidase and SOD (superoxide dismutase). Presence of tannins; flavonoid glycosides; flavonoids and sterols building the anti – oxidant property of bael in it (Singh et al., 2000).

• Acting against cancerous growth

The bael hydro alcoholic leaf extract showing anticancer action in animals. Consumption of extract at a dose 400 milligram per kilogram has shown the best activity against cancer cells. Action against cancer such as Beta-Lymphoid Raji; Lecukenic K562; Erythro Leukemic HEL; T-Lymphoid Jurat human tumor growth cell lines inhibited (Lampronti et al, 2003).

• Antimicrobial activity

Because of the phytochemicals present in bael which can act against various microbes bael based extracts showing a antimicrobial activity. Bael based antimicrobial components can act against microbes such as *Xanthomonas vesicatoria; Psuedomonas salanacearum; E.coli* and *Aeromonas spp* (Ruhil et al, 2011).

• Antifungal activity

Seed extract of bael can act against fungus like *Trichophyton rubrum; T.terrestrese; Aspergillus fumigatus: Aspergillus niger: A.flavus* and *Epidermophyton floccosum* (Ruhil et al, 2011).

• Constipation

Ripe bael fruit is a good laxative. Consumption of ripe bael fruit cleans and tones up intestines which gives relief from constipation. Continuous consumption is effective in removal of old and accumulated fecal matter from bowels (Panda et al, 2011).

Bael Pulping and Further Product Development

Extraction bael pulp (mesh)

Aged bael fruits are utilized for separating pulp. The imperative or basic factors in thought for maximum separation water binding property of pulp, heat inactivation of proteins and acidity or pH change (Sharma et al, 2014). Separation process of pulp from fruit gotten adequately by the water expansion effectively with an extent ratio 1:1 and 2:1 to pulp. By the process of centrifugation @ 4000 rpm for 10 minutes a reasonable bael juice or beverage was obtained, centrifuged pulp or mash was fruit and water taken in a ratio of 1:2 respectively. In case of pulp lingering chemical action was resolved and squeeze. In case of juice Pectin methyl esterase action was least that was about 1.163 U and it was greater for pulp that was about 1.375 U (Sharma et al, 2014).

In processing the fundamental hindrance to face will be the separation pulp from fruit. Bael pulp separation carried out by using sieving method, without addition of water fruit is passing through the sieve it will give sticky pulp. Because of this sticking nature it is difficult to take care, loss of about 10% pulp during extraction, adhering in the sieves. Adhesive substance present in the fruit is the cause behind this loss (Shrestha, 2000).

The qualities of bael organic product hydrolysate exhibited that bael organic product or fruit hydrolysed accordingly duration as hours, 2 & 4 hours longer process resulting more prominent

exercise in cell reinforcement. Hydrolysis of bael natural product at 6 hour brought about the littlest molecule estimate at 79.92 μ m (Sharma et al, 2014). The hydrolysis of bael organic product brought about higher solvent dietary fibre and unstable mixes, however it didn't influence the prebiotic action score when contrasted with non-treated specimen. Aftereffects evaluation of phyto-chemical present in fluid concentrate uncovered presence of flavonoids; terpenoid; lignin: tannins; saponin and steroid. Accessibility of alkaloids demonstrated by alcoholic concentrate (Sujata et al, 2011)

Bael fruit juice:

Because of energetic effect and medicinal properties of bael fruit, bael natural products can be commercialized. Juice separation from bael fruit by utilizing pectinase compounds which is unrefined can make the product advanced or better (Singh et al. 2013). As juice extraction is troublesome because of the pectin that hold the water present in juice, utilization of enzymes or proteins adequately increment the yield and clearness of juice. Studies based on yield related to the time taken for incubation and the effect developed, temperature in which incubation done and the enzyme concentration weather it is crude or not, juice properties got by utilizing bael natural fruit pulp that is thickness and clearness of the juice (Singh et al. 2013). 475 minutes incubation time and 45°C temperature used to examine the conditions for treatment in case of enzymes and bael fruit contains enzyme in crude form with a concentration 0.20 ml per 25 gram of mash separated from fruit. Clearness and viscosity of the juice were 21.32 percentage T and 1.41 cps separately. To look towards the changes in comparison of different juices treated with enzymes yield and lucidity factors having major role.

Bael fruit beverages:

Because of expanding interest of enriched drinks in light of indigenous organic products, this natural product can be handled for making drinks. Different specialists have effectively formulated several drinks from bael natural product. A whey protein enhanced bael organic product drink was formulated by including carboxy methyl cellulose; concentrate form of whey protein and pectin with desired level (Singh and Nath 2004). The drink with protein percentage of 1.75%; TSS 16°Brix; pH of 3.9 level and mash from fruit of 25% found to be the best in case overall acceptability in case of overall quality. In case of guava and bael mixed drinks formulated with process of separating the pulps of the two organic products (Nidhi et al. 2008). Pulp of bael was removed from fruit by adding water in same level to the fruit mass and increasing temperature by warming it to 80°C for 1 min and allowing it to go inside pulper. Guava pulp was gotten by taking natural product cuts on heating for five minutes at 80°C with 25 percentage water and to get mash without strands, seeds pulping using pulper which

improve homogeneity also. Pulp mixing with different ratios done to find the better ratio for formulating juice. Ratios for mixing pulp are 0:100; 25:75; 50:50; 75:25 and 0:100. Ready to serve drinks of 15 and 20 % fruit pulp; 15 % total soluble solids and 0.26 % acidity were formulated and developed by using the available fruit pulp mixes (Nidhi et al. 2008).

Ready to serve drink, nectar and squash from bael organic product were formulated (Verma and Gehlot 2006). To set up these products, separation pulp is necessary for that at 70°C for one minute heating of each kilogram of mass with one litre water and further filtering followed by cooling with muslin fabric. Filtered pulp tested to determine acidity and total soluble solids. To maintain the particulars and respective characteristics in final product addition of sugar and citric acid must be in definite amount. So that measure of citric acid and sugar is essential to maintain the characteristic properties of the product. Like in most of the products homogenization and purification processes done in nectar & RTS also after formulation. Sodium benzoate is used as a preservative compound in squash developed from bael. After the process of homogenization 1 gram per litre squash sodium benzoate is added followed by the dissolving of sodium benzoate in required quantity of water. Squash packaging done in bottles mostly glass bottles not in metal type packaging material. Purification and sterilization processes also done after packaging. (Verma and Gehlot 2006).

Restorative drinks from bael natural product were produced (Verma and Gehlot 2007). Mostly matured but not ripened fruit product of bael help to cure diarrhoea with blood and curing activity of ripened fruit of bael includes activity as laxative and as tonic in case of brain and heart also capable to cure dyspepsia. Analysts working on different aspects of bael to formulate and to prepare ready to serve beverages, syrup, nectar and squash from fruit product of bael. To make ready to serve, as a fruit part 1 litre bael fruit pulp along with sugar 1.2 kilo gram to maintain taste and acid TSS ratio of the product; 7.7 litre water and citric acid weighed 28 gram added. To make nectar as a fruit part 1 litre bael fruit pulp along with sugar 650 gram to maintain taste and acid TSS ratio of the product; 3.3 litre water; citric acid weighed 15 gram were added. To make squash as a fruit part 1 litre bael fruit pulp along with sugar 1.5 kilo gram to maintain taste and acid TSS ratio of the product; 1 litre water; citric acid weighed 20 gram and 1 gram per litre sodium benzoate added (Verma and Gehlot 2007). Total soluble solids 40% and 25% of fruit pulp maintained. Formulated syrup will be of fruit portion at least 25% and total soluble solids 65%. To make syrup sugar; bael fruit pulp; water and citric acid were used in definite amount 2 kilogram; 1 litre; 500ml; 20 gram respectively. Even though normally no additives used in syrup then also sodium benzoate is added to increase the keeping quality.

Bael fruit grown in wild condition having more nutrients in it so that to develop enriched products these types of bael is incorporated (Kenghe et al. 2009). Through changing the total soluble solids level of pulp and adding important additives formulated squash from fruit. Fruit pulp separation done by applying heat for 1 minute at 80°C followed by the addition of water in same quantity as pulp and the heated solution is filtered by using muslin fabric (Kenghe et al. 2009). Bottled and capped squash pasteurized for 30 minutes at 80°C temperature afterwards cooling also done.

Beal RTS can be developed in mixed manner also (Kenghe and Zambare 2009). Prior to mixing process fruit preparation done. Well matured, good quality and fresh fruits were taken and washed, cutting in to halves by means of blade. Pulp and seeds from peel also collected and boiled for a time frame with little amount water. Pulp sifted during this time. To make ready to serve drink with particular rate of pulp and total soluble solids to meet the standards with sensory acceptability adding of citric acid; pulp and estimated amount of sugar syrup has been done. With particular standards and sensory acceptability tamarind ready to serve drink also prepared. To make mixed RTS of bael and tamarind mixing of RTSs made respectively mixed with extends 1:1; 1:5; and 1:9 has been done to get good quality mix in every aspect. To the RTS mix KMS (potassium metabisulfite) and jalgerra added in different level of 15 ml and 0.9 mg respectively per litre of the RTS (Kenghe and Zambare 2009). From the further study of the mixes with different extents found that mix made with proportion level of 1:9 shown best quality and as an organic food additive jalgerra can be utilized.

The RTS drinks from bael and citrus natural products mixes was formulated (Nagpal and Rajyalakshmi 2009). Pulp separation is the basic step in all types of RTS related to the particular fruit used. To make RTS of bael incorporated with citrus fruits pulp separated, adding same level measured water to crude or unrefined mass of fruit and mixed and sifted with a sifter of 20 mesh made up of stainless steel to get pulp of uniformly thick and free from filaments and seeds. Good quality citrus fruits such as sweet orange and lime were selected and cutting in to equal halves. With the help of a juice extractor and also by using a wooden squeezer juice extracted from the equal halves of fruits. In case of mandarin oranges to extract the juice first the fruit peeled and by using screw type extractor juice is extracted. To get the pineapple juice extract after the selection process the fruit is peeled and cutting in to uniform pieces. Pieces of pineapple mixed together and sifted by using sifter. Collecting the different fruit juices such as sweet orange; lime; pineapple and mandarin orange. Mixing of the bael juices with the collected juices with different extents. To make the ready to serve drinks with characteristic level of acidity or pH; total soluble solids and concentration incorporating citric acid; water and sugar. Sterilized glass packaging is done in case of fully cooked drinks. Filling in to glass

bottles, pasteurized and stored at desired temperature followed by cooling (Nagpal and Rajyalakshmi 2009).

Bael wine:

Bael wine is a fermented ready to serve drink with numerous medical advantages. To make wine bael fruit with particular sugar level is selected (Singh et al. 2006). Change in the total soluble solids to 24°B present in pulp separated out from bael fruit. TSS change done during the making of must by the incorporation of sugar, KMS alongside 0.4 % cyclodextrins. Inclusion of these done with yeast starter culture of 10%. Starter culture prepared with yeast extract and ammonium phosphate of definite concentration of 0.05% and 0.1 % respectively at pH 5 by adding in to the bael pulp boiled and cooled. Fermentation or maturation temperature taken is 25°C (Singh et al. 2006). Alcohol level maintained maximum up to 10.08 % on fermented product at pH 5 for time taken 88 hours.

Bael preserve:

To make preserve (murabba) from bael not ripened green bael is used. Murabba from bael having imperative therapeutic properties and capable to act against wide range of stomach related inconveniences. Preserve was prepared by utilizing green bael fruits (Kaushik et al. 2002). For the peeling process a blade used to make an opening in the fruit at the stellar end, splitting and removing the skin. Cutting the peeled fruit with around 2 cm thickness transversely. Washing and by using a fork of stainless steel both sides of the cuts were pricked. Blanching for 5 minutes or to that point needed cuts to be delicate in boiling water. Citric acid 0.6 % is added to 40% sugar syrup made and this sugar syrup is filled in to the cans were fruits filled after treating (Kaushik et al. 2002). Afterwards in following day concentration of the syrup was increased by incorporating 300 gram of extra to that my means of heat. This extra addition of sugar repeated on fourth and sixth day and to obtain the sugar concentration to 70 % the syrup was heat treated these preserve at ambient temperature stored in containers made with glass.

Formula to make bael preserve was explained in other ways also (Rakesh et al. 2005). Ingredients used includes cuts of matured bael fruit (2 centimetre thick); sugar; citric acid and water in definite amounts 1 kilogram; 1.25 kilogram; 2 to 3 grams and water respectively. By using a fork of stainless steel both sides of the cuts were pricked and immersed in 2 % lime solution for 2 - 3 hours. From the lime water fruit cuts were taken out and washed completely with moving water afterwards to make it delicate dipping in water boiled. Sugar syrup prepared by using 700 gram sugar and cuts softened immersed in the 40 % sugar syrup prepared. The sugar syrup separated out in the followed day and from rest of the sugar half added to the removed syrup, again concentrated and again fruit cuts

in the can were filled with this. Following 2 days the process of removing syrup and concentrating with addition of sugar repeated and 70 % of total soluble solids level of syrup maintained (Rakesh et al. 2005).

Bael candy:

Candies also can be prepared by using bael fruit (Rakesh et al. 2005). The formula used in production of preserve is same in case of candies also. In case of preserve last cuts were dipped in 70 % syrup made of sugar. In case of candies taking those fruit cuts immersed in syrup and dried for 8 - 10 hours by using oven at 55 - 60° C. To make candies cultivar NB – 9 bael was used and after filled in jars made up of glass or in polythene packets were stored at ambient condition. Tests during monthly intervals used determine the quality parameters of the candies. An increase in percentage of browning of candy; total soluble solids in the candy and also the acidity seen during storage. This storage changes seen because of the decrease in ascorbic acid content on going storage (Rakesh et al. 2005). Storage of candies in packets made up of polythene helps to prevent spoilage organoleptic quality maintained for 4 months (Mishra et al., 2013).

Some confectionary products can be prepared by non - enzymatic browning methods. Toffee is a kind of confectionary product made with non - enzymatic browning methods involved molasses or sugar caramelization along with butter. Heating of this blend until the point when the temperature achieves 100 - 154° C (Rakesh et al. 2005). Ingredients used in production of bael fruit toffee includes bael fruit pulp; glucose; butter; skim milk powder and sugar. Measured amount of pulp and desired amount of water also added, for 1 kilogram pulp 750 ml water added and heating at 80°C followed by well mixing. With screening process fine pulp was collected. Cooking of 1 kilogram pulp to the point of 33 % volume of its remained. To the processing mix 100 gram of each glucose and butter and 500 gram sugar was added. End point of cooking reached when the mass cooking leaves the sides of the pan. After cooking spreading the mass consistently with thickness ranging from 0.5 mm to 0.75 mm. cooling, cutting and wrap packing by using paper with barrier property against moisture transmission (Rakesh et al. 2005).

Bael slab:

Bael fruit slab can be prepared by using various methods. Water addition of measured quantity 200 – 300 ml done for every kilogram crude weight of fruit. Sifter made up of stainless steel is used sifting is done to test the proper extraction. In to the pulp extracted potassium metabisufite; sugar and citric acid were incorporated. Pulp contains 0.07 % potassium metabisulfite (K2S2O5); total soluble solids of 35 % and total acidity of 0.5 %. At that point boiling also the spreading of pulp on a wide

aluminium plate already rubbed with butter take place. To maintain the moisture content level of cut chunks to 14.5 % drying process for 15 - 16 hours done at 55 - 60° C. Dried product collected in packs made up of polyethylene followed by the process of butter paper covering of the chunks (Rakesh et al. 2005).

Bael fruit jam:

Making of bael used jam can be prepared as single fruit also as mixed fruit with mango (Mishra and Chopra 2006). To separate pulp out addition of some level of water in to the crude fruit mass done. Temperature increased for one minute at 80° C and sifted by using stainless steel sifter. Jam composed of 45 % pulp (1: 1 ratio addition of bael and mango pulp); 70 % total soluble solids and acidity of 0.5 %, in light of considered organoleptic qualities it was the overall accepted blend.

Accordingly with desired product needed at last, formulation process will also vary (Rakesh et al. 2005). Jam preparation using other formula that is incorporation citric acid 3 - 4 grams and sugar of 750 gram to the 1 kilogram pulp extracted and strained having no filaments and seeds in it. Cooking the jam and till the last second of cooking ceaseless mixing with a spoon was done. By the help of sheet test end point determined. After reaching end point cooking was stopped. Then the hot jam was filled into perfect and very much cleaned bottles. For the complete sterilization jam filled bottles inverted. 10 minutes like inverted sterilize bottle caps.

Dehydrated bael:

Drying or dehydration is used as a process of preservation of bael fruit. To make dried or dehydrated product well developed, fresh green fruit of bael was used. (Rakesh et al. 2005). Fruit to process by using drying methods cutting in to pieces sizing from 1 to 1.5 cm in thickness and SO2 fumigation in sulphur box for 30 minutes and drying the cuts at $55 - 60^{\circ}$ C by using oven till it achieves a consistent weight (Rakesh et al. 2005).

Bael powder:

Bael fruit cuts drying and further grinding using various machine will produce bael powder. Bael powder produced packed in polythene packs and stored under dry condition followed by the tight sealing (Rakesh et al., 2005). Bael powder can be prepared by using spray dryer. In this method bael fruit pulp is sprayed to form powder. Streaming property is good in powder made in spray drier it is also having yellow – red colour giving higher timeframe of useful life. Bael panjiri:

This item is profoundly nutritive and providing lots of health benefits. bael powder 1 kilogram, butter 1 kilogram, Wheat flour, 1.5 kilogram of powdered sugar and dry fruits were utilized in the preparation of bael panjiri (Rakesh et al. 2005). Bael powder roasting was done by using butter ghee .In butter ghee according to the taste roasted bael powder is added, mixing of other ingredients also done (Rakesh et al. 2005).

Bael processing waste utilization:

Utilization of processing waste will help to decrease the contamination chances. Peel and the pomace left were the main waste materials produced during the processing period. In a bael fruit normally 35 to 40 % consist of peel and pomace only. This wastage can be prevented by giving this peel and pomace to cattle as feed (Saini et al. 2002). During processing dry wastes are also produced. About 22 to 27 quintals waste in dried form is produced from one hectare bael plantation. Drying at 60°C and grinding done in case of peel and pomace. This bael peel and pomace powder used in various studies related to different biochemical compounds. Peel contains calcium; phosphorus; crude fibre and crude protein in different levels 1.00 %; 0.14 %; 27.34 % and 31.85 % respectively. Pomace composed of fibre of 9.98 %; phosphorus of 0.10 %; calcium of 1.20 % and 10.50 % crude protein (Saini et al. 2002). Waste utilization reducing contamination through this pollution prevented.

OBJECTIVES

- 1. To standardize RTS beverages prepared by the process of incorporation of bael into various fruit blends of different proportions for optimum acidity and TSS.
- 2. To evaluate the RTS beverage organoleptically for finding acceptable proportion of the blends.
- 3. To determine the physico-chemical properties and microbial load in the selected fresh RTS beverages.
- 4. To study storage quality of the RTS beverage through physico-chemical, microbial and organoleptic evaluation for a time period.

MATERIALS AND METHODS

Selection of bael fruit

The fully matured, fir ripe, healthy fruits of bael and mango were selected for the preparation of blended RTS.

Preparation of bael fruit

The selected fruits were washed in running tap water for removing the adhering dirt after washing of fruits, preliminary trial was conducted to standardize the method of extraction of pulp. The pulp was extracted using the following procedure.

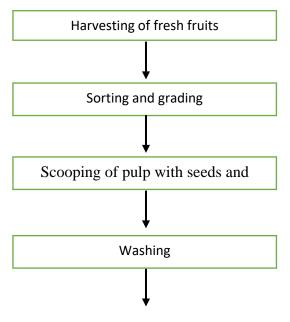
Extraction of bael pulp

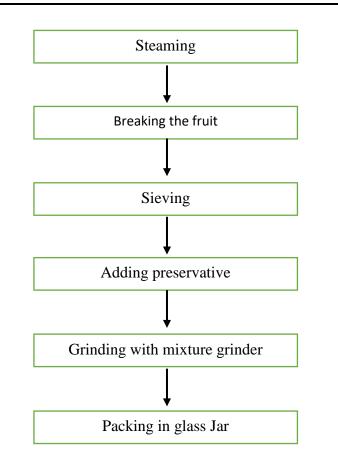
Available literature says that in ripe fruits, the seeds and gum substances are found merged with the pulp whereas fruits just beginning to ripe or semi-ripened fruits have more gummy substances present in the seed sacs which can be easily removed. (Wealth of India, 1948; Gayatri, 2002).

Therefore in the present study only semi-ripened fruits were used for the extraction of pulp so that the pulp obtained is less astringent. The fruits were washed in running water to remove dirt and extraneous matter. It was broken by striking with a hammer and the seed sacs were removed using a stainless steel knife. The edible portion was scooped out with a stainless steel spoon.

The fruit pulp along with its seeds and fibres was scooped with the help of a stainless steel spoon. 200 ml water was added to each one kg of bael fruit pulp. The mixture of fruit pulp and water was then kneaded, heated up to 60 to 65°C temperature for 10 minute and allowed to cool. The cooled pulp was passed through muslin cloth to obtain seed and fibre free pulp.

Flow-chart for extraction of bael pulp





Combination of Bael & Other fruit pulp

| | Comb | ination of Bael & Mango pulp | | |
|----------|-----------------|--|--------|--|
| Sr. No. | Treatment | Quantity of pulp per litre of RTS (Bael: Mango) | Pulp % | |
| 1 | T_1 | 00 + 100 | 10 % | |
| 2 | T_2 | 10 + 90 | 10 % | |
| 3 | T ₃ | 20 + 80 | 10 % | |
| 4 | T_4 | 30 + 70 | 10 % | |
| | Combin | nation of Bael & pineapple pulp | | |
| Sr. No. | Treatment | Quantity of pulp per litre of RTS (Bael: pineapple) | Pulp % | |
| 1 | T5 | 00 + 100 | 10 % | |
| 2 | T ₆ | 10 + 90 | 10 % | |
| 3 | T ₇ | 20 + 80 | 10 % | |
| 4 | T ₈ | 30 + 70 | 10 % | |
| . | Comb | pination of Bael & apple pulp | | |
| Sr. No. | Treatment | Quantity of pulp per litre of RTS (Bael: apple) | Pulp % | |
| 1 | T9 | 00 + 100 | 10 % | |
| 2 | T_{10} | 10 + 90 | 10 % | |
| 3 | T_{11} | 20 + 80 | 10 % | |
| 4 | T ₁₂ | 30 + 70 | 10 % | |

Procurement of raw materials

Will be purchase from local market.

Fruits

Bael fruit

Chemical composition: (AOAC, 2000)

Moisture Content

Moisture content of the sample was determined by following the oven drying method. 5g of each sample was taken in a previously weighed, dried aluminium moisture cups. These cups were kept in a hot air oven at $60(\pm 5^{\circ}C)$ for 8 hrs. The aluminium cups were taken out from oven and kept in desiccators for cooling for 30 min, for attaining a constant weight. After cooling, samples were weighed with aluminium cups. The loss in weight represented the moisture content of the sample.

Weight of empty aluminium cup = W1 (g)

Weight of sample = W2 (g)

Weight of aluminium cup + sample before drying = X (g)

Weight of aluminium cup + sample after drying = Y (g)

Moisture content (%) =
$$\frac{\text{Loss in weight (g)}}{\text{Weight of sample (g)}} \times 100$$

Ash content

The weighed amount of sample (1g) was taken and put in previously dried and weighed silica crucibles. Samples were first incinerated over an electric hot plate followed by ashing in muffle furnace at a temperature of $550(\pm 25^{\circ}C)$ for 6 hrs (until a pale white residue was obtained). These ashed samples were taken out from the muffle furnace and kept in desiccator for 2 hrs for cooling. After cooling samples were weighed again and percent ash content was calculated as follows:

Weight of empty crucible = W(g)

Weight of crucible + sample before ashing = W1 (g)

Weight of crucible + sample after ashing = W2(g)

Ash content (%) =
$$\frac{\text{Weight after ashing (g)}}{\text{Weight of sample (g)}} \times 100$$

(Or)

Ash content (%) =
$$\frac{(W2 - W)}{(W1 - W)} \times 100$$

Physio-chemical analysis of bael juice

Total soluble solids (TSS)

TSS was determined with the help of hand refractometer of range 0-32°B (Model ERMA). The TSS was recorded by placing 1-2 drops of juice sample on the prism of a hand refractometer. The results were expressed as ° Brix (Ranganna, 2007).

Titratable acidity

Titratable acidity was estimated by titrating a known volume of the sample against standard 0.1 N NaOH solution by using phenolphthalein as an indicator up to the end point (pink colour). The titratable acidity was expressed as per cent malic/citric acid (AOAC, 2004).

| | Titre × Normality of | f × volume > | × equivalent weight | | |
|--------------------------|----------------------|-----------------|--------------------------|---|-----|
| Titratable acidity (%) = | alkali | made up | of acid | × | 100 |
| | Volume of sample | \times volume | of aliquot $\times 1000$ | | |

pН

pH was taken with ELTOP-3030 pH meter. Prior to pH measurement, the instrument was standardized with the buffer solutions of pH 4, 7 and 9. The pH of the samples was estimated directly. **Sugars**

Estimation of sugars in juice blends

A known weight of sample (25 g) was taken in a 250 mL volumetric flask and 100 mL water was added to it. Solution was neutralized with 1 N NaOH and 2 mL of 45% lead acetate was added to it and kept for 10 min. Excess of lead acetate was removed from the sample by using 2 mL of 22% potassium oxalate in 250 mL volumetric flask. After diluting it up to the mark, the solution was filtered and clear filtrate was taken to estimate reducing sugars by titrating against a known quantity of Fehling's A and Fehling's B solution using methylene blue as an indicator (Lane and Eynon, 1923). Reducing sugars were estimated as per cent and calculated as given below:

Reducing sugars (%) =
$$\frac{\text{Factor } \times \text{ Dilution}}{\text{Titre value } \times \text{ Weight of sample taken}} \times 100$$

Total sugars were estimated by adding 5 g of citric acid to 50 mL calibrated sample solution and heating it for 10 min. For complete inversion of sugars, neutralizing with NaOH and making volume 250 mL in volumetric flask was done. The total sugars were estimated as per cent and calculated as given as under:

Total sugars as invert sugars (%) = $\frac{\text{Factor} \times \text{Dilution}}{\text{Titre value} \times \text{Weight of sample}} \times 100$

% Sucrose = (% total invert sugars - % reducing sugars) \times 0.95

% Total sugars = (% reducing sugars + % sucrose)

Total proteins

Protein in different samples was determined by Lowry's method as described by Sadasivam and Manickam (1991). A known volume of (0.1 mL) was taken in separate test tubes and volume was made up to 1 mL with distilled water. Then 5 mL of alkaline copper solution was added to each test tube and incubated at room temperature for 10 min. After that 0.5 mL of Folin-Ciocalteu reagent was added to each test tube and again incubated at room temperature in dark for 30 min. Optical density of the sample was measured at 660 nm with a UV-viz-spectro-photometer. The concentration was determined as per the standard procedure from the standard curve which was prepared using different concentrations of bovine serum albumin (BSA; 8-32 μ g/mL) using the same procedure. The results were expressed as mg per 100 ml on volume/volume basis and calculated as given below:

| Protein (%) = | O D of unknown sample | × | Protein value from standard curve (µg) | × | Total volume of extract | × 100 |
|---------------|-----------------------------|---|--|---|-------------------------------|-------|
| | Aliquot i.e. juice used | × | Weight of sample taken | × | 1000 | - |

Total phenolics

The amount of total phenolics in the sample was determined with the Folin-Ciocalteu reagent according to the method of Bray and Thorpe (1954) using catechol as a standard. One gram of sample was taken and grinded with 10 mL of 80 per cent ethanol in pestle and mortar, and centrifuged for 20 min at 1000 rpm and filtered. Filtrate was evaporated in oven up to dryness and dried extract was dissolved in 5 mL distilled water. 0.2-2.0 mL aliquot was taken in separate test tubes and volume was made up to 3 mL. Then 0.5 mL Folin-Ciocalteu reagent was added. After 3 min 2 mL of Na₂CO₃ (20%) was added and mixed. Test tubes were placed in boiling water bath for one min and then cooled. Optical density of the sample was recorded at 650 nm with the help of UV-Vis spectrophotometer (Model Shimadzu, Japan). The concentration was determined as per the standard procedure from the

standard curve. The standard curve was prepared using different concentrations (8-32 μ g/mL) of catechol and results were expressed as mg per 100 g on fresh weight basis.

Ascorbic acid

Ascorbic acid content was determined as per AOAC (2004) method using 2, 6- dichlorophenol indophenol dye. A known volume of the sample extracted in 3% m-phosphoric acid was titrated with dye to pink colour end point. Results were expressed as mg per 100 g of sample and calculated by using the following formula:

Ascorbic acid $\left(\frac{\text{mg}}{100}\text{g}\right) = \frac{\text{Titre} \times \text{Dye factor} \times \text{Volume made up}}{\text{Aliquot of extract taken} \times \text{Weight of sample taken}} \times 100$

Antioxidant activity

Antioxidant activity (Free radical scavenging activity) was measured as per the method of Brand-Williams *et al.* (1995). DPPH (2, 2-diphenyl-1-picrylhydrazyl) was used as a source of free radical. A quantity of 3.9 mL of $6x10^{-5}$ mol/L DPPH in methanol was put into a cuvette with 0.1 mL of sample extract and the decrease in absorbance was measured at 515 nm for 30 min or until the absorbance become steady. Methanol was used as blank. The remaining DPPH concentration was calculated using the following equation:

Antioxidant activity (%) =
$$\frac{Ab(B) - Ab(S)}{Ab(B)} \times 100$$

Where,

Ab (B) = Absorbance of blank

Ab (S) = Absorbance of sample

Sensory evaluation

Nine point Hedonic scale method as given by Amerine *et al.* (1965) was followed for conducting the sensory evaluation of juice blends. The panel of 7 judges comprising of faculty members and post-graduate students of the department of Food Technology and Nutrition, Lovely Professional University was selected with care to evaluate the blends for sensory parameters such as colour, consistency, taste and overall acceptability. Efforts were made to keep the same panel for sensory evaluation throughout the entire period of study. The samples were presented to judges and plain water was given to them to rinse their mouth in between the evaluation of samples. No discussion during evaluation was allowed.

| Like extremely | → | 9 |
|--------------------------|----------|---|
| Like very much | → | 8 |
| Like moderately | → | 7 |
| Like slightly | → | 6 |
| Neither like nor dislike | → | 5 |
| Dislike | - | 4 |
| Dislike moderately | → | 3 |
| Dislike very much | → | 2 |
| Dislike extremely | → | 1 |

Total tannin content (AOAC)

Tannin content can be estimated by colorimetric method using folin-denis reagent. The folin-denis reagent is specific for compounds containing the oxy-phenyl group. Take 5ml of sample, filter it. Add 5ml of folin-denis reagent and 10 ml sodium carbonate solution and make volume up to 100ml with water. Mix well and measure the colour after 30 min at 760nm. Make a standard curve with standard tannic acid. Calculate the % tannin content by using formula:

% Tannin content= _____ Mg of tannin content x dilution x 100ml

Weight of sample taken for colour development × weight of sample × 1000

EXPECTED RESEARCH OUTCOME

Commendable flavour and activity as resuscitating refreshment of bael incorporated beverages increases its importance in the field of RTS beverages. Big scale production of bael used products is not yet happened only unorganized sectors are there in case of bael related product processing. Bael used product development and its business sides as value added products also impressive. To find the unexplored facts based on bael and bael incorporated products efficiently engaged research is required.

Outcomes from this research based on the production of blended RTS with bael and other fruits will be the production of a value added drink that can provide various benefits. I hope this research will give an impact on bael incorporated RTS processing.

PROPOSED WORK WITH PLAN TIMELINE

| Work plan | Jan | Feb | March | April | May | June | July | Aug | Sept | Oct | Nov |
|-------------------------|-----|--------------|--------------|--------------|-----|------|--------------|--------------|------|-----|--------------|
| Review of Literature | | \checkmark | \checkmark | | | | | | | | |
| Report Submission | | | | \checkmark | | | | | | | |
| Product standardization | | | | | | | \checkmark | | | | |
| Product development | | | | | | | | \checkmark | | | |
| Product Analysis | | | | | | | | | | | |
| Result compilation | | | | | | | | | | | \checkmark |

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