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RESEARCH PAPER

Antioxidant and reducing activities of bael (*Aegle marmelos* Linn.) extracts

■ S.J. PRASHANTH, D. SURESH, V.H. POTTY AND P. SADANANDA MAIYA

SUMMARY : Bael has been known to India from prehistoric times. A study was undertaken to assess various solvent extracts from different parts of bael for their antioxidant and reducing power activities. The crude extracts of three different parts such as pulp, rind and seed with five solvents namely hexane, chloroform, ethyl acetate, acetone and methanol were examined for the antioxidant activity by DPPH radical scavenging method. It was found that methanol extract has given highest activity when compared to other extracts. Chloroform extracts have shown least activity and hexane extracts have shown no activity. Between the freeze-dried and oven dried extract of methanol, freeze dried extracts showed good activity (53.09%) compared to oven dried extracts (51.09%). IC_{50} value of DPPH radical scavenging activity of methanol extract has showed the highest activity and lowest concentration was found in freeze dried pulp (83.82 µg / ml), rind (109.52 µg/ ml) and seed (121.32 µg/ ml) when compared to oven dried pulp, rind and seed. The crude extracts of ethyl acetate, acetone and methanol which have shown good antioxidant activity were subjected to total reducing power assay. All the extracts have shown increased reducing power when concentration was increased. Among the drying methods *i.e.*, freeze and oven, freeze dried extracts shown good activity.Total reducing power of the methanol extract of oven dried and freeze dried pulp, rind and seed, exhibited increase in total reducing power with increase in concentration. There was no significant difference in total reducing power between oven dried and freeze dried extracts. The total reducing power of these samples could be due to the presence of high poly phenols.

Key Words : Oranges, Bael, Extracts, Antioxidant, Reducing power activity

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Bael (*Aegle marmelos* Linn.) is the underutilized fruit used in India dates back to several thousand years. Mention has been made in Ayurveda for it's therapeutic uses as on astringent and in treatment of diorrhoea, dysentery etc. There has also been ample of evidence in literature proving the efficacy of pulp, leaves, roots, stem bark against diabetes, cancer, ulcers, fertility control etc., The ripe fruit is laxative and unripe fruit is prescribed for diarrhoea and dysentery. Dyspepsia it has a great demand from Indian system of medicine such as Ayurveda (Kirtikar and Basu, 1933 and Satyavati *et al.*, 1976). Bael root was used for its anti diorrhoetic activity (Pitre and Srivastava, 1987). Aqueous decoction of bael root is reported to have hypoglycemic activity (Karunanayake *et al.*, 1987). Root bark of this plant has been used particularly in intermittent fevers and also as a fish poison (Basu and Sen, 1974). The juice of leaves along with black pepper is given for diabetes. The leaves are also given in Jaundice (Chakraborthi, 1988; Alam, 1990 and Ponnachan, 1993). With respect to clinical applications, roots are astringent, bitter and febrifuge. They are useful in diarrhoea, dysentery, dyspepsia, stomachalgia (Shoba and Thomas, 2001), cardioplamus, seminal weakness, vomiting, intermittent fever and swelling. The leaves are useful as laxative

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febrifuges and expectorant, and also in opthalmia, deafness, inflammations, cataract, diabetes, asthamatic and antifungal complaints (Rana *et al.*, 1997). The effects of all these extracts were examined in the resolution of hyperthyroidism (Kar *et al.*, 2002). The root bark extract is used to cure intermittent fever, mental diseases, peri carditis and angina pectoris (Nadkarni, 1976). The present investigation, a study has been undertaken to know the bioactivities of bael fruit pulp, rind and seed with respect to antioxidant and reducing power activities.

EXPERIMENTAL METHODS

Statistical analysis:

The experiments were carried out in triplicates and significant differences ($P < 0.05$) were determined by Duncan's Multiple Range Test (DMRT). Duncan. (1955).

Chemicals:

All the organic solvents used for extraction (Hexane, Chloroform, Ethyl acetate, Acetone and Methanol) were of AR grade from E.Merk (Mumbai, India). Tris HCl and 1, 1- Diphenyl-2- Picrylhydrazyl (DPPH) was from Sigma-Aldrich, St. Louis, USA. Trichloro acetic acid from Qualigens Fine Chemicals, India. Potassium Ferricyanide and ferric chloride were purchased from M/s Sisco Research Laboratories Mumbai, India. 2,6, dichloro benzenone indophenol was purchased from Sigma chemicals. Co. (St. Louis, MI, USA). All other chemicals were of extra pure analytical grade were purchased locally.

Proximate composition analysis of bael pulp:

Moisture content:

Relative moisture content in the pulp was determined using a modified standard procedure (AOAC, 1990). About 5g of pulp was placed in pre-dried and weighed glass Petridish (100mm) and dried in a vacuum oven at 90^0C for 48 hours at a pressure of 200 mm Hg. Dried samples were placed in a desiccator and cooled for 30 min. to room temperature. The weight was recorded and percentage moisture content based on initial wet weight was calculated. Each measurement was replicated thrice.

Total soluble solids:

^{0}Brix, a measure of soluble solid was determined by placing 3-4 drops of bael pulp juice over a prism of the digital refractometer (ATAGO. RX – 5000). Calibrated with triple distilled water. The ^{0}Brix value was recorded directly from the digital readout of the refractometer as per cent sucrose content. Each measurement was replicated thrice.

Titrable acidity:

Titrable acidity was determined by AOAC method (1990). About 2-3 g of bael juice was titrated against 0.1 N NaoH with phenolphthalein as indicator until pink colour persisted. Titrable acidity was calculated using formula and expressed as per cent citric acid extent.

$$\text{Titrable acidity (\% citric acid)} = \frac{64 \times N_{NaOH} \times \text{Titre value}}{\text{Wt. of sample} \times 1000} \times 100$$

Total sugars and reducing sugars:

Total sugars and reducing sugars were estimated (Lane and Eynon, 1923). In principle, reducing sugars reduces the Copper Fehling's solution to red insoluble cuprous oxide. The sugar content can be estimated by determining the volume of unknown sugar solution required to completely reduce a measured volume of Fehling's solution.

Determination of factor for Fehling's solution:

About 40 ml Fehling's solution (Fehling's A: Fehling's B: water, 1:1:2) was pipetted in to a 250 ml conical flask, heated to boiling. Under boiling condition, standard invert sugar solution prepared by inverting aliquot quantity of sucrose with 5 ml HCl was titrated. Methylene blue was used as indicator and formation of brick red colour was considered as endpoint. Burette reading was recorded and factor for Fehling's solution was calculated using following formula.

$$\text{Factor for Fehling's solution (g. of invert sugar)} = \frac{\text{Titre} \times 2.5}{1000}$$

Estimation of reducing sugars:

Preparation of sample:

About 10 g of filtered bael juice sample was transferred to 250 ml volumetric flask, diluted to 100 ml and neutralized with 1N NaOH in presence of phenolphthalein indicator. Volume was raised to 250 ml. 50 ml burette was filled with sample solution for titration.

Titration:

About 40 ml of Fehling's solution (Fehling's A: Fehling's B: water, 1:1:2) was pipetted in to a 250 ml conical flask, heated to boiling. Under boiling condition sample juice was titrated with methylene blue as indicator. Formation of brick red colour was considered as endpoint, and burette reading was recorded. Reducing sugar content was calculated using the following formula and expressed as per cent content

$$\text{Reducing sugars (\%)} = \frac{\text{Factor} \times \text{dilution}}{\text{Titre} \times \text{weight of sample}} \times 100$$

Estimation of total sugars:

Preparation of sample:

About 10 g of filtered bael juice sample was transferred to 250 ml volumetric flask, diluted to 25 ml. Sample was allowed

for inversion by adding about 5ml of concentrated HCl and keeping it overnight. Inverted sample juice was neutralized with 1N NaOH in the presence of phenolphthalein indicator and volume was raised to 250 ml. 50 ml burette was filled with sample solution for titration.

Titration:

Titration was carried out as described in estimation of reducing sugars. Total sugar content was calculated as per the following formula and expressed as per cent content.

$$\text{Total sugars (\%)} = \frac{\text{Factor x dilution}}{\text{Titre x weight of sample}} \times 100$$

Ascorbic acid content determination:

The ascorbic acid content was determined by 2,6 dichlorophenol indophenol visual titration method. The dye, which is blue in alkaline solution and red in acidic solution, is reduced by ascorbic acid to a colour less form. (Ranganna. 1977.)

Reagents:

Metaphosphoric acid (MPA, 3%):

Prepared by dissolving the sticks or pellets of HPO_3 in a distilled water.

Standard ascorbic acid (0.1%):

100 mg. L- ascorbic acid was dissolved and volume was made up to 100 ml with 3 per cent metaphosporic acid. 10ml of this solution was taken and made to 100 ml with 3 per cent HPO_3 (1ml = 0.1 mg of ascorbic acid)

Dye solution:

50 mg of sodium salt of 2,6 dichlorophenolindophenol was dissolved in approximately 150 ml of hot glass distilled water containing 42 mg of sodium bicarbonate. This was cooled and diluted with distilled water to 200 ml and stored in a refrigerator and standardized every day.

Procedure:

Standardization of dye:

5 ml of standard ascorbic acid solution was taken and added 5ml of 3 per cent HPO_3, micro burette was filled with dye. The dye solution was titrated with standard ascorbic acid to get a pink colour as an end point. The dye factor was determined *i.e.*, mg of ascorbic acid per ml of the dye, using formula.

Dye factor = 0.5 / titre

Preparation of sample:

10-15 mg of sample was weighed and blend with 3 per cent HPO_3 in case of solid sample and volume is made up to 100ml with 3 per cent HPO_3. This solution was filtered through Whatman No. 4 filter paper.

Assay of extract:

An aliquat of 5 ml of the HPO_3 extract of the sample was taken and titrated with the standard dye, to get a pink end point, which persisted, for at least 15 sec. All the measurements were made in three replicates.

Calculation:

$$\text{Ascorbic acid (mg/ 100g)} = \frac{\text{Titre x Dye factor x Volume made up to}}{\text{Aliquot of extract taken for estimation} \times \text{Wt. of sample}} \times 100$$

Total phenolics:

Estimation of total phenolics was done according to the method described by Singleton and Rossi (1965). To a 250 mg of fresh sample, 50ml double distilled water was added and the mixture was transferred to a rotary shaker for 12 hours to ensure full extraction. The mixture was thereafter filtered and filtrate was made upto 50ml. Total phenolics was determined by Folin – ciocalteu method. 200ml diluted filtrate was added to 1ml of 1: 10 diluated Folin - ciocalteu reagent. After 4 mins, 800ml of sodium carbonate (75 gm/lit) were added. After 2 hour of incubation at room temperature the absorbance at 750 nm were measured. Gallic acid (0-100 mg/lit) was used for calibration of a standard curve. The resulted were expressed as gallic acid equivalents (GAE) / 100g fresh weight of plant material. All the measurements were made in three replicates

Preparation of bael powder:

Freshly harvested matured and ripened fruits were obtained from the orchard. Fruits were washed and pulp, rind and seeds were separated and they were subjected to hot air oven drying at 40-48^{0}C and also freeze-drying at - 28^{0}C. The dried fruit parts are powdered by apex grinder of 60-120 mesh size.

Solvent extraction of powder:

Dry powder of plant material was considered for extraction material to avoid interference of water. For isolating bioactive molecules from a plant material, the samples were extracted with a variety of solvents, sequentially starting from low polarity to high polarity. Most preferred solvent sequence used for extracting the plant material with unknown composition is as follows, which is based on the polarity of solvents (George *et. al.*, 2001). Hexane < chloroform < ethyl acetate < acetone < methanol. The extract is subjected to distillation by flash evaporation and it is lyophilized to get concentrated powder.

DPPH radical scavenging activity:

The antioxidant activity of all the solvent extracts of bael pulp, rind and seed on DPPH radical was measured according

to the method of Moon and Terao (1998). About 100 µl of test sample was mixed with 0.9 ml of Tris HCl buffer (pH. 7.4), to which 1 ml of DPPH (250 µM) in ethanol was added. The mixture was shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm in a UV-Visible spectrophotometer. All measurements were made in triplicates with a BHA as positive control. IC_{50} represents the concentration of test sample, which scavenged 50 per cent radicals. The per cent antioxidant activity was calculated by the following formula.

$$\% \text{ Antioxidant activity} = 1 - \frac{A_{\text{sample (517 nm)}}}{A_{\text{control(517 nm)}}} \times 100$$

Total reducing power:

The reducing powers of ethyl acetate, acetone and methanol extracts were quantified by the method described by Yen and Chen (1955), with minor modifications. Reaction mixture, containing test samples at different concentrations (10-100 µl) in phosphate buffer (0.2M. pH 6.6), was incubated with potassium ferricyanide (1% v/v) at 50^0 C for 20 min. The reaction was terminated by the addition of TCA solution (10% w/v) and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant was mixed with distilled water and ferric chloride (0.1 % w/v) solution and the absorbance was measured at 700 nm. All measurements were made in triplicates. Increased absorbance of the reaction mixture indicated increased reducing power.

EXPERIMENTAL FINDINGS AND ANALYSIS

The results of the present study as well as relevant discussions have been presented under following sub heads:

Composition of bael fruit pulp:

Freshly harvested bael fruit pulp was considered for the analysis of chemical composition. It contains a TSS of 36.8^0 Brix, moisture content 74.90 per cent with 1.28 per cent acidity. The ascorbic acid content was found to be 66.65 mg per 100 g. Total and reducing sugars were found to be 11.16 per cent and 6.84 per cent, respectively. The phenol content of the pulp was 1.76 g / 100 g (Table 1). Thus bael fruit pulp can be a rich source of phenols and also ascorbic acid.

Table 1: Proximate composition of bael fruit pulp

Particulars	Composition
TSS (°Brix)	36.8
Moisture (%)	74.9
Acidity (%)	1.28
Ascorbic acid (mg/100g)	66.65
Total sugars (%)	11.16
Reducing sugars (%)	6.84
Non-reducing sugars (%)	4.32
Phenols (g/100g)	1.76

Per cent contribution of different parts of bael fruit:

Freshly harvested fruit was manually separated in to pulp, rind and seed. The pulp percentage was found to be high (64.18%) compared to rind (32.84%) and seed (2.98%) (Fig. 1).

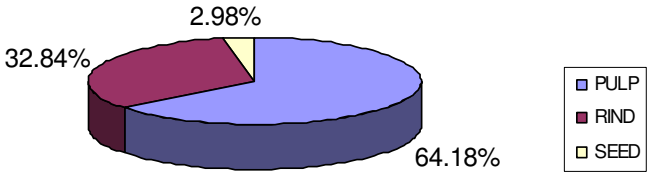


Fig. 1 : Per cent contribution of different parts of bael fruit

Per cent yield of dried powder of bael fruit parts:

The per cent yield of the freeze dried powder of pulp was higher (25.05%) compared to oven dried powder of pulp (25.00%). Similarly freeze dried powder of rind and seed was found to be high when compared with their respective oven dried powder (Table 2). This is due to less loss during freeze-drying compared to oven drying.

Table 2 : Percentage yield of dried powder of bael fruit parts

Sample preparation	Yield (%)
Oven dried	
Pulp	25.00
Rind	27.92
Seed	63.90
Freeze dried	
Pulp	25.05
Rind	29.81
Seed	65.10

Solvent extraction of dried powder and their per cent yield:

The powder of both oven and freeze-drying method were subjected to sequential solvent extraction. The yield of hexane extract of freeze-dried pulp, rind and seeds were 0.61 per cent, 0.35 per cent and 0.10 per cent w/w, respectively. The yield of hexane extract of oven dried pulp, rind and seed were 0.58 per cent, 0.33 per cent and 0.09 per cent w/w, respectively. The chloroform extract of freeze-dried pulp, rind and seed were found to be 1.3 per cent, 0.40 per cent and 0.21 per cent w/w, respectively. The chloroform extract of oven dried pulp, rind and seed were found to be 1.10 per cent, 0.40 per cent and 0.20 per cent w/w, respectively. The ethyl acetate extract of freeze dried pulp, rind and seeds were 1.90 per cent, 1.10 per cent and 0.45 per cent w/w, respectively, and the oven dried pulp, rind and seed were found to be 1.70 per cent 1.0 per cent and 0.43 per cent w/w, respectively. The acetone extract of freeze dried pulp, rind and seeds were found to be 4.50 per cent, 2.10 per cent and 0.90 per cent w/w, respectively and the oven dried pulp, rind and seed were found to be 4.20 per cent, 1.80 per

Table 3 : Percentage yield of different solvents extracts of bael fruit parts

Sample preparation	Solvent extracts				
	Hexane	Chloroform	Ethyl acetate	Acetone	Methanol
Oven dried (% by w/w)					
Pulp	0.58	1.10	1.70	4.20	40.70
Rind	0.33	0.40	1.00	1.80	18.30
Seed	0.09	0.20	0.43	0.60	3.10
Freeze dried (% by w/w)					
Pulp	0.61	1.30	1.90	4.50	43.00
Rind	0.35	0.40	1.10	2.10	20.50
Seed	0.10	0.21	0.45	0.90	4.30

Table 4 : Antioxidant activity of bael fruit parts extracted with different solvents by DPPH method

Sample Preparation	Solvent extracts				
	Hexane	Chloroform	Ethyl Acetate	Acetone	Methanol
Oven dried			DPPH activity (%)		
Pulp	NA	2.92a	36.25b	26.10c	51.89d
Rind	NA	6.76a	16.33b	24.30c	48.15d
Seed	NA	0.0a	13.75a	2.58b	45.97c
Freeze dried					
Pulp	NA	2.78a	36.65b	28.90c	53.09d
Rind	NA	6.37a	18.34b	26.73c	48.09d
Seed	NA	0.0a	14.93a	7.76b	46.88c

Concentration of sample: 2mg/ml

cent and 0.60 per cent w/w, respectively. The methanol extract of freeze dried pulp, rind and seed were 43 per cent, 20.50 per cent and 4.30 per cent w/w, respectively and the oven dried pulp, rind and seed were found to be 40.70 per cent, 18.30 per cent and 3.10 per cent w/w, respectively (Table 3). This indicates that there will be high yield in freeze-dried extracts compared to oven dried extracts.

DPPH radical scavenging activity:

DPPH radical scavenging potential of different solvent extracts of bael fruit parts at the concentration of 2 mg/ml was measured. The high percentage DPPH radical scavenging activity was found in freeze dried extract of methanol solvent of pulp (53.09%), rind (48.09%) and seed (46.88%) compared to methanol extract of oven dried pulp (51.89%), rind (48.15%) and seed (45.97%) at 100µg concentration (Table 4). The ethyl acetate, acetone and chloroform extracts have shown low activity compared to methanol extract, but seed extract of chloroform, and hexane extract of pulp, rind and seed in both freeze dried and oven dried method were found to have no activity. Methanol extract of freeze dried pulp, rind and seed showed IC_{50} value for DPPH radicals, at the concentration of 83.82 µg/ml, 109.52 µg/ml and 121.32 µg/ml, respectively, when compared to oven dried methanol extract of pulp (84.87 µg/ml), rind (111.37µg/ml) and (123.5 µg/ml) (Table 5).

Higher antioxidant activities have been found in the freeze-dried pulp, rind and seed extracts compared to the oven dried pulp, rind and seed extracts. This antioxidant activities of the pulp, rind and seed was due to the presence of high poly phenols.

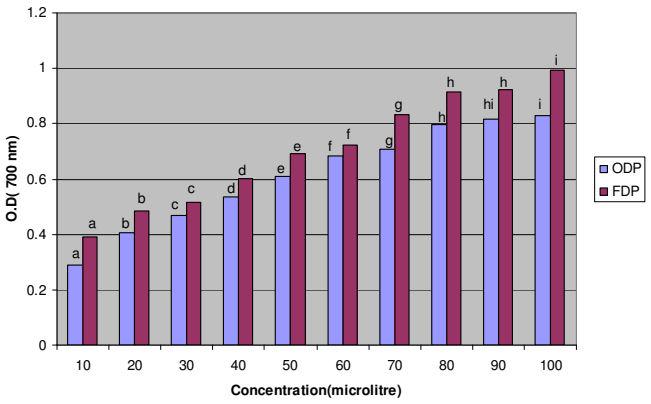
Table 5 : IC_{50} Values of DPPH radical scavenging activity of methanol extract of bael fruit parts

Sample preparation	IC_{50} value (µg/ml)
Oven dried	
Pulp	84.87a
Rind	111.37b
Seed	123.51c
Freeze dried	
Pulp	83.82a
Rind	109.52b
Seed	121.32c

Sample concentration: 2mg/ ml
Each value represents mean of three different observations. Mean values in rows with different subscripts (a,b,c…) differ significantly at $P< 0.05$.

Total reducing power of solvent extracts (Ethyl acetate, Acetone and Methanol) of bael fruit:

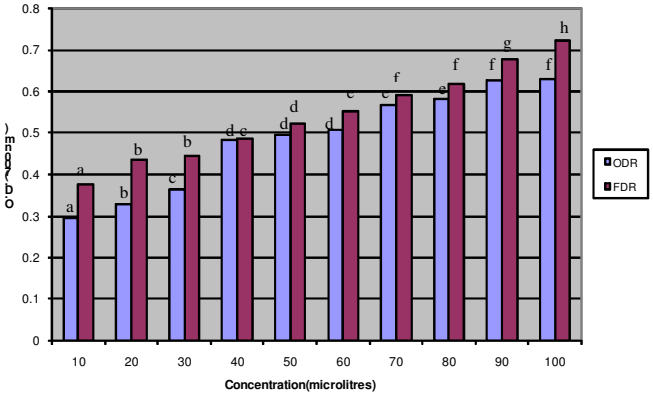
Total reducing power of the ethyl acetate extract of oven dried and freeze dried pulp (Fig. 2), rind (Fig. 3) and seed (Fig. 4), depicts increase in reducing power with increase in



Concentration of sample: 2 mg/ ml

Each value represents mean value of three different observations. Mean values with different superscripts ($^{a,b,c,\cdots}$) differ significantly at P< 0.05.

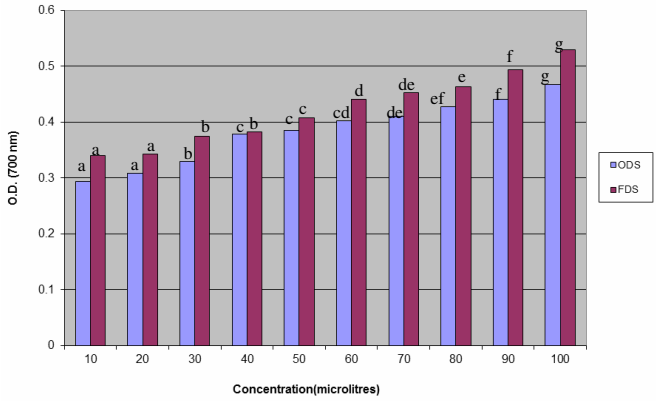
Fig. 2 : Total reducing power of ethyl acetate extract of oven dried pulp (ODP) and freeze dried pulp(FDP) of bael fruit



Concentration of sample: 2 mg/ ml

Each value represents mean value of three different observations. Mean values with different superscripts ($^{a,b,c,\cdots}$) differ significantly at P< 0.05.

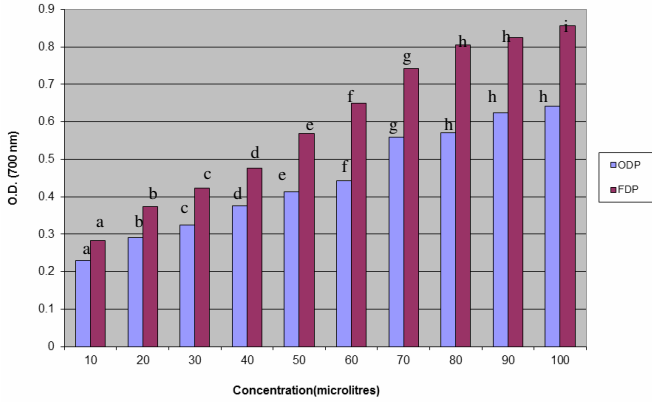
Fig. 3 : Total reducing power of ethyl acetate extract of oven dried rind (ODR) and freeze dried rind (FDR) of bael fruit



Concentration of sample: 2 mg/ ml

Each value represents mean value of three different observations. Mean values with different superscripts ($^{a,b,c,\cdots}$) differ significantly at P< 0.05.

Fig. 4 : Total reducing power of ethyl acetate extract of oven dried seed (ODS) and freeze dried seed (FDS) of bael fruit



Concentration of sample: 2 mg/ ml

Each value represents mean value of three different observations. Mean values with different superscripts ($^{a,b,c,\cdots}$) differ significantly at P< 0.05.

Fig. 5 : Total reducing power of acetone extract of oven dried seed (ODS) and freeze dried seed (FDS) of bael fruit

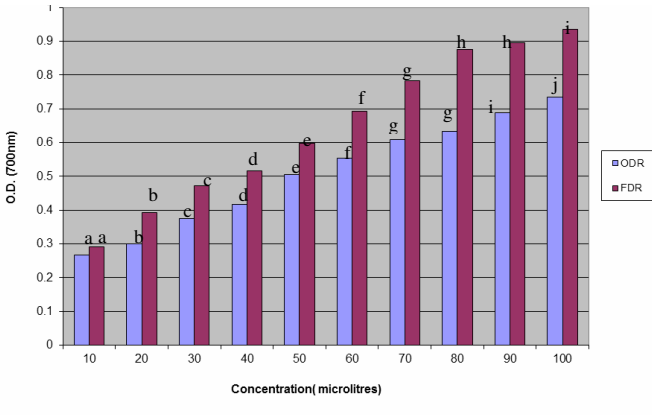
concentration of extracts. Freeze dried samples showed higher total reducing power activity when compared with the oven-dried samples. This may be due to high retention of phenols in freeze-dried samples.

The total reducing power of the acetone extracts of oven dried and freeze dried pulp (Fig. 5), rind (Fig. 6) and seed (Fig. 7) demonstrated increase in reducing power with increased concentration. Freeze-dried extracts of pulp, rind and seed has shown higher activity compared to oven dried extracts. This may be attributed to the high retention of phenols under freeze-dried condition.

Total reducing power of the methanol extract of oven dried and freeze dried pulp (Fig. 8), rind (Fig. 9) and seed (Fig. 10), exhibited increase in total reducing power with increase in concentration. There was no significant difference in total reducing power between oven dried and freeze dried extracts.The total reducing power of these samples was may be due to the presence of high poly phenols.

Summary and conclusion:

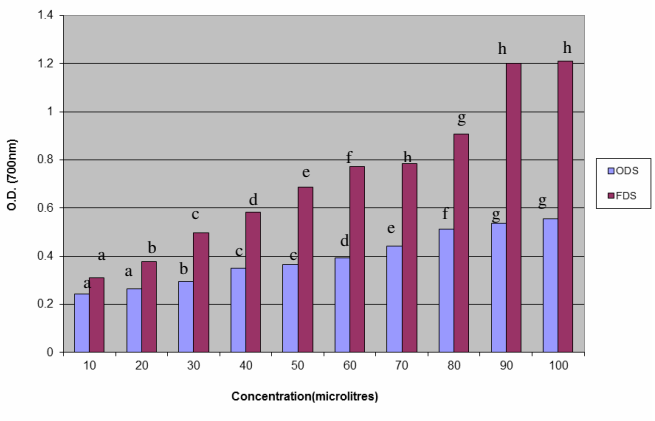
Bael is a perennial fruit crop of Rutaceae family. It has been widely used as a fruit crop since time immemorial. Its medicinal uses in traditional system of medicine are well documented in the ancient literature.Almost all the parts like pulp, root, bark and leaves have shown nutraceutical properties, which includes hypoglycemic, regeneration of damaged



Concentration of sample: 2 mg/ ml

Each value represents mean value of three different observations. Mean values with different superscripts ($^{a,b,c,\cdots}$) differ significantly at P< 0.05.

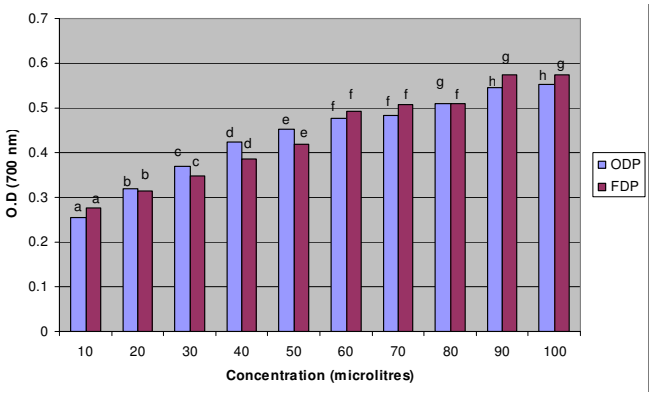
Fig. 6 : Total reducing power of acetone extract of oven dried seed (ODS) and freeze dried seed (FDS) of bael fruit



Concentration of sample: 2 mg/ ml

Each value represents mean value of three different observations. Mean values with different superscripts ($^{a,b,c,\cdots}$) differ significantly at P< 0.05.

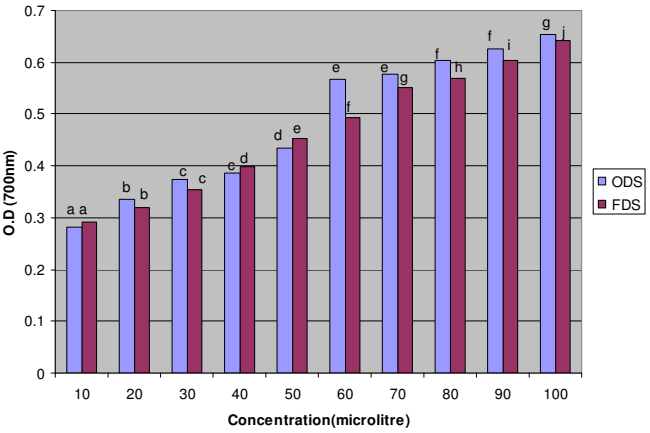
Fig. 7 : Total reducing power of acetone extract of oven dried seed (ODS) and freeze dried seed (FDS) of bael fruit



Concentration of sample: 2 mg/ ml

Each value represents mean value of three different observations. Mean values with different superscripts ($^{a,b,c,\cdots}$) differ significantly at P< 0.05.

Fig. 8 : Total reducing power of Methanol extract of oven dried seed (ODS) and freeze dried seed (FDS) of bael fruit



Concentration of sample: 2 mg/ ml

Each value represents mean value of three different observations. Mean values with different superscripts ($^{a,b,c,\cdots}$) differ significantly at P< 0.05.

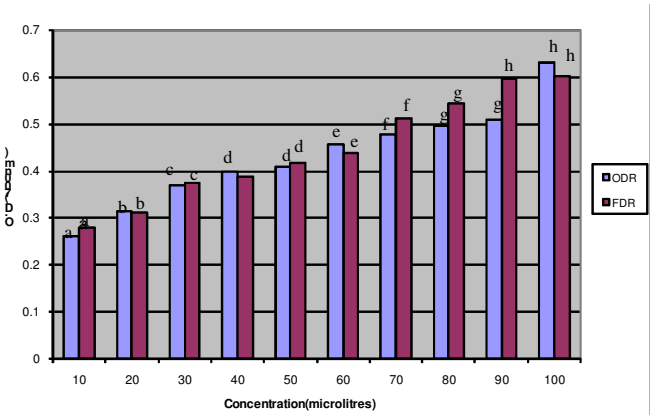
Fig. 9 : Total reducing power of methanol extract of oven dried seed (ODS) and freeze dried seed (FDS) of bael fruit

pancreas, fertility control, anticancer, antioxidant etc. Thus bael is emerging as a food / fruit with myriad applications and biological activities, besides it's traditional functions as fruit and also medicinal uses.In the present study the bioactivity of the different solvent extracts of bael fruit pulp, rind and seeds were examined.

The ripened fruits were freshly harvested and they are manually separated in to pulp, rind, and seed. The pulp was taken for the estimation of the physico-chemical properties and it was found that pulp is a rich source of ascorbic acid (66.65 mg/ 100 g) and phenols (1.76 g / 100 g).The different parts of bael fruits (pulp rind and seed) after manual separation they were subjected to drying by hot air oven (at 48-50^{0}C) and freeze drying (at – 20^{0}C). The dried parts were subjected to powdering by the apex grinder of mesh size 60. After powdering the freeze-dried and oven dried pulp, rind and seed were examined for the per cent powder yield, which showed high yield in freeze dried pulp, rind and seed compared to oven dried.

The freeze-dried and oven dried pulp, rind and seed were subjected to sequential solvent extractions separately by Hexane < chloroform < ethyl acetate < acetone < methanol based on the polarity *i.e.*, from non polar solvent to polar solvent. The obtained extracts from each solvent was subjected to concentration by flash evaporation followed by lyophillization to obtain crude extract.

The highest yield of the crude extract was obtained in



Concentration of sample: 2 mg/ ml

Each value represents mean value of three different observations. Mean values with different superscripts ($^{a,b,c,\cdots}$) differ significantly at P< 0.05.

Fig. 10 : Total reducing power of methanol extract of oven dried seed (ODS) and freeze dried seed (FDS) of bael fruit

the methanol extracts compared to other four solvent extracts. Among the methanol extract freeze-dried pulp had given highest extract (43.00% w/w) compared to oven dried pulp (40.70% w/ w), and also freeze-dried rind and seed extracts of methanol was higher compared to oven dried rind and seed extract.

The extracts of all the five solvents were examined for the antioxidant activity by DPPH radical scavenging method. It was found that methanol extract has given highest activity when compared to ethyl acetate and acetone extracts. Chloroform extracts have shown very less activity. Hexane extracts do not have any activity. Between the freeze-dried and oven dried extract of methanol, freeze dried extracts showed good activity (53.09%) compared to oven dried extracts (51.09%). IC_{50} value of DPPH radical scavenging activity of methanol extract was done and it showed that, highest activity with low concentration was found in freeze dried pulp (83.32 µg / ml), rind (109.52 µg/ ml) and seed (121.32 µg/ml) when compared oven dried pulp, rind and seed.

The crude extract of ethyl acetate, acetone and methanol which have shown good antioxidant activity were subjected to total reducing power assay. All the extracts have shown increased reducing power when concentration was increased. Among the both typesof drying methods *i.e.*, freeze and oven, no significant difference was found, but compared to oven dried, freeze dried extracts shown good activity.Bioactivity assay of different parts of bael fruit indicated highest activity in pulp, an edible portion of the fruit, compared to rind and seed.The bioactivity of sequential solvent extraction of bael fruit indicated that, polar solvents like methanol, acetone and ethyl acetate, had shown higher bioactivity compared to chloroform and hexane. Comparative evaluations of sample preparation methods showed that there was no significant difference in antioxidant activities between freeze dried and oven dried samples of bael fruit.

The results of this investigation have clearly indicated that various parts of bael have potential nutraceutical properties and can be considered for further explorations.

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