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Effect of Incorporation of Fresh *Basella alba* Leaves on Sensory Attributes and Antioxidant Potential of a Traditional Indian Product 'Biwadi'

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ABSTRACT

In recent years antioxidants derived from natural sources mainly plants have been intensively used to prevent oxidative damage because of its advantages over synthetic ones; as they are easily obtained, economical and have slight or negligible effects. Basella alba leaves (BAL) used for medicinal purpose since ages. This study was conducted, to study the effect of addition of fresh BAL on sensory characteristics, total phenols, flavonoid and total antioxidant capacity (TAC) of the Indian traditional product 'Biwadi'. Control and experimental biwadi (the one with highest total phenolic content, fresh BAL added a level of 5%) were used for estimation of total phenols, flavonoid and TAC using FRAP, DPPH, ABTS and RPA assay. Addition of fresh BAL did not show any significant change ($p \leq 0.05$) in the various sensory attributes. Addition of 5% fresh BAL to biwadi significantly increased ($p \leq 0.05$) the total phenols and flavonoid content. TAC using FRAP, ABTS and RPA assays increased with the addition of fresh BAL in the experimental biwadi compared to the control. No significant difference was observed in TAC using DPPH assay. Protein content did not show any significant change by the addition of fresh BAL in experimental product (9.80 gm%) compared to control (9.13 gm%). Addition of fresh BAL in experimental biwadi showed a significant decrease ($p \leq 0.05$) in the L value and hardness of the experimental biwadi. Based on the results reported herein fresh BAL can be considered to be a good source of bioactive compounds and natural antioxidants as evident from results of the total phenols and total antioxidant capacity.*

Key words: *Basella alba leaves, Biwadi, Antioxidant*

INTRODUCTION

Oxidative stress results due to enhanced formation of free radicals, is now a days becoming a major pathophysiological substance in occurrence of many disease. It results due to imbalance between the prooxidants and antioxidants system of the body. Free radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are neutralized by the enzymatic and nonenzymatic antioxidants of the body.

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Free radicals are reactive oxygen species (ROS) and reactive nitrogen species (RNS) that are neutralized by the enzymatic and nonenzymatic antioxidants of the body. Antioxidants play a crucial role in the defense mechanism against ROS which are the harmful by products produced during various metabolic processes. Antioxidants are responsible in inhibition or prevention of the hazardous effects of oxidative stress because of free radical scavenging capacity. Green leafy vegetables contain various phytochemicals as well as vital compounds that are necessary for improving the health status of a person. The fiber present in them add bulk to the diet, improves the gastrointestinal function, helps in the prevention of the constipation and reduce risk of metabolic diseases, type-II diabetes mellitus, cardiovascular disease etc[1]. Green leafy vegetables are very good source of micro nutrients, many are rich in iron and other important nutrients. Many of them are not used properly for human consumption.

BAL is one such green leafy vegetable which is often ignored by humans and not included in the daily diet though each part of BAL is used for medicinal purpose since ages. It is also known as Ceylon spinach, Malabar spinach, Indian spinach, Mayalu leaves and Poi[2]. It is a wildly cultivated plant which has a climbing growth habit. It has two types of verities in stem: a) purplish colored b) green colored. The leaves of BAL are oval and heart shaped, fleshy in texture and tapering to a pointed tip and is 5 to 12 cm long. It has a potent antioxidant activity and antimutagenic, CNS depressant activity and anti-inflammatory activity[1]. The leaves are used as an antibiotic as well as for hypertensive patients. It is also used for treating inflammation, headache and ulcer[3]. The leaf juice is used to treat boils[4]. It is a good source of vitamins A, B and C as well as minerals like iron and calcium. Kaempherol is the flavonoid present in BAL[1].

'Biwadi' is a traditional Indian dehydrated product of Gujarat state prepared by incorporating pearl millet, green garlic, fresh coriander leaves along with various spices. Before consumption it is deep fried. Since antioxidants have been proven to combat against various degenerative diseases and that fresh BAL are a good source of various phytochemicals like saponins, diterpenes, alkaloid, terpenoid, glycoside, tannins, phenols and flavonoids that possess antioxidant capacity[1], an attempt was made in the present research to optimize fresh BAL in the development of a traditional food product named "biwadi" and to assess the sensory, antioxidant and textural properties from the developed product.

MATERIAL AND METHODS

Development of control and experimental biwadi:

Procurement of raw materials:

It deals with procurement of raw materials like broken rice, pearl millet, green garlic, coriander leaves and spices from the local market of Anand. fresh BAL was procured from ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand, Gujarat.

Preparation of control and experimental biwadi

Broken rice was soaked for 24 hrs while pearl millet was soaked for 48 hrs. Half dried pearl millet was pounded. All ingredients were mixed and pressure cooked. Small round balls were flattened between palms, sun dried and fried before consumption.

Experimental biwadi was prepared same as control by incorporating fresh BAL in different proportions to the control biwadi (Table 1).

Studying organoleptic characteristics of biwadi

Color, appearance, texture, crispiness, flavor and overall acceptability were studied from control and experimental biwadi using Composite Scoring Test. **The experimental biwadi containing highest phenolic compound was chosen for chemical and physical analysis.**

Chemical analysis of biwadi

Sample preparation

Known amount of powdered sample was taken, to it 15 ml of methanol:water solvent

(80:20, pH-2) was added. The sample was crushed in mortar pestle till a fine paste is formed. The mixture was kept in a shaker for 30 minutes. The content was centrifuged for 10-15 min at 6000 rpm. The supernatant was collected in a sugar tube. To the residue add 10 ml of methanol:water solvent followed by crushing it again in a mortar pestle. It was then kept in a shaker for 30 min followed by centrifugation for 10-15 min at 6000 rpm. This process was done for three times and the supernatant was collected. The supernatant was pooled and made the volume to 40 ml with methanol:water solvent.

Total Phenols

Total phenols were determined by using Folin-Ciocalteu method given by Singleton and Rossi[6]. Known amount of sample was taken as a sample aliquot. Volume was made up to 1.5 with D/W. To this 0.5 ml of Folin-Ciocalteu (FC) reagent (diluted 1:1) was added. After that 10 ml sodium carbonate was added. Content was made up to 12 ml mix properly and allowed to incubate for 30 minutes at room temperature. The color intensity was measured at 750 nm on a spectrophotometer. For blank 1.5 ml of distilled water was taken followed by addition of 0.5 ml of FC reagent (1:1) and 10 ml of sodium carbonate then treated same as sample. Standard series of known concentration of gallic acid (5-20µg) were prepared and treated in same way as sample.

Flavonoid

The total flavonoids content was measured by using colorimetric assay, used by Singleton et al[7]. Known amount of sample aliquot was taken and volume was made up to 5 ml with distilled water. At 0 minute, 0.3 ml of sodium nitrite, at 5 minutes 0.6 ml of 10% aluminum chloride and at 6 minutes 2 ml of 1N sodium hydroxide were added to the mixture. This was followed by the addition of 2.1 ml of distilled water to it. The solution was mixed well and the intensity of pink color was measured at 510 nm in a spectrophotometer against blank. For blank 5 ml of distilled water was taken and treated same way as sample. Standard series of known concentration of Rutin (20-80 µg) was prepared and final volume was made up to 5 ml with distilled water and there after treated in same way as sample.

Total Antioxidant Capacity using –

Ferric Reducing Antioxidant Power (FRAP)

The procedure described by Benzie and strain[8] was used to evaluate the Total Antioxidant Capacity (TAC). The principle of this method is based on the reduction of a ferric 2,4,6-tripyridyl-striazine complex (Fe3+-TPTZ) to its ferrous colored form (Fe2+-TPTZ) in the presence of antioxidants. Known amount of sample aliquot was taken and volume was made up to 300 µl with distilled water. 1.8 ml of FRAP reagent was added and allowed to incubate at 37^{o}C for 10 minutes. The color complex was measured at 593 nm using spectrophotometer. For blank, to 300 µl of distilled water, 1.8 ml of FRAP reagent was added. Standard series of known concentration of trolox (1-4 µg) was taken and the volume was made up to 300 µl with distilled water. There after all tubes were treated in the same way as sample.

2, 2-diphenyl-1-picrylhydrazyl-radical scavenging activity assay (DPPH-RSA)

The antioxidant activity was determined by the ability of extract to scavenge DPPH radicals. This method was described by Brand-Williams *et al* [9]. The assessment of antioxidant activity is a free radical colorimetry that relies on the reaction of specific antioxidant with a stable free radical DPPH dissolved in methanol. As a result of reduction of DPPH by antioxidant, the optical absorbance at 517 nm of this purple colored solution of DPPH in methanol decreases. This change is detected by spectrophotometer. Known amount of sample aliquot was taken and volume was made up to 1 ml with methanol. 3ml of DPPH reagent was added followed by vigorous shaking and incubation for 20 minutes at 37^{o}C. The reduction of the DPPH radical was determined by measuring the absorption at 517 nm. To 1ml of methanol 3 ml DPPH was added and used as control. Methanol was used as a blank.

ABTS (2, 2, azinobis, 3 ethyl benzo-thiazolin, 6- sulphonic acid assay)

ABTS was carried out by the method performed by Miller & Rice-Evans[10]. Different aliquots of sample and volume made up to 1

ml with ethanol. Added 3 ml of ABTS reagent to it. Incubated for 10 min at 37^{o}C. Read the absorbance of the resulting oxidized solution at 734 nm against ethanol as blank. For control, 1.0 ml ethanol and treated same as sample. Standard - standard series was prepared with known concentration of trolox (1.0-4.0 µg).

Reducing power assay (RPA)

Reducing power assay (RPA) is based on the principle to reduce Fe+3/ferricyanide complex to Fe+2 form by antioxidants at pH 6.6 which is monitored by measuring the formation of Perl's Prussian blue at 700 nm[11]. Known amount of methanolic extracts of the sample was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50^{o}C for 20 min. After incubation, 2.5 ml of 10% trichloroacetic acid was added to it and centrifuged at 3000 rpm for 10 min. 2.5 ml of the supernatant was mixed with 2.5 ml distilled water and 0.5 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm. Different aliquots of Trolox were treated as standard. The total antioxidant capacity of sample measured by RPA is expressed in terms of mg Trolox equivalents/ 100 g (mg TE/ 100 g).

Protein

Protein content was measured using kjeldahl automatic nitrogen distillation system, model: Supra LX VA

Physical Measurement of biwadi

Color (Hunter L*, a*, b* Color scale)

Color measurement was done by Hunter L*, a*, b* color scale. The hunter L*, a*, b* color space is organized in cube form. The 'L*' axis runs from top to bottom. The maximum for 'L*' is 100, which would be black. The 'a*' and 'b*' axis have no specific numerical limits. Positive 'a*' is red. Negative 'a*' is green. Positive 'b*' is yellow. Negative 'b*' is blue.

Texture score (Brookfield CT3 texture analyzer, version 2.1, 50000 gm unit)

A textural analyzer (CT3-100, Brookfiled, USA) using a texture analyzer system "texture pro CT software" equipped with a 50 kg load cell was used. The analyzer was set a " return to start" cycle, a pre-test, test and post-test speed of 1.00 mm/s, 0.5 mm/s and 10.00 mm/s respectively the distance of 10 mm was kept for snap test.

Statistical Analysis

The observation obtained were compared using paired 't' test: Two-sample assuming equal variances and regression analysis using M. S. Excel. One-way ANOVA for sensory score was carried out using SPSS (Version 20). Scatter graph was prepared using M. S. Excel.

RESULTS

Table 2 represents the mean values of different sensory attributes of control and experimental biwadi. It was observed that addition of fresh BAL at different levels (5, 10, 15 and 20 %) in biwadi preparation did not show any significant change in the color, appearance, crispiness, flavor, over acceptability scores of experimental biwadi as compared to the control samples. Incorporation of fresh BAL at a level of 10 % non-significantly improved the texture score of experimental biwadi.

Table 3 represents mean values of total phenols (mg GAE/100gm) and flavonoid (mg RE/100gm) content of biwadi. Total phenols and flavonoid content of experimental biwadi were 164.81 mg GAE/100gm and 127.16 mg RE/100gm respectively which were found to be significantly higher ($p \leq 0.05$) than that of control biwadi (94.02 mg GAE/100gm and 85.12 mg RE/100gm respectively).

Table 4 represents mean values of total antioxidant capacity of biwadi. Total antioxidant capacity of experimental biwadi using FRAP, ABTS-RSA and RPA were found to be 12.65, 11.01 and 434.56 mg TE/100gm respectively which were found to be significantly higher ($p \leq 0.05$) than that of control biwadi (7.13, 6.18 and 253.49 mg TE/100gm respectively). DPPH-RSA of experimental biwadi (50.78 mg TE/100gm) was non-significantly higher than control biwadi (49.61 mg TE/100gm).

Regression analysis indicated a positive and significant correlation ($R^2 = 0.99$, $P = 0.001$) between total phenols and FRAP

assay (Figure : 2), positive and significant correlation (R^2 = 0.99, P = 0.003) between total phenols and DPPH assay (Figure : 3), positive and significant correlation (R^2 = 0.97, P = 0.01) between total phenols and ABTS assay (Figure : 4) of control biwadi.

Regression analysis indicated a positive and nonsignificant correlation (R^2 = 0.57, P = 0.83) between total phenols and total antioxidant capacity (using ABTS assay) of experimental biwadi (Figure 5).

Regression analysis indicated a positive and nonsignificant correlation (R^2 = 0.62, P = 0.57) between total phenols and total antioxidant capacity (using RPA assay) of experimental biwadi (Figure 6).

Table 5 represents mean values of protein content of control and experimental biwadi. The protein content of experimental biwadi was 9.80 gm% which was nonsignificantly higher than the control biwadi (9.13 gm%).

Table 6 represents the mean values of hunter lab (L*, a*, b*) of control and experimental biwadi. From the results it can be interpreted that the L* value which shows the lightness, was significantly higher ($p \leq 0.05$) in control biwadi (53.24) as compared to experimental biwadi (44.73). The positive value of a* (redness) and b* (yellowness) depicted a nonsignificantly higher ($p \leq 0.05$) value of control biwadi (7.59, 28.99 respectively) compared to the experimental biwadi (7.09, 27.55 respectively). On the basis of L*, a*, b* value of control and experimental biwadi it can be concluded that addition of fresh BAL darkened the experimental biwadi.

Table 7 represents the mean values of texture scores of control and experimental biwadi. The samples were analyzed for hardness, deformation at hardness and fracturability score. From the results it can be interpreted that the hardness score was significantly higher ($p \leq 0.05$) in control biwadi (1318.75 gm) as compared to experimental biwadi (665 gm). The deformation at hardness score was nonsignificantly higher ($p \leq 0.05$) in experimental biwadi (2.67 mm) compared to the control biwadi (2.03 mm). Control biwadi (382.5 gm) had nonsignificantly higher ($p \leq 0.05$) fracturability compared to experimental biwadi (198.75 gm).

DISCUSSION

The present study revealed that biwadi incorporated with fresh BAL at varying levels were at par with the control biwadi in terms of all sensory attributes. Parota, herbal tea, cookies, paneer and dosa were prepared by incorporation of BAL by Meti[2] who carried out sensory evaluation with the help of 5 point rating scale with reference to appearance, taste, texture and flavor showed that the prepared recipes were found highly acceptable. Many scientists have reported the presence of total phenols and flavonoid content in BAL[12]. BAL contains basella saponins[13] and peptide and also contains phenolic compounds[14]. BAL has flavonoid compounds namely flavonols, anthocyanins[12], luteolin (0.099 mg/ml), apigenin (0.165 mg/ml) and naringin (0.180 mg/ml)[15]. Incorporation of fresh BAL, which is a rich source of favonoids as reported by many authors, might have increased the flavonoid content of experimental biwadi in the present study. BAL contains flavonoids, ascorbic acid, phenolic compounds, carotenoids (like lutein, zeaxanthin, β-carotene), organic acids, water soluble polysaccharides, bioflavonoid[12,16,17] and Basella saponin A, B, C and D[13]. Olagire and Azeez[17] have reported that BAL has DPPH antioxidant activity. In addition to this BAL also contains antioxidant enzymes like catalase (0.56 units/g), peroxidase (1.25 units/g), polyphenol oxidase (0.35 units/g), glutathione reductase[12]. It has minerals and vitamins such as zinc, copper, iron, magnesium, vitamin A, E, C and K that also adds to total antioxidant capacity[15,16,18,19]. BAL contains many phytochemicals including tannins, saponins, alkaloids, diterpenes, terpenoid[3,15,20]. These compounds together act as oxygen derived free radical scavenger[2]. Presence of antioxidants like phenolic compounds, phytochemicals, enzymes, pigments, minerals and vitamins as in BAL by many authors could justify the higher level of TAC of experimental biwadi in

the present study. Areekul and Phomkaivon[21] found a close correlation between TPC and all antioxidants activities (FRAP, DPPH and ABTS). A good correlation between DPPH values and total phenolic contents of the *Salvia* species indicated that phenolic compounds could be responsible for ability of reducing oxidants[22]. As shown by Adegoke and Ojo[20], there was a positive correlation between the phenolic and flavonoid at $r^2 = 0.645$ (0.01 levels). The author in the present study also found a positive and significant correlation of phenols with FRAP ($R^2 = 0.99$, P = 0.001) and DPPH ($R^2 = 0.99$, P = 0.003).

The total protein content of BAL was found to be 3 gm^2. The plants consist of essential amino acids such as arginine, isoleucine, leucine, lysine, threonine and tryptophan[16]. Amino acids are essential for the growth and development, reproduction and health of all human[24]. Amino acids play an important role in cell signaling involving protein kinase, G protein-coupled receptors and gaseous molecules[25].

The dark color of experimental biwadi may be due to fresh BAL incorporation which is evident from a lower 'L*' value as compared to the control biwadi. Gala *et al*[23]. found that addition of spinach powder in biscuits darkens the experimental product than control.

Table 1: Composition of control and experimental biwadi (per 100 gm)

Ingredients	C	E-I	E-II	E-III	E-IV
Broken rice	14.28	14.28	14.28	14.28	14.28
Pearl millet	42.85	42.85	42.85	42.85	42.85
FRESH BAL	-	5	10	15	20
Green garlic	18.57	15.71	13.57	11.07	8.57
Coriander leaves	18.57	15.71	13.57	11.07	8.57
Spices	9.56	9.56	9.56	9.56	9.56

- C :- Control product; E- I, II, III, IV :- Experimental products

- Plate 1: Appearance of Biwadi
- A) Control biwadi, B) E-I (5gm%), C) E-II (10gm%), D) E-III (15gm%), E) E-IV (20gm%)

Table 2: Sensory scores of control and experimental 'biwadi'

	Color	Appearance	Texture	Crispiness	Flavor	Overall acceptability	Total
C	7.83 ^{a} ± 1.12	7.63 ^{a} ± 0.92	7.83 ^{b} ± 1.12	16.08 ^{a} ± 3.28	23.71 ^{a} ± 4.42	16.00 ^{a} ± 2.65	79.13 ^{a} ± 10.96
E-I	7.33 ^{a} ± 1.09	7.29 ^{a} ±0.90	7.71 ^{b} ± 0.95	15.88 ^{a} ± 2.77	22.42 ^{a} ± 5.94	15.67 ^{a} ± 2.66	76.25 ^{a} ± 11.58
E-II	7.25 ^{a} ± 1.22	7.29 ^{a} ± 1.19	7.92 ^{ab} ± 0.97	16.79 ^{a} ± 2.28	22.67 ^{a} ± 5.49	16.08 ^{a} ± 2.76	78.00 ^{a} ± 11.48
E-III	7.67 ^{a} ± 1.67	7.58 ^{a} ± 1.06	8.17 ^{b} ± 0.91	16.71 ^{a} ± 2.91	23.13 ^{a} ± 5.20	15.96 ^{a} ± 2.67	79.21 ^{a} ± 11.27
E-IV	7.54 ^{a} ± 0.97	7.33 ^{a} ± 0.86	7.21 ^{a} ± 0.88	15.58 ^{a} ± 3.22	21.96 ^{a} ± 6.04	15.33 ^{a} ± 2.49	74.96 ^{a} ± 11.60
F – value	1.08^{ns}	0.65^{ns}	3.16*	0.78 ^{ns}	0.36 ^{ns}	0.32 ^{ns}	0.64 ^{ns}

- Values are Mean ± SD of three trials, *Indicates significant difference (p≤0.05), ^{ns}Indicates non significant difference (p≤0.05)
- Mean values with different superscripts within the column differ significantly (p≤0.05)

Table 3: Total phenols and flavonoid content of control and experimental 'biwadi' (5 gm%)

	Total phenols (mg GAE/100gm)	Flavonoid (mg RE/100gm)
Control biwadi	94.02 ± 8.64	85.12 ± 5.46
Experimental biwadi	164.81 ± 3.83	127.16 ± 1.82
T – value	-12.97*	-12.65*

- Values are Mean ± SD of four observations, *Indicates significant difference (p≤0.05)

Table 4: Total antioxidant capacity (using FRAP, DPPH, ABTS, RPA) of control and experimental 'biwadi' (5 gm%)

	FRAP (mg TE/ 100gm)	DPPH (mg TE/ 100gm)	ABTS (mg TE/ 100gm)	RPA (mg TE/ 100gm)
Control biwadi	7.13 ± 0.13	49.61 ± 2.04	6.18 ± 0.09	253.49 ± 1.42
Experimental biwadi	12.65 ± 0.15	50.78 ± 2.04	11.01 ± 0.27	434.56 ± 39.40
T – value	-46.53*	-0.71 ^{ns}	-28.56*	-7.93*

- Values are Mean ± SD of four observations, * Indicates significant difference (p≤0.05), ^{ns}Indicates non significant difference (p≤0.05)

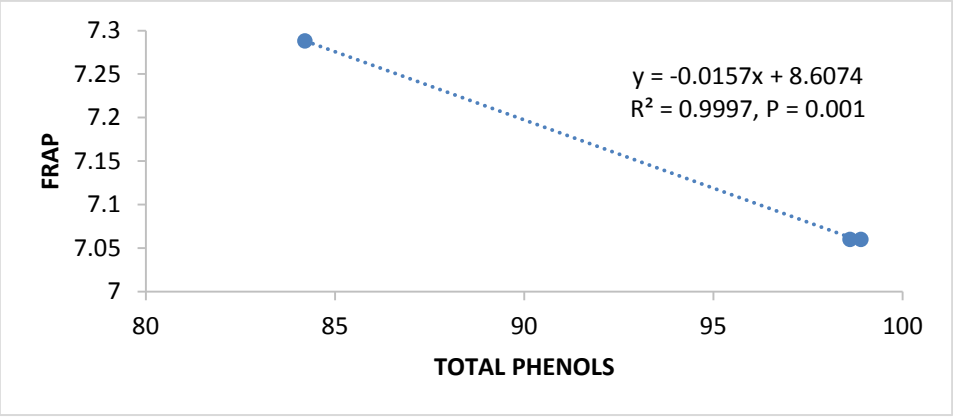

Fig. 2: Regression analysis between total phenols and total antioxidant capacity (using FRAP assay) of control biwadi

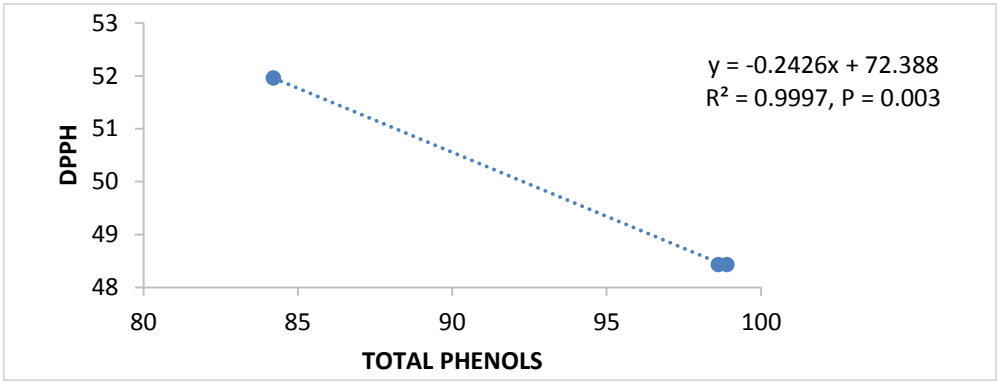

Fig. 3: Regression analysis between total phenols and total antioxidant capacity (using DPPH assay) of control biwadi

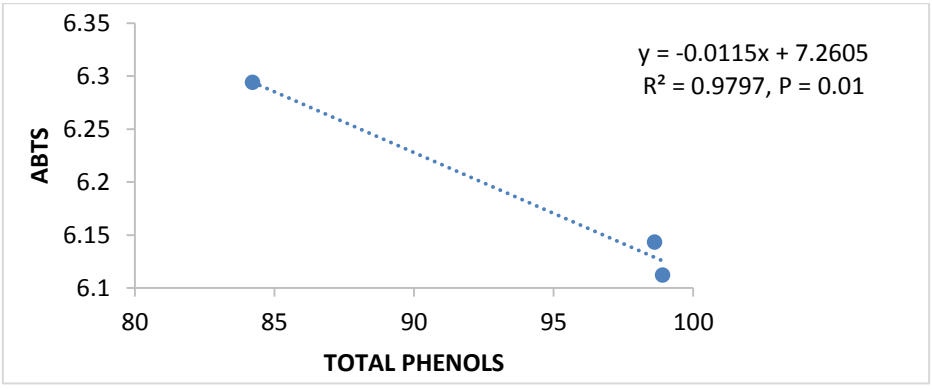

Fig. 4: Regression analysis between total phenols and total antioxidant capacity (using ABTS assay) of control biwadi

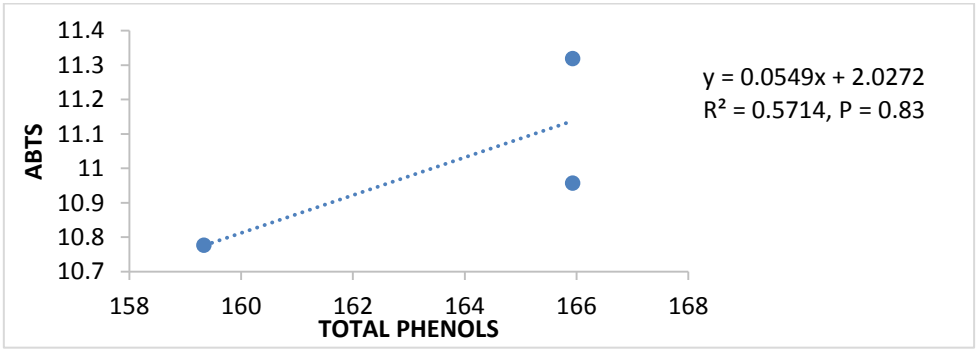

Fig. 5: Regression analysis between total phenols and total antioxidant capacity (using ABTS assay) of experimental biwadi (5 gm%)

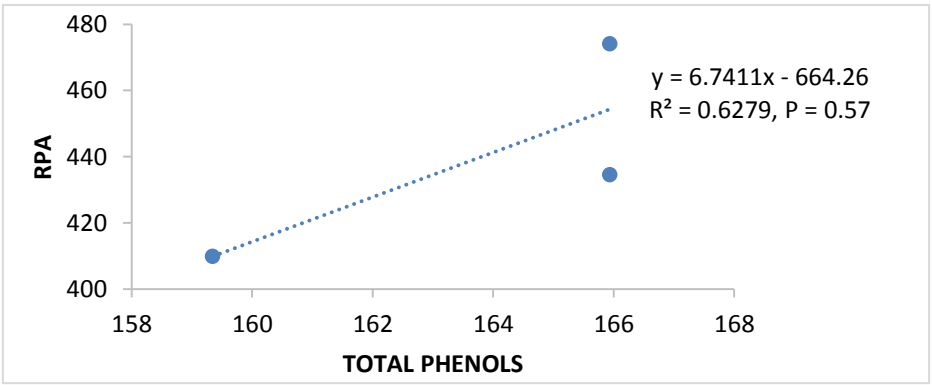

Fig. 6: Regression analysis between total phenols and total antioxidant capacity (using RPA assay) of experimental biwadi (5 gm%)

Table 5: Protein content of control and experimental 'biwadi'

	Protein (gm%)
Control biwadi	9.13 ± 0.22
Experimental biwadi	9.80 ± 1.20
T- value	-0.94^{ns}

➢ Values are Mean ± SD of three observations, ^{ns}Indicates non significant difference ($p \leq 0.05$)

Table 6 Color values (L*, a*, b*) of control and experimental 'biwadi'

Sample	**L***	**a***	**b***
Control biwadi	53.24 ± 1.50	7.59 ± 0.46	28.99 ± 0.95
Experimental biwadi	44.73 ± 2.69	7.09 ± 1.51	27.55 ± 2.06
T-value	5.50*	0.62^{ns}	1.26^{ns}

➢ Values are Mean ± SD of four observations, *Indicates significant different ($p \leq 0.05$), ^{ns}Indicates non significant difference ($p \leq 0.05$)

Table 7: Texture scores of control and experimental 'biwadi'

Sample	**Hardness (gm)**	**Deformation at hardness (mm)**	**Fracturability (gm)**
Control biwadi	1318.75 ± 119.53	2.03 ± 1.22	382.5 ± 294.63
Experimental biwadi	665 ± 189.69	2.67 ± 1.47	198.75 ± 223.06
T-value	5.83*	-0.66^{ns}	0.99^{ns}

➢ Values are Mean ± SD of four observations, *Indicates significant different ($p \leq 0.05$), ^{ns}Indicates non significant difference ($p \leq 0.05$)

CONCLUSION

Based on the results reported herein fresh BAL can be considered to be a good source of bioactive compounds and natural antioxidants as evident from results of the total phenols and total antioxidant capacity. Experimental biwadi having 5 gm% fresh BAL had higher antioxidant capacity, higher protein and crispier texture than the control biwadi.

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