Evaluation of antioxidant potentials in seeds and sprouts of *Eleusine coracana L.*

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ABSTRACT: : *The present investigation was carried out to evaluate the phytochemicals and antioxidant capacities of dried seeds and sprouts of important cereal grain – Eleusine coracana L., commonly called ragi. The dried seeds and germinated seeds (24 hrs, 48 hrs and 72 hrs) coarse powder were extracted separately with 70% ethanol and the extracts were rigorously subjected for phytochemical analysis and seven different antioxidant assays. Phytochemical analysis indicate the presence of major phytochemicals viz, Phenolics, flavonoids, tannins, saponins, terpenoids, etc., in all the stages of germination and dry ragi seeds. Quantitave estimation of total phenolics, flavonoids, tannins in the extracts imply the presence of high concentration in dried seeds compared to the extract from the different germination periods. Similarly, among seven different antioxidant assays, the dry seeds extract showed best quenching ability in DPPH free radical with IC-50 value 246.65µg followed by NO scavenging (IC-50 260.9µg), lipid peroxidation inhibition (IC-50 261.5µg), ABTS (IC-50 272.2µg), reducing power (IC-50 273.9µg), OH radical (IC-50 275.5µg) and metal chelating (IC-50 484.7µg) assay. Among four different samples dry seeds extract found to have best scavenging ability when compared with different time germinated seed extracts. The results were discussed.*

Key Words: *Eleusine coracana L; antioxidants; finger millets; sprouts*

Introduction

Oxygen plays a key role in the existence of life on earth. Living organisms utilize oxygen for their metabolic needs during which, around 5% of the oxygen gets reduced to free radicals, collectively called as Reactive Oxygen Species (ROS) that are known to exert oxidative stress on the cells (Mondal *et al,* 2006). The oxidative stress is said to be an imbalance between **prooxidants**, and **antioxidants**, which turned in favour of the prooxidants resulting in major diseases like aging, diabetes, cancer, cardiovascular diseases, etc (Jain and Agrawal, 2008 and Jacqueline, 2000). Antioxidants block oxidation by neutralizing ROS. In doing so, the antioxidants themselves become oxidized because of which there is definitely a constant need to replenish our antioxidant resources (Shetti *et al*, 2009). Although cells are equipped with superlative defense system comprising of various antioxidant enzymes and small antioxidant molecules, these may not always be sufficient enough to neutralize oxidative stress. Therefore, in order to restore normal cellular antioxidant system, supplementing exogenous antioxidants is very much necessary (Swargiary *et al*, 2015). Synthetic antioxidants have proven to be a bad choice in the view of health concerns. And this fact has shifted the attention towards plant-derived antioxidants.

The notable primary sources of natural antioxidants are whole grains, fruits and vegetables (Prakash, 2001). However, it has to be noted that grains have largely been ignored as important source of dietary antioxidants, despite the known fact that they contribute as the staple dietary components in every part of the world (Sridevi *et al*, 2008). Major antioxidants found in whole grain foods are minerals (Ca, Mg, K, P, Na and Fe) and phytochemicals like phytates and phenolic compounds (Sridevi *et al*, 2008). Millets, one of the major groups of cereal grains are small grained, annual, warm weather crops consisting 8000 species within 600 genera, of which only 35 species of 20 genera are known to be domesticated (Pradhan *et al,* 2010).

Finger millet (*Eleusine coracana* (L.) is an important millet cultivated in India and several African countries (Hilu, 1988). It is very commonly known as ragi or mandva in Indian subcontinent (Pradhan *et al,* 2010).

Ragi contains various phenolic compounds including tannins that contribute to its excellent antioxidant activity (Sripriya *et al,* 1996). The US National Research Council in 1996 report states that 'Despite its importance, finger millet is grossly neglected both scientifically and internationally and it is more nutritious than most cereal grains with respect to minerals, dietary fibre and amino acids (Siwela, 2009)'. Malt is viewed as having a better food value than unmalted grain (Chavan and Kadam, 1989).

Germination is a set of processes that leads to the birth of a plant from seed (Coulibaly and Chen, 2011). Malting is the limited germination of cereal grains under controlled conditions (Briggs, 1998). Seed germination and post-germination seedling development are well-regulated processes that involve high metabolic activity and generation of ROS in the cell (Kiran *et al,* 2012). ROS are known to have a dual role as both toxic byproducts of aerobic metabolism and key regulators of growth, development and defense pathways (Kiran *et al,* 2012). Germination has a profound change on nutritional quality of the cereal and protein bioavailability is said to be increased. Since the consumption of seeds and sprouts is gaining popularity among people interested in improving and maintaining their health status (Ramesh *et al,* 2011), there is a necessity to study the impact of traditional method of soaking and germination in improvement of the nutritive value of cereal grains (Coulibaly and Chen, 2011). The seeds and sprouts are excellent examples of 'functional food', which lowers the risk of various diseases and promoting health benefits in addition to its nutritive value (Ramesh *et al,* 2011). Germinated ragi is said to have better bioavailability of zinc and iron (Sarkar *et al,* 2015). In view to this, the present investigation is aimed to evaluate the antioxidant benefits of seeds and different stages of sprouts of ragi, in terms of phytochemicals and antioxidant capacities.

Materials and Methods

Collection of Seeds

The dried seeds of ragi - *Eleusine coracana* L. var., GPU-8 were procured from University of Agricultural and Horticultural Sciences, Shivamogga.

Sample Preparation

The ragi sample was cleaned manually to remove dust and other impurities. 500 g of dried seeds were grounded into fine powder using a grinder and stored in an airtight container. One and half kilogram of dried seeds was divided into 3 batches of 500 g each, and soaked in distilled water in ratio of 1:3 (p/v) for 24hrs and the grains were spread on a white cotton fabric and kept wet by spraying distilled water frequently according to the method described by Coulibaly and Chen (2011). One batch comprising 500 g of seeds was allowed for 24 hrs for germination, the second batch of seeds was allowed for 48 hrs germination and the last batch for 72 hrs germination. After germination, the seeds were dried separately at 45^0C using hot air oven and were grounded into fine powder and stored in airtight container for further use.

Extraction

The extraction of sample was performed according to the method described by Jamuna *et al*, 2015. Exactly 200 g of each ground sample was weighed and extracted in 70% ethanol for 7 days in dark at room temperature with intermittent shaking. After 7 days, the whole extracts were filtered using muslin cloth and concentrated using a rotary evaporator (Buchi, Flaweli, Switzerland). The yield of crude extract is noted, stored in desiccators for 3-4 days, and later stored in deep freezer in separate containers. The extracts were labeled as R1 (ethanolic extract of dried seeds), R2 (ethanolic extract of 24hrs germinated seeds), R3 (ethanolic extract of 48hrs germinated seeds) and R4 (ethanolic extract of 72hrs germinated seeds).

Qualitative Phytochemical Analysis

The qualitative phytochemical analysis was performed with all the four samples for the presence of different chemical groups. The method for photochemical analysis was followed as described by Makari *et al*, 2009.

Quantitative Phytochemical Analysis

Total Phenolic Content

Total phenolics in the extracts were determined by the method described by Gulcin *et al*. (2002) using Folin-Ciocalteu reagent with gallic acid as standard and expressed as equivalent to gallic acid in mg/g.

Total Flavonoid Content

Total flavonoid content of all the extracts of ragi were determined by Aluminium chloride colorimetric method as described by Basniwal, *et al* (2009) and were expressed as equivalent to rutin in mg/g.

Total Tannin Content

The total tannin content of all the four extracts were determined by the method as described by Price and Butler, (1977) and were expressed as µg tannic acid equivalents/mg dry weight.

Total Antioxidant Capacity (TAC)

The TAC of all the four ethanolic extracts of ragi dried seeds and sprouts of different intervals, was performed according to the spectrophotometeric method of Yen and Chen, (1995) and were expressed as equivalents of ascorbic acid in µg/mg of extract.

Evaluation of *in vitro* antioxidant activities

The antioxidant assays provide an estimate of antioxidant ability of ragi seeds and sprouts in the varied oxidation scenarios. All the antioxidant assays were performed in triplicates and the results were reported as IC50, which is the amount of antioxidant necessary to scavenge the 50% of ROS.

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

The DPPH radical quenching ability of ragi extracts was determined by the colorimetric method as described by Brand-Williams *et al*, (1995). Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula,

% Inhibition = ((A0-A1)/A0 × 100)

Where, A0 is the absorbance of the control (in absence of test sample), and A1 is the absorbance of test samples.

Reducing power assay

The Fe^{3+} reducing power of the extracts was determined by the method of Oyaizu, (1986) with slight modifications.

ABTS Assay

The ABTS assay for all the four extracts of ragi was carried out by the method as described by Roberta Re *et al*, (1999). The formula used was

% Inhibition = ((A0-A1)/A0 × 100)

Where, A0 is the absorbance of the control (in absence of test sample), and A1 is the absorbance of test samples.

Hydroxyl radical scavenging assay

The scavenging activity for hydroxyl radical in the given extracts of ragi was measured by Fenton reaction as explained by Halliwell, *et al.*1987. The formula used was

% Inhibition = ((A0-A1)/A0 × 100)

Where, A0 is the absorbance of the control (in absence of test sample), and A1 is the absorbance of test samples.

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity for the given ragi extracts was determined according to the method reported by Garrat, (1964). The formula used was

% Inhibition = ((A0-A1)/A0 × 100)

Where, A0 is the absorbance of the control (in absence of test sample), and A1 is the absorbance of test samples.

Lipid Peroxidation assay

The assay was performed as explained by Dehpour *et al,* 2009. The formula used was

% Inhibition = ((A0-A1)/A0 × 100)

Where, A0 is the absorbance of the control (in absence of test sample), and A1 is the absorbance of test samples.

Fe^{2+} chelating assay

The chelating activity for the extracts for ferrous ions Fe^{2+} was measured according to the method of Dinis *et al*., 1994. The formula used was

% Inhibition = ((A0-A1)/A0 × 100)

Where, A0 is the absorbance of the control (in absence of test sample), and A1 is the absorbance of test samples.

Statistical analysis

All the experiments were carried out in triplicates. The result of the triplicates was pooled and expressed as mean±standard error.

Results

Qualitative phytochemical analysis

The preliminary qualitative phytochemical investigation documented that all four extracts of Ragi showed the presence of many bioactive compounds, namely, alkaloids, flavonoids, tannins, glycosides, triterpenes and saponins. The results of the analysis are shown in Table 1.

Table 1: Qualitative Phytochemical Analysis of Seeds and sprouts of Ragi

Sample	Alkaloids	Flavonoids	Tannins	Glycosides	Triterpenes	Saponins
R1	+	+	+	+	+	+
R2	+	+	+	+	+	+
R3	+	+	+	+	+	+
R4	+	+	+	+	+	+

R1-Ragi Dry seeds, R2- Ragi 24hrs germinated, R3- Ragi 48hrs germinated & R4- Ragi 72 hrs germinated

Quantitative phytochemical analysis

The result analysis of all the Quantitave phytochemical estimations is depicted in Table 2 and Fig 1.

Total Phenolic Content

The total phenolic content present in ethanolic extract of dried ragi seeds was found to be 8.45 mg GAeq/g (Table 2), and in 24hr germinated seed extract it was 6.13 mg GAeq/g followed by 5.84 mg GAeq/g in 48hr germinated seed extract, and 5.90 mg GAeq/g in 72hr germinated seed extract. Among the four different extracts, the highest total phenolic content was found to be in the dried seeds which started to decrease upon germination. The order of total phenolic content in the examined extracts was found to be dried seeds>24 hrs germinated seeds>48 hrs germinated seeds. However, at 72 hrs germination, the concentration was found to increase. Totally, the dried seeds of ragi were found to be rich in phenolic compounds.

Total flavonoid content

In the ethanolic extract of dried ragi seeds, total flavonoid content was found to be 5.89 mgReq/g (Table 2) and it was found to be decreasing in 24hr germinated seed extract to 3.38 mgReq/g and further reduced after subsequent germination to 2.19 mgReq/g (48 hrs) and 2.01 mgReq/g (72 hrs). The flavonoid content in Ragi was found to decrease upon malting and germination.

Total Tannin Content

The tannin content in the ethanolic extract of dried seeds of ragi was found to be 5.12 TAeqmg/g (Table 2). The tannins were observed to decrease upon germination initially and was found to be 3.78 TAeqmg/g for 24hrs germinated seed extract, and 2.19 TAeqmg/g for 48hr germinated seed extract. But the amount of tannin was found to increase upto 3.07 TAeqmg/g in the 72 hrs germinated seed extract. The tannins in ragi was high in dried seeds and begins to decrease upon germination till 72hrs in which, an increase in the concentration was observed.

Total Antioxidant Capacity (TAC)

The total antioxidant capacity is expressed as equivalents of ascorbic acid in nmolAA/g (Table 2). The total antioxidant capacity of ethanolic extract of dried seeds of ragi was found to be maximum among the four samples and was found to be 711.67 nmolAA/g while in the 24 hrs germinated seed extract it was found to be 517.67 nmolAA/g, in 48 hrs germinated seed extract it was 411.33 nmolAA/g and in 72 hrs germinated seed extract it slightly increased upto 413.67 nmolAA/g. The results clearly state that dried seeds of ragi exhibit high total antioxidant capacity in comparison with the germinated seeds.

Table 2. Quantitative Phytochemical evaluation of raw and different stage germinated ragi

Sample	Total Phenolics (mg GAeq/g)	Total Flavonoids (mg Req/g)	Total Tanin (mg TAeq/g)	Total Antioxidant (nmolAA/g)
R1	8.45±0.24**	5.89 ± 0.23**	5.12± 0.12**	711.67±34.20
R2	6.13±0.12**	3.38 ± 0.28**	3.78± 0.18**	517.67±9.39
R3	5.84±0.20**	2.19 ± 0. 10**	2.19± 0.12**	411.33±8.65
R4	5.90±0.10	2.01 ± 0.09	3.07± 0.15	413.67±32.38

*=p<0.05 **=p<0.01,

GAeq= Gallic acid Equivalent ; # Req = Rutin Equivalent; TA eq= Tannic Acid equivalent, AA=Ascorbic acid.

R1-Ragi Dry seeds, R2- Ragi 24hrs germinated, R3- Ragi 48hrs germinated & R4- Ragi 72 hrs germinated.

Evaluation of *in vitro* Antioxidant activity

DPPH radical scavenging activity

All the four ethanolic ragi extracts showed the excellent DPPH radical scavenging activity along with standard, ascorbic acid and the results are depicted in the **Fig 1A and Fig 3**. The DPPH radical scavenging activity was increased with the increase in dose of the extract; therefore, the scavenging activity is dose

dependent. The standard ascorbic acid showed the IC50 value at very low concentration (below 10µg) where as the dry seeds extract showed IC50 at 246.65 µg and it is followed by R2 (384.5µg), R3 (479.5µg) and R4 (437.1µg).

Reducing power assay

The ability of reductive capacity of ragi extracts were compared with the standard ascorbic acid. Among all the four extracts, R1 performed best with dose dependent pattern where 273.9 µg of was required to reduce 50% of Fe^{3+} molecule when compared to 386 µg, 387.5 µg and 379.1 µg values of R2, R3 and R4 respectively. The values show a decrease upon germination till 48 hrs and an increase at 72hrs germination. The results were shown in **Fig 1B and Fig 3**.

ABTS Assay

All the four ethanolic extracts of Ragi were evaluated for the ABTS assay along with standard, Ascorbic acid. The IC 50 value for R1 sample was found to be 272.2 µg which is higher than R2 (398.3 µg), R3 (474 µg) and R4 (450.4 µg). The antioxidant activity was dose dependent; higher the concentration of sample, higher is the reduction of oxidant molecule. The values begin to decrease upon germination till 48 hrs (R3 sample) and a rise was observed in the values for 72hrs germinated sample (R4). The results are depicted in **Fig 1C and Fig 3**.

Hydroxyl radical scavenging assay

Hydroxyl radical scavenging assay results show a maximum activity in R1 with IC50 275.5 µg compared to the germinated seed extracts. The antioxidant activity was found to decrease upon germination up to 48 hrs and shows an increase in 72 hrs germinated seed extract. The result is shown in **Fig 1D and Fig 3**.

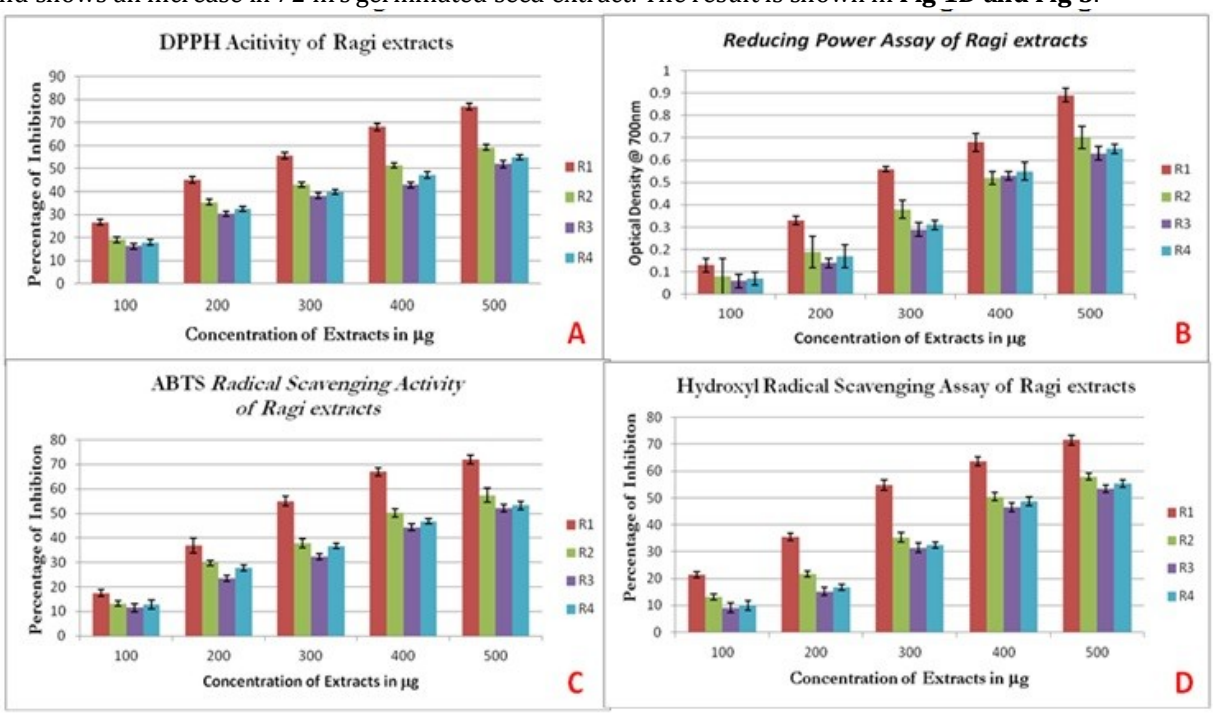

Figure 1. Graphical representation of quenching A) DPPH, B) Reducing power assay, C) ABTS Radical scavenging assay and D) Hydroxyl radical scavenging assay

Nitric oxide radical scavenging activity

Dose dependent nitric oxide scavenging assay was shown by ragi extracts. The maximum activity was in dried seed extract R1 (IC50 260.9µg) compared to germinated seed extracts R2 (IC50 - 485.8µg), R3 (IC50 - 460.4µg) and R4 (IC50- 407.2µg) respectively. IC-50 value shows minimum for R1 and increase upon germination till 72hrs. The results are shown in **Fig 2A and Fig 3**.

Lipid peroxidation Inhibition assay

All the four ragi extracts were subjected to lipid peroxidation inhibition assay. Ethanolic extract of dried seeds showed maximum activity compared to the germinated seed extracts. The activity was found to decrease in 24 hrs and 48 hrs germination; however, an increase in the activity was observed at 72 hrs germination. The IC-50 values pattern shows that R1 has comparatively less value than R2, R3 and R4 as in **Fig 3.** The values are depicted in the **Fig 2B**.

Fe^{2+} chelating assay

Among the different antioxidant activities, the Fe^{2+} chelating assay consumed higher concentration of extract which is almost double than any other activity. Among four samples the dried seed extract of ragi shows a maximum activity in Iron chelating assay with IC50 value 484.7 µg. The values are found to decrease upon germination with a slight rise in 72 hrs (IC50 - 806.25 µg) germination seed extract. The results are depicted in the **Fig 2C and Fig 3**.

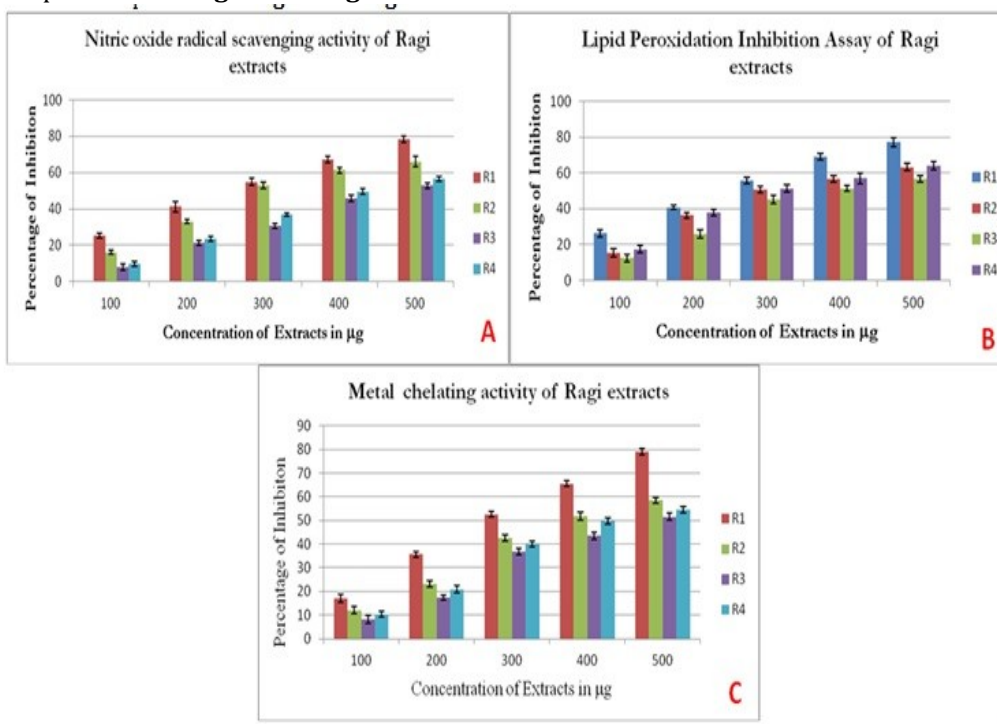

Figure 2. Graphical representation of quenching A) Nitric oxide scavenging activity B) Lipid peroxidation inhibition assay and C) Iron chelating assay

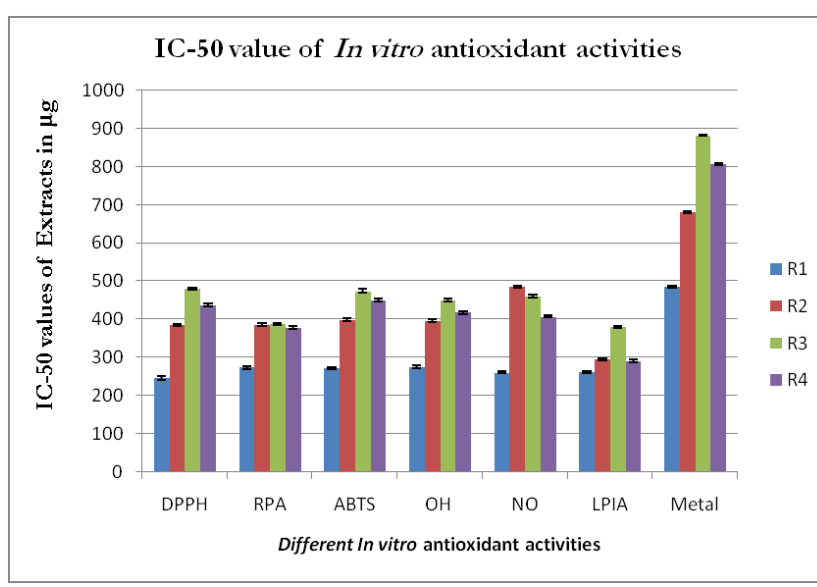

Figure 3. IC-50 values of different extract for different antioxidant assays

Discussion

Antioxidant foods like cereal grains represent a premier area of research which needs to be worked on to provide better nutrition with medicinal benefits to the population. Utilization of whole grain cereals in food formulations is increasing worldwide and so it becomes necessary to assess the quality of grains in different

growth stages. Ragi is a major cereal grain that exhibit many medicinal properties. The presence of phytochemicals *viz* alkaloids, tannins, glycosides, saponins, is attributed to the medicinal properties of ragi. Phenolic acids and their derivatives, flavonoids and tannins present in ragi seed coat have multiple functions and can act as reducing agents (free radical terminators), metal chelators, and singlet oxygen quenchers (Palanisamy *et al*, 2014).

The present investigation is aimed towards the comparison of antioxidant capacities of ethanolic extract of dried seeds and sprouts of ragi using various methods. Our attempt was to rank antioxidant capacities of ragi extracts of different growth stages by pooling the overall results of seven assays. Regardless of the method selected, high antioxidant activity was found in the ethanolic extract of dried seeds of ragi. Similar works report the decrease in the content of phytic acid upon germination of ragi (Sripriya *et al,* 1997). Tannin content is also said to be reduced by 54% upon malting (Rao, 1994). All these findings can be attributed to the decrease in the antioxidant activity in the germinated seeds reported in the present investigation. The germination is said to decrease the antinutritional factors of the seeds and improve the digestibility (Sripriya *et al,* 1997). The obtained results clearly show the decrease in antioxidant activity upon malting and germination initially but at 72hrs germination, the activity was found to increase. Malting process is found to exert negative effect on the antioxidant capacities of ragi which increases in the further stages of germination. Focused research is further required to identify and isolate compounds that are contributing to the changes in the antioxidant capacities in ragi at different stages of growth.

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