



COMPARATIVE ANTIOXIDANT AND PHYTOCHEMICAL ACTIVITY OF RAW AND BOILED TUBER OF *DIOSCOREA BULBIFERA* COLLECTED FROM TRIBAL FOREST OF SUNDARGARH DISTRICT, ODISHA, INDIA

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ABSTRACT

In the present study, we have investigated the differences in the composition of proximate minerals, vitamins bioactive compounds, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging activity between the raw and boiled tubers of *Dioscorea bulbifera*. The results showed that both the raw and boiled tubers have rich sources of carbohydrates (31.62% and 23.94%), proteins (3.48% and 2.25%), starch (8.6% and 11.67%), and free amino acids (1.45% and 0.59%); but have low-fat content (0.19% and 0.14%). Vitamin profiling of the tubers contained a substantial amount of ascorbic acid, vitamins B1, B2, B3, and B6. Further, the raw and boiled tuber of *Dioscorea bulbifera* had a very high amount of bioactive compounds like phenolics, flavonoid, diosgenin, tannin, and saponin. Phenolic and flavonoid content positively correlated with free radical scavenging activity of tuber and performed better scavenging activity compared to ascorbic acid and butylated hydroxytoluene (BHT). Thus, the tuber of *Dioscorea bulbifera* is a better food supplement to meet the calorie requirement of the tribal people and a rich source of antioxidants.

1. Introduction

Wild tubers *Dioscorea bulbifera*, known as air potato or yam, is primarily found in the tropical, subtropical, and temperate region of the world (Abara, 2011). *Dioscorea bulbifera* produces both underground and aerial tubers. However, underground tubers are very rich in starch hence mostly consumed. The tribal people used it as food as well as medicine (Kumar *et al.*, 2013). The tubers are composed of a high amount of carbohydrates, fibers, and low fats and protein content with a good proportion of different amino acids, making them an excellent dietary source and could be consumed, boiled, steamed, baked, or fried (Osman, 1990). Due to lack of good nutritional information, the broad

utilization of the yam is very limited; Studies of the nutritional composition of the locally available *Dioscorea bulbifera* is very important since it may help the tribal people to fulfill their dietary requirements. Besides excellent nutritional properties, *Dioscorea bulbifera* reported exhibiting antimicrobial, antioxidant, plasmid curing, analgesic, anti-inflammatory, antihyperglycemic, antihyperlipidemic, antidiabetic, antinociceptive, and antitumor activities (Ghosh *et al.*, 2015). *Dioscorea bulbifera* is also reported to have good radical scavenging and singlet oxygen quenching ability hence used as potential herbal therapeutic agents for various diseases that occurred due to

oxidative stress (Ghosh *et al.*, 2013). *Dioscorea bulbifera* (*D. bulbifera*) exhibits higher antioxidant capacities with lower IC₅₀ values than the other species (Padhan *et al.*, 2020). Detailed information on the profiling of tubers of *D. bulbifera* from the state of Odisha is very rare. In this regard, the present study is therefore aimed to evaluate the comparative phytochemical and antioxidant properties of the boiled and raw tuber of wild yam *D. bulbifera*.

2. Materials and methods

2.1. Collection and preparation of sample

The tubers specimen and the plant were collected from the forest of Sundargarh, Odisha, and identified at the Centre of Excellence in Natural Products and Therapeutics, Dept. of Biotechnology and Bioinformatics, Sambalpur University. Tubers were washed thoroughly, peeled, and sliced approximately 1-2 mm of thickness. Some of the sliced tubers were boiled and dried in a hot air oven at 80 °C until a constant weight was obtained. In contrast, the other portion was taken fresh, without boiled, and dried in a hot air oven at 80 °C until a constant weight was obtained. After drying, the samples were powdered and sieved through a 1mm sieve. Both raw and boiled tuber powder samples were preserved in an airtight glass bottle for further analysis.

2.2. Comparative Nutritional analysis

Nutritional analyses of both raw and boiled tuber were determined. Moisture, ash, and fat contents were determined using a hot air oven, muffle furnace and Soxhlet apparatus following the standard method described by Ranganna (2007). Total carbohydrate and starch content were estimated using the Anthrone reagent method (Thayumanavan and Sadasivam, 1984). Reducing sugar content of both the raw and boiled tuber samples was estimated by the Dinitrosalicylic acid method (Sadasivam and Manickam, 2008). The protein content of both the raw and boiled tuber samples was estimated following the standard method of Lowery *et al.* (1951). The total free amino acid of the tuber samples was determined using Ninhydrin

reagent (Sadasivam and Manickam, 2008). The amino acid profiling of the raw and boiled tuber samples was determined using 5.54 SP 5LAB solutions software after detection through Shimadzu LC-30 AD HPLC (Pal *et al.*, 2016). Vitamins (B1, B2, B3, and B6) in tuber samples were analyzed in HPLC (Shimadzu HPLC and photodiode array detector. Supelcosil LC 17 DB column 250 mm×4.6 mm, 5µm; Sigma, USA) following the method of Perales *et al.* (2005). The mineral compositions were analyzed using an Inductively Coupled Atomic Adsorption Spectrometer (Perkin-Elmer Optical Emission Spectrometer, Optima 7000 DV) as per the methods of Kalra (1998). The ascorbic acid content was estimated as the standard method described by Sadasivam and Manickam (2008).

2.3. Comparative Bioactive compounds analysis

Total free phenolics and Total flavonoid content were determined in methanol, acetone, and water extract of tuber using modified Folin-Ciocalteu method (Ordon Ez *et al.*, 2006) and the aluminum chloride colorimetric assay (Pallab *et al.*, 2013), respectively. Tannin was estimated by following the methods of Schanderl (1970). The method of Obadoni and Ochuko (2001) was used for the determination of saponin content. Diosgenin was determined following the methods of Uematsu *et al.* (2000).

2.4. DPPH scavenging activity

Tuber extracts were concentrated to dryness under reduced pressures at 40 °C using a rotary evaporator and dissolved in methanol to make a stock solution of 50 mg/ml and used for different antioxidant activities. The effect of the extracts on DPPH (1,1-diphenyl-2-picrylhydrazyl) radical was determined following the standard method described by Liyana- Pathiranan and Shahidi (2005). The quantity of DPPH radical scavenged was calculated using the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(\text{AbsControl} - \text{AbsSample}) / (\text{AbsControl})] \times 100}{\dots} \text{Eq}^{\text{p}} \dots \quad (1)$$

Abs Control = absorbance of DPPH radical+methanol; (2)

Abs Sample = absorbance of DPPH radical+sample extract/standard. (3)

Where Abs = Absorbency

2.5. Statistical Analysis

The results obtained were subjected to statistical analysis as mean and standard deviation (Zar, 1984). The mean values and standard deviations were calculated from the data obtained from three different experiments. The statistical difference at $p < 0.05$ was considered to be significant.

3. Results and discussions

3.1. Nutritional content

In the present study, a comparative analysis of nutritional composition between the raw and boiled tubers has been carried out, and the proximate content of the raw and boiled tubers is presented in Table-1. The moisture content of the raw tuber was found to be relatively low ($74.89 \pm 0.54\%$) compared to the boiled tuber ($80.48 \pm 1.18\%$). The ash content of the raw tuber was found to be high ($2.57 \pm 0.04\%$) compared to the boiled tuber ($1.66 \pm 0.34\%$). We found a very low amount of fats in both raw tubers ($0.19 \pm 0.01\%$) and boiled tuber ($0.14 \pm 0.012\%$), which are not significantly different. Total Carbohydrate ($31.62 \pm 0.46\%$) and reducing sugar content ($0.018 \pm 0.008\%$) of raw tuber was found to be relatively high compared to the boiled tuber ($23.94 \pm 0.50\%$ and $0.012 \pm 0.008\%$). In contrast, it was found that the boiled tuber contained an albeit high amount of starch

($11.67 \pm 0.65\%$) compared to the raw tuber ($8.6 \pm 0.54\%$). The protein content of the raw and boiled tuber was found to be $3.48 \pm 0.92\%$ and $2.25 \pm 0.16\%$, respectively. The total free amino acid content was found to be slightly high in the raw tuber ($1.45 \pm 0.05\%$) compared to the boiled tuber ($0.59 \pm 0.13\%$). HPLC analysis of both the raw and boiled tubers for different amino acids revealed that out of nine essential amino acids, six amino acids (Histidine, methionine, lysine, phenylalanine, threonine, valine) are observed in the raw tuber and four amino acids (lysine, threonine, valine, and phenylalanine) in the boiled tuber (Table 1). Phenylalanine was present in the highest amount in both raw and boiled tuber, followed by valine. Histidine, methionine, and cysteine amino acids were found in minimum quantity in the raw tuber and were not detected in the boiled samples. The observations of the mineral compositions of the raw and boiled tubers are presented in Table 1. The sodium, potassium, phosphorus, iron, and calcium content of the raw tubers were observed in the range of 316 ± 27.78 , 677.33 ± 21.38 , 153.20 ± 17.17 , 6.16 ± 0.89 , 290 ± 4.13 mg/100g dry mass, respectively. In contrast, the estimated value of the above mineral contents in boiled tuber were 119.36 ± 16.25 , 232.33 ± 12.50 , 60.43 ± 1.72 , 3.24 ± 1.06 , 180 ± 2.28 mg/100g dry mass, respectively. The other essential elements such as magnesium, zinc, manganese, and copper were found to be 203 ± 6.42 , 0.45 ± 0.95 , 4.2 ± 2.16 , and 0.79 ± 0.62 mg/100g dry mass, respectively in the raw tuber. In contrast, the boiled tuber contained a lower amount of magnesium, zinc, manganese, and copper elements (102 ± 4.14 , 0.18 ± 0.83 , 0.89 ± 1.86 , and 0.12 ± 0.37 mg/100g dry mass, respectively).

Table 1. Nutritional composition analysis of raw and boiled tubers of *Dioscorea bulbifera*

Nutrient content	Raw tuber	Boiled tuber
Proximate		
Moisture (%)	74.89±0.54	80.48±1.18
Ash (%)	2.57±0.04	1.66±0.34
Total carbohydrate (%)	31.62±0.46	23.94±0.50
Starch (%)	8.6±0.54	11.67±0.65
Reducing sugar (%)	0.018±0.008	0.012±0.008
Fat (%)	0.19±0.01	0.14±0.012
Protein (%)	3.48±0.92	2.25±0.16
Free amino acid (%)	1.45±0.05	0.59±0.13
Amino acid quantity (mg/100g)		
Glutamic acid	14.27±0.51	10.24±0.35
Glutamine	8.95±0.11	7.6±0.29
Histidine	1.033±0.19	Nd
Arginine	3.9±0.23	3.34±0.15
Alanine	5.2±0.15	3.29±0.32
Serine	6.54±0.03	5.85±0.25
Tyrosine	4.18±0.17	3.58±0.24
Cysteine	0.37±0.13	Nd
Methionine	1.16±0.03	Nd
Proline	4.01±0.01	3.46±0.03
Glycine	5.5±0.03	6.3±0.08
Lysine	3.54±0.43	2.32±0.07
Threonine	4.42±0.27	2.05±0.05
Valine	5.19±0.11	4.43±0.03
Phenylalanine	5.29±0.35	4.83±0.21
Mineral element (mg/100g)		
Sodium	316±27.8	120±16.3
Potassium	677±21.4	232±12.5
Phosphorus	153±17.2	60.4±1.72
Iron	6.16±0.89	3.24±1.06
Calcium	290±4.13	180±2.28
Magnesium	203±6.42	102±4.14
Zinc	0.45±0.95	0.18±0.83
Manganese	4.2±2.16	0.89±1.86
Copper	0.79±0.62	0.12±0.37
Vitamin mg/100g		
Vitamin C	99.5±0.94	70.7±1.19
Vitamin B1	0.007±0.0008	0.005±0.0004
Vitamin B2	0.027±0.007	0.014±0.003
Vitamin B3	27.38±1.42	14.65±1.25
Vitamin B6	0.128±0.028	0.084±0.006

Not detected

The details of vitamin analysis of *Dioscorea bulbifera* are shown in Table 1. The ascorbic acid content of the raw tuber was found to be 99.5 ± 0.94 mg/100g dry mass. In contrast, the ascorbic content of the boiled tuber was found to be 70.7 ± 1.19 mg/100g dry mass. The content of vitamin B1, vitamin B2, vitamin B3, and vitamin B6 for the raw tuber was found to be 0.007 ± 0.0004 , 0.027 ± 0.007 , 27.38 ± 1.42 , and 0.128 ± 0.028 mg/100g dry mass, respectively. In contrast, the boiled tuber's vitamin B1, vitamin B2, vitamin B3, and vitamin B6 content was found to be comparatively in lesser quantity than that of the raw tubers (Table 1). Among all the contents analyzed, it has been observed that in comparison to the present finding, a higher amount of fat, starch, protein, potassium, calcium, zinc, and copper has been reported earlier by other researchers (Polycarp *et al.*, 2012). In contrast, carbohydrate and sodium contents reported earlier have been lower than the present findings (Sanful *et al.*, 2013). A very similar amount of ash, protein, phosphorus, manganese, magnesium, ascorbic, and vitamin contents has been observed compared to the earlier report (Okwu and Ndu, 2006). In comparison to the boiled tuber, raw tubers are found rich in nutrition (Table-1) which might be due to the leaching and degradation of most of the vitamins and mineral contents during boiling. In comparison to the previous reports, the variation observed in the nutritional content of the tuber in the current report might be due to the soil Physico-chemical properties, soil fertility, geographical and climatic condition, genetic variation, etc. (Bhandari and Kawabata, 2004). Overall the results revealed that the tuber of *Dioscorea bulbifera* are good sources of minerals and could be used as food supplements

3.2. Bioactive compounds

Bioactive compounds are secondary metabolites of the plant that have pharmacological or toxicological effects in humans and animals. Tannin is one of the phenolic compounds which gives an astringent and bitter taste. Tannins act as antidiarrheal, haemostatic and anti hemorrhoidal, anti-

inflammatory, antiviral and antibacterial, antiseptic, antioxidant. Diosgenin content plants are grown for steroid preparation (Behera *et al.*, 2010). Saponins are responsible for the reduction of cholesterol levels in animals along with other animals. (Desai *et al.*, 2017). The analysis showed that raw tuber contained 160.2 ± 0.84 mg/100g while boil tuber contained 12.5 ± 0.11 mg/100g of diosgenin. Tannin content was found to be 180.11 ± 0.32 mg/100g and 12.09 ± 0.12 mg/100g for raw and boil tuber, respectively, in this present study. The saponin content of raw and boil tuber was found to be 150.34 ± 0.67 mg/100g and 21.26 mg/100g, respectively. Ghosh *et al.* (2014) reported diosgenin exhibited potent inhibition against both porcine pancreatic alpha-amylase and alpha-glucosidase as well as against crude murine amylase and glucosidase and acts as lead candidate in managing Type II Diabetes Mellitus. Behera *et al.* (2010) reported 1383 mg/100g of diosgenin for *Dioscorea bulbifera* tuber. Polycarp *et al.* (2012) reported 10.98 mg/100g of tannin in *Dioscorea bulbifera*. Princewill-Ogbonna and Ibeji (2015) found 8.49- 14.03 mg/100g of saponin content of three cultivars of *Dioscorea bulbifera*.

3.3. Antioxidant properties

Phenols and flavonoids are compounds having antioxidant activity hence can absorb and neutralize free radicals, quenching singlet and triplet oxygen, or decompose peroxides (Luis *et al.*, 2012). Among boiled and raw tuber, the latter one showed excellent radical scavenging activity, which is significantly higher than that of ascorbic acid and BHT (Butylated Hydroxytoluene). Methanolic extracts of raw and boiled tubers of *Dioscorea bulbifera* were used in the present study to determine the DPPH radical scavenging activity, and results were compared with standard ascorbic acid and BHT (Figure 1). The IC_{50} value (the concentration of the sample that reduced 50% of the absorbances of DPPH) of raw and boiled tuber, ascorbic acid, and BHT are included in Figure 2. Higher the IC_{50} value signifies less antioxidant activity and *vice-versa*. It was found that the methanolic

extract of the raw tuber has significantly higher antioxidant activity (IC_{50} value is $46.11 \mu\text{g/ml}$) compared to the ascorbic acid (IC_{50} value is $92.86 \mu\text{g/ml}$), BHT (IC_{50} value is $54.35 \mu\text{g/ml}$) and boiled tuber extract (IC_{50} value is $455.37 \mu\text{g/ml}$). Phenolic content of the raw tuber extracted with different solvents such as acetone, methanol, and water was found to be 95.92 ± 11.78 , 121.11 ± 13.71 , and 205.19 ± 15.91 mg GAE/100g dry weight, respectively (Table 2). In contrast, the boiled tuber's acetone, methanol, and water extract contained 12.71 ± 5.28 , 23.04 ± 5.60 , and 54.18 ± 10.40 mg GAE/100g dry weight, respectively of total phenols. Previously, Bhandari et al. (2003) have reported 166 mg GAE/100g fresh weight of total phenols for the acetone extract of *Dioscorea bulbifera* tuber. Total flavonoid content with the methanol, acetone, and water extract of raw tuber was 359.82 ± 18.10 , 232.10 ± 34.22 , and 387.71 ± 9.96 mg quercetin equivalent/100 g dry weight (Table 2). In contrast, the methanol,

acetone, and water extract of boiled tuber possessed a quite low flavonoid content viz. 75.91 ± 12.63 , 54.83 ± 7.11 , and 64.13 ± 11.04 mg quercetin equivalent/100 g dry weight. Okwu and Ndu (2006) reported 8.04 mg quercetin equivalent /100g, and Adeosun et al. (2016) reported 5.36 mg quercetin equivalent/100g of flavonoid contents of *Dioscorea bulbifera* tuber on the dry weight basis. TPC (total phenol content) of tuber positively correlated with DPPH scavenging activity at the 0.01 level ($R^2=0.9762$) (Figure 3). TFC (total flavonoid content) and DPPH also positively correlated at 0.01 level ($R^2=0.9717$) (Figure 4). The result of the analysis showed phenols are potent antioxidant and scavenging agents.

Overall, for treating radical-related pathological damage, the tuber of *Dioscorea bulbifera* can be used as a therapeutic agent. The current report also suggests that in *Dioscorea bulbifera*, polyphenol is essential component responsible for its antioxidant activities.

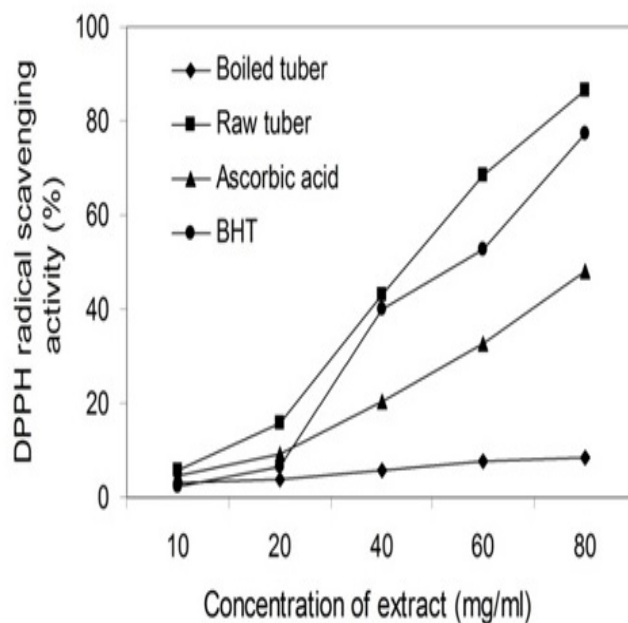


Figure 1. Comparison of DPPH radical scavenging activity of methanolic extract of the raw and boiled tuber of *Dioscorea bulbifera* with ascorbic acid and BHT.

Table 2. Phenols and flavonoid content of raw and boiled tubers of *Dioscorea bulbifera*

Polyphenol content	Raw tuber			Boiled tuber		
	Methanol extract	Acetone extract	Water extract	Methanol extract	Acetone extract	Water extract
Phenols (mg/100 g dry mass)	121.1±13.71	95.92±11.78	205.2±15.91	23.04±5.60	12.71±5.28	54.18±10.40
Flavonoid Mg/100 g dry mass) DPPH	359.8±18.10	232.1±34.22	387.7±9.96	75.91±12.63	54.83±7.11	64.13±11.04

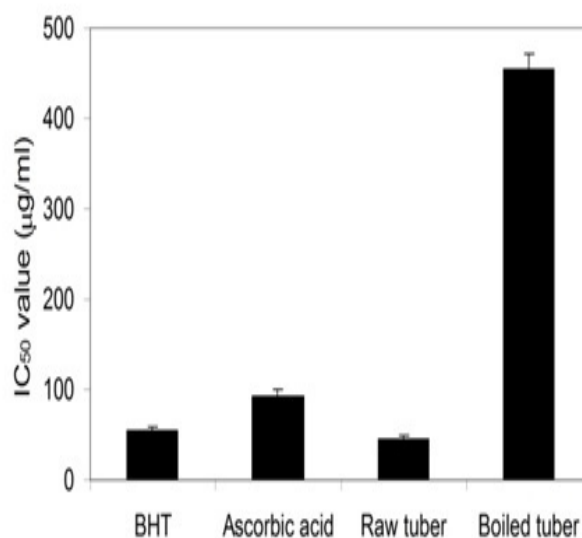


Figure 2. The IC₅₀ value of raw and boiled tuber, ascorbic acid, and BHT

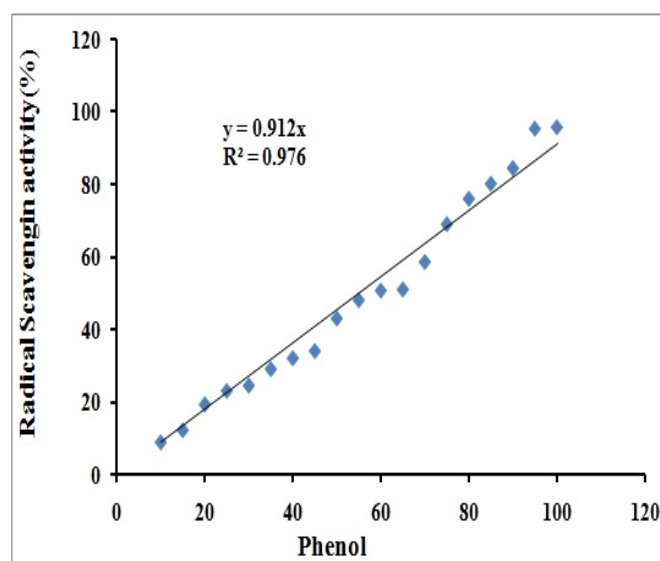


Figure 3. Correlation between TPC and DPPH

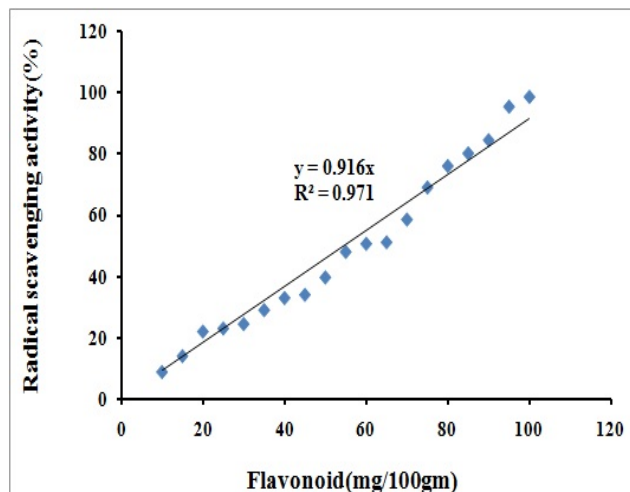


Figure 4. Correlation between TFC and DPPH

4. Conclusions

We have evaluated the nutritional composition and antioxidant activity of the tuber of *D. bulbifera*, both in raw and in boiled form. We found that the nutritional composition of the raw form is very rich than the boiled form of the tubers. The antioxidant activity of the raw tuber was found to be significantly very high compared to the ascorbic acid and BHT. The total phenolics and flavonoids are found to be significantly high in both raw and boiled tubers. The present study also emphasizes phytochemical analysis. Hence the tuber *D. bulbifera* could be a good candidate for functional foods.

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