Nutritional potential of selected species of *Arisaema* Mart. from Nepal

**LAXMI DHAKAL**,1,*** BIVA ARYAL,**2,3** GIRI PRASAD JOSHI,1 DEEPAK RAJ PANT1,***

1Central Department of Botany, Tribhuvan University. 44613 Kirtipur, Nepal.  
*email: laxmidhakal704@gmail.com, **deepak.pant@cdb.tu.edu.np, dr.pant@cdbtu.edu.np  
2Department of Botany, Amrit Campus, Tribhuvan University. 44600 Kathmandu, Nepal  
3Science Hub Nepal. Balaju-15, 44600 Kathmandu, Nepal

Abstract. Dhakal L, Aryal B, Joshi GP, Pant DR. 2020. Nutritional potential of selected species of *Arisaema* Mart. from Nepal. *Biodiversitas* 21: 5703-5709. Species of *Arisaema* Mart. are believed to be poisonous, but few species are used as food in different communities. Therefore, an assessment of nutritional properties of six selected taxa of *Arisaema* including three edible taxa was made in order to find out their nutritional potential. Methanolic or aqueous extracts of samples (rhizomes and aerial parts) were analyzed for various nutritional and anti-nutritional parameters. On nutritional basis, the total starch content and total reducing sugar were found highest (25.71±0.04 mg/g powder) and (46.73±0.06 mg/g extract), respectively in aerial parts of *A. echinatum*. Total protein and phosphorus content was highest in rhizome of *A. tortuosum* var. *curvatum*. Similarly, extracts of aerial parts of *A. tortuosum* var. *curvatum* also possessed highest amount of total phenolic content (52.67±0.24 mg GAE/g), total flavonoid content (14.13 ± 0.03 mg QE/g), and also demonstrated best antioxidant activity (IC50, 108.59±0.54 µg/mL) in terms of DPPH radical scavenging activity among the selected taxa. Inhibition of α-amylase and a-glucosidase *in vitro* was found to be highest (78.82 %) in rhizome extracts of *A. echinatum* and extracts of aerial parts of *A. concinnum* (34.82 %), respectively.

Keywords: Antioxidant activity, anti-nutrients, *Arisaema*, nutritional value

INTRODUCTION

The global population at present is nearly 8 billion (Worldometers 2020) and is expected to reach 9-11 billion by 2050 (Roos et al. 2017). At the same time, the urban population will reach 70% as compared to 56% now (Worldometers 2020). There is a need to increase cereal production substantially to reach over 3 billion tons per year by 2050 (FAO 2009). These facts indicate a bleak future for the people living in the third world in terms of food security. It will be mostly the rural poor and marginalized populations who may have difficulty in having access to proper nutrition as they do not have resources to pay for their nutritional needs. In this context, use of wild edibles for human food or animal feed can be one of the approaches to address the problem of food security in these areas.

People in different parts of the World have been using wild edible plants to cater to their various needs. About 150 species representing nearly one-fifth of the number of wild species consumed as food in India, Malaysia, and Thailand have been identified as sources of emergency food crops (FAO 1992). Aryan et al. (2018) have reported about 99 species of wild edible non-cultivated plants from western Himalaya. These resources are available freely in nature and can help supplement the nutritional requirements of the local people as well as guarantee food security during difficult times.

*Arisaema* Mart. is one of the large and diverse genera of the flowering plant family Araceae. It comprises 151 species collected from Asia, Eastern Africa, and Eastern North America (Li et al. 2010). It is represented by 16 species in Nepal (Rajbhandari and Rai 2017). There is perception among the general public in Nepal that all species of *Arisaema* are toxic. However, there are certain ethnic groups that are using different species of *Arisaema* as food. In upper Mustang, Nepal, *A. jacquemontii* is used as vegetables after processing (Lama et al. 2001). Similarly, Manandhar (2002) reported five different species of *Arisaema* including *A. consanguineum*, *A. flavum*, *A. jacquemontii*, *A. tortuosum*, and *A. utile* being used as food in Nepal. In addition, various species of *Arisaema* are also used in traditional medicine as anthelmintic (Balami 2004; Manandhar 2002), laxative (Bhattarai 1992), in skin infections (Gewali 2008; Manandhar 2002), etc.

Foodstuffs derived from plants including vegetables also contain different types of anti-nutritional factors which reduce nutrient availability in food used for human or animal consumption (Sahu et al. 2020). Plants of the family Araceae including *Arisaema* are rich in raphides (Keating 2004) which also act as toxin, affect calcium metabolism, and also form kidney stones (Petroski and Minich 2020). Similarly, anti-nutrients like amylase inhibitors that affect carbohydrate metabolism are reported to be present in food plants of Araceae like *Colocasia* (Kumari et al. 2012). In this background, present study aims to analyze nutritional potential and anti-nutrient (anti-amylase and anti-glucosidase) activities of selected taxa of *Arisaema* from Nepal to find out differences, if any, in selected nutritional parameters among different taxa. The findings of the study will help to find out the possibility of using other taxa as well for human food and/or animal feed.
MATERIALS AND METHODS

Collection and identification of plant samples

The list of taxa taken for study and their respective collection sites is presented in Table 1 and the photographs are presented in Figure 1. Samples (rhizomes and aerial parts) of Arisaema tortuosum var. curvatum were collected from Surkhet Valley (Western Nepal) while that of all other species were collected from different parts of Kathmandu valley, Nepal. Voucher specimens for Herbarium preparation were collected along with the samples and deposited at the National Herbarium, Kathmandu, Nepal (KATH), Nepal. Field notes along with GPS co-ordinates (Garmin eTrex-30) were also noted while collecting the samples.

Table 1. Collection sites of plant samples

| Species | Site | Alt. (m.) | Location |
|---------|------|-----------|----------|
| A. concinnum Schott | A | 1792 | 27°34'78" N, 85°23'97" E |
| A. consanguineum Schott | B | 1627 | 27°38'71" N, 85°15'83" E |
| A. echinatum (Wall.) Schott | C | 1712 | 27°39'38" N, 85°14'89" E |
| A. erubescens (Wall.) Schott | C | 1635 | 27°39'32" N, 85°14'88" E |
| A. tortuosum var. tortuosum (Wall.) Schott | D | 1380 | 27°39'48" N, 85°15'85" E |
| A. tortuosum var. curvatum (Roxb.) Engl. | E | 953 | 28°34'08" N, 81°39'28" E |

Note: *A: Phulchoki, Lalitpur; B: Champadevi, Kathmandu; C: Machchhegoun, Kirtipur; D: Bhatkepati, Kirtipur; E: Lekhbas, Surkhet*

**Figure 1.** Photographs of selected species of Arisaema: A. A. concinnum, B. A. consanguineum, C. A. echinatum, D. A. erubescens, E. A. tortuosum var. tortuosum, F. A. tortuosum var. curvatum
Preparation of plant extracts
The collected plants were chopped into small pieces and shade dried until constant weight. Dried samples were ground to fine powder separately by using mixer grinder. The powdered sample was mixed with 10 times the volume of solvent (methanol or water) and subjected to sonication at 40 kHz for 2 hours at 40-60°C. Afterward, the mixture was filtered through Whatman No. 1 filter paper. The filtrate was collected and residue was mixed again with 10 times the volume of respective solvent. The mixture was subjected to sonication for one and half hours under similar conditions to ensure complete extraction. Both filtrates were mixed and concentrated under reduced pressure at 80°C. The dried extracts were scraped off the Petri plates and stored in 2 mL polypropylene tubes at -20°C.

Estimation of nutritional components
Reducing sugar
Reducing sugar in aqueous extracts of rhizome and aerial parts of Arisaema species was determined by dinitro salicylic acid (DNS) method (Bailey 1988). Glucose was used as standard and reducing sugar was expressed as mg of glucose equivalent per gram dry weight of plant powder. In order to minimize the interference of other substances present in extracts of different species, negative controls were used for every sample and their absorbance readings deducted from that of samples from respective species.

Total starch
A half-gram (0.5 g) of plant powder was washed with 80% (v/v) hot ethanol (Changshu Hongsheng Fine Chemicals, Changshu, China) twice to remove sugar, and residue was collected. Then excess ethanol was dried off by evaporation in hot air oven. After complete drying 5 mL of distilled water and 6.5 mL of perchloric acid (Qualigens chemicals, Mumbai, India) was added to the residue and mixed well. Then the samples were left to stand at 4°C for 20 min. Afterward supernatant was filtered twice for further tests by DNS method to calculate the amount of sugar as glucose equivalent. Starch content was obtained by multiplying the glucose content by a factor of 0.9 following the method described by Chow and Landhausser (2004).

Total protein
Total protein content of the aqueous extract of plant samples was quantified as per Bradford assay (He 2011).

Total phosphorus
The powdered plant sample (0.5 g) was burnt to ash in a muffle furnace at 550°C. Total phosphorus content present in the ash was determined following the method of Bertramson (1942).

Total phenolic and flavonoid contents
Total phenolic and flavonoid content were determined only from methanolic extracts. Total phenolic content (TPC) was determined using Folin-Ciocalteu Method (Ainsworth and Gillespie 2007) and total flavonoid content (TFC) was determined using the Aluminum Chloride (AlCl₃) colorimetric method (Roy et al. 2011).

Antioxidant activity
The free radical scavenging activity of samples and standard ascorbic acid solution in methanol was determined based on their ability to react with stable methanolic solution (0.2 mM) of DPPH (Fisher Scientific India Limited) in dark (Singh et al. 2002). The absorbance (abs.) of the solution was measured at 517 nm using UV-Visible Spectrophotometer (CT8600, E-chrome Tech, Taiwan). The percentage of free radical scavenging activity (RSA) of the plant sample was calculated by using the following formula:

\[
\text{% Radical Scavenging activity (RSA)} = \frac{100\times (\text{control abs.} - \text{sample abs.})}{\text{control abs.}}
\]

A curve was obtained by plotting the percentage RSA against concentration. Based on the standard curve, IC₅₀ was calculated by using linear equation of the curve obtained.

\[
Y = a\times X + b \\
\text{IC}_50 = \frac{(50 - b)}{a}
\]

Where, \(X\) = Concentration, \(Y\) = % RSA, \(a\) and \(b\) are the coefficient and constant, respectively, of the linear equation.

Evaluation of anti-amylase and anti-glucosidase activity
Anti-amylase and anti-glucosidase activity of the methanolic plant extracts in vitro was measured using inhibition assays for the enzymes porcine pancreatic amylase and α-glucosidase (Sigma Aldrich, Germany) using protocols described by Ahmed et al. (2009) and Si et al. (2010).

Data analysis
All the data are presented as mean ± SD of three samples. The data were statistically analyzed by using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Nutritional components
Nutritional value of different species of Arisaema is presented in Table 2. The highest amount of total reducing sugar (46.73±0.06 mg/g extract) was found in aerial parts of A. echinatum and the lowest (18.30±0.46 mg/g extract) was in rhizome of A. concinnum. The highest starch content (25.71±0.04 mg/g powder) was found in aerial parts of A. echinatum and the lowest (18.13±0.04 mg/g powder) was in aerial parts of A. tortuosum var. tortuosum. The total protein content ranged from 0.90±0.16 mg BSA/g in rhizome of A. tortuosum var. tortuosum to 30.78±0.37 mg BSA/g in A. tortuosum var. curvatum. The highest phosphorous content (176.48±3.36 μg/g) was found in rhizome of A. tortuosum var. curvatum and the lowest (16.48±1.68 μg/g) was in rhizome of A. consanguineum.
Table 2. Nutritional value of different species of Arisaema. The highest and lowest values in each parameter are underlined.

| Extract | Total starch content (mg/g powder) | Total reducing sugar (mg/g extract) | Total protein content (mg BSA/g) | Total phosphorous content (µg/g) |
|---------|-----------------------------------|------------------------------------|---------------------------------|---------------------------------|
| ACC-A   | 25.39 ± 0.06                      | 42.07 ± 0.35                      | 3.00 ± 0.05                    | 19.39 ± 5.55                   |
| ACC-R   | 21.69 ± 0.07                      | 18.30 ± 0.46                      | 12.77 ± 0.07                   | 114.72 ± 5.60                  |
| ACG-A   | 21.86 ± 0.07                      | 30.13 ± 0.32                      | 8.98 ± 0.04                    | 38.79 ± 4.20                   |
| ACG-R   | 19.80 ± 0.07                      | 29.60 ± 0.10                      | 11.30 ± 0.11                   | 16.48 ± 1.68                   |
| AEC-A   | 25.71 ± 0.04                      | 46.73 ± 0.06                      | 1.10 ± 0.18                    | 41.21 ± 4.20                   |
| AEC-R   | 19.36 ± 0.07                      | 31.20 ± 0.60                      | 3.33 ± 0.14                    | 65.94 ± 4.44                   |
| AER-A   | 24.62 ± 0.04                      | 44.67 ± 0.31                      | 23.40 ± 1.29                   | 43.64 ± 7.27                   |
| AER-R   | 18.93 ± 0.05                      | 26.63 ± 0.47                      | 25.58 ± 0.09                   | 28.12 ± 4.44                   |
| ATC-A   | 18.63 ± 0.05                      | 39.43 ± 0.45                      | 8.98 ± 0.15                    | 99.39 ± 4.20                   |
| ATC-R   | 19.15 ± 0.08                      | 33.63 ± 0.38                      | 30.78 ± 0.37                   | 176.48 ± 3.36                  |
| ATT-A   | 18.13 ± 0.04                      | 20.93 ± 0.47                      | 12.07 ± 0.23                   | 80.00 ± 7.27                   |
| ATT-R   | 19.56 ± 0.08                      | 28.30 ± 0.26                      | 0.90 ± 0.16                    | 89.21 ± 6.06                   |

Note: ACC: A. consanguineum, ACG: A. consanguineum, AEC: A. echinatum, AER: A. erubescens, ATC: A. tortuosum var. curvatum, ATT: A. tortuosum var. tortuosum. The letter separated by hyphen represents the parts used: aerial parts (A) or rhizome (R)

Total phenolic and total flavonoid content

Total phenolic content (mg gallic acid equivalent per gram) in methanolic extracts were found to be higher in aerial parts than that in rhizomes in all the taxa except A. consanguineum (Figure 2). The highest TPC content (52.67±0.24mg GAE/g) was found in aerial parts of A. tortuosum var. curvatum while the lowest TPC content (8.02±0.3mg GAE/g) was found in aerial parts of A. consanguineum (Figure 3). The highest amount of TFC (14.13 ± 0.03 mgQE/g extract) was found in aerial parts of A. tortuosum var. curvatum and lowest (2.82 ± 0.03mg QE/g extract) in rhizome of A. tortuosum var. tortuosum. The values of other species were between these extremes.

Antioxidant activity

The antioxidant activities of crude extracts of different species of Arisaema expressed in terms IC₅₀ value of DPPH radical scavenging activity is shown in Figure 4. The minimum IC₅₀ value was reported in A. tortuosum var. curvatum (108.59 ± 0.54µg/mL) and the maximum IC₅₀ value was reported in A. consanguineum (820.59 ± 2.59µg/mL). The extract with lowest IC₅₀ value i.e., A. tortuosum var. curvatum was found to show highest antioxidant activity.

Figure 2. Total phenolic content present in methanolic extract of different taxa of Arisaema. Note: ACC: A. consanguineum, ACG: A. consanguineum, AEC: A. echinatum, AER: A. erubescens, ATC: A. tortuosum var. curvatum, ATT: A. tortuosum var. tortuosum

Figure 3. Total flavonoid content present in methanolic extract of different taxa of Arisaema. Note: ACC: A. consanguineum, ACG: A. consanguineum, AEC: A. echinatum, AER: A. erubescens, ATC: A. tortuosum var. curvatum, ATT: A. tortuosum var. tortuosum

Figure 4. IC₅₀ value (µg/mL) of DPPH radical scavenging activity in extracts of selected species of Arisaema. Note: ACC: A. consanguineum, ACG: A. consanguineum, AEC: A. echinatum, AER: A. erubescens, ATC: A. tortuosum var. curvatum, ATT: A. tortuosum var. tortuosum
Anti-amylase and anti-glucosidase activity

The anti-amylase and anti-glucosidase activities of methanolic extracts of different species of *Arisaema* are shown in Figures 5 and 6. Inhibition of α-amylase *in vitro* was found to be highest (78.82 %) in rhizome extracts of *A. echinatum* while that of α-glucosidase was highest (34.82 %) in extracts from aerial parts of *A. concinnum*. At the same concentration, Acarbose showed 94.12 % of inhibition in α-amylase and 16.79 % of inhibition in α-glucosidase, respectively.

Discussion

The *Diplazium esculentum* is a wild edible vegetable with having reducing sugar content of 21.5 mg/g (Archana et al. 2013), which is comparable with that of aerial parts of *A. tortuosum*. Similarly, some leafy vegetables like mint, coriander, curry leaves have a reducing sugar range of 3-5% (Vyanakatrao 2015). The reducing sugar in aqueous extracts of different species of *Arisaema* lied between that of Archana et al. (2013) and Vyanakatrao (2015).

Total carbohydrate content as high as 26.8% on fresh weight basis has been reported in rhizomes of *Colocasia esculenta* (Temesgen and Retta 2015). However, the carbohydrate content (starch equivalent) in rhizome of *Arisaema* is much lower. Since the starch content in plant tissues varies with species, parts used, and maturity, some of these factors might have contributed to lower carbohydrate content in present investigation.

The crude protein in *Colocasia esculenta* was 307 gm/kg dw (Ejoh et al. 1996). The total protein content in *A. tortuosum var. curvatum* is higher than other species used in present study but much lower than that of *Colocasia esculenta*. The lower protein content in comparison to *C. esculenta* may be attributed to the fact that wild plants grow under nutrient stressed conditions compared to cultivated ones.

The phosphorus content in common edible plants varies from 32 to 138 mg/100g (Jaarsveld et al. 2014). The total phosphorus content in rhizomes and aerial parts of different species of *Arisaema* in present investigation was found to be much lower than that reported for *C. esculenta*. Since the nutritional value varies with cultivated and wild plants, cultivated plants get additive nutrition which may responsible for having more carbohydrates, protein, and phosphorous content than wild plants. Meanwhile, the starch content, protein content, and phosphorous content in plant tissues varies with species, parts used, nutritional status of the plants, etc., some of these factors might have contributed to low nutrient contents in present investigation.

Phenolic compounds are a large class of plant secondary metabolites having structure range from simple (phenolic acids) to diverse (polyphenols) forms. Phenolic compounds are important for the quality of plant-based food. These compounds also contribute to health benefits associated with dietary consumption of fruits and vegetables (Cheynier 2012). Baba and Malik (2015) reported that methanolic extracts from the rhizome of *A. jacquemontii* had phenolic content 45.17± 1.70mg GAE/g. Similarly, Nile and Park (2013) reported that the TPC value of 86.2 mg catechin equivalent/g in extracts from rhizomes of *A. tortuosum*. The value of TPC in methanolic extracts of rhizomes of all taxa of *Arisaema* in present study is much lower than that reported by Baba and Malik (2015). However, the highest amount of TPC in *A. tortuosum var. curvatum* among the studied taxa reaffirms its selection as one of the edible species by the hills tribe in Surkhet valley (Karnali, Nepal).

Similarly, flavonoids are a diverse group of phytochemicals found in almost all fruits and plants parts. Along with carotenoids, they are responsible for the vivid colors in fruits and vegetables. Flavonoids also have powerful antioxidants, anti-inflammatory and immune system benefits. They are also used in prevention of neurodegenerative diseases (Spagnulo et al. 2017). Baba and Malik (2015) reported that the TFC value of 35 ± 2.20 mg rutin equivalent/g in tuber extracts of *A. jacquemontii* while Nile and Park (2013) reported TFC value of 175.5 mg rutin equivalent/g in *A. tortuosum* tuber extract. Since the flavonoid content in present study is expressed in quercetin equivalents, direct comparison with earlier reports is not possible. Similar to TPC, the TFC was also
found to be higher in aerial parts than in rhizomes. This may be attributed to high synthetic activities in aerial parts due to the presence of chloroplast, photosynthetic activity and Reactive oxygen species (ROS).

Antioxidants are compounds that prevent oxidation and prolong the life of oxidizable matters. Extracts of different species of *Arisaema* also possess different antioxidant compounds similar to other plants. Though, the overall trend of antioxidant activity of extracts of *Arisaema* in methanol extract are similar to those reported earlier, the values are quite different. Previously, methanolic extract of rhizomes of *A. tortuusum* was reported to have higher (852± 1.3 µg/mL) IC₅₀ value (Nile and Park 2013). In present study, the highest IC₅₀ (820.59± 2.59 µg/mL) was found in methanolic extract of aerial parts of *A. concinnum* and lowest (108.59± 0.54 µg/mL) in aerial parts of *A. tortuusum* var. *curvatum*. The IC₅₀ value of methanolic extracts of rhizomes for both varieties of *A. tortuusum* is much less than that reported by Nile and Park (2013).

The present study revealed that the species which have been used as vegetable had higher antioxidant potential than the rest of other species. Similarly, the extracts of aerial parts were found to possess higher percentages of Radical Scavenging Activity than that of underground parts. This is because chloroplast and mitochondria are two main powerhouses and sites of ROS generator units in plant cells. These parts are also involved in maintaining the fine balance between energy-linked function and control of ROS production (Kasote et al. 2015).

Pancreatic amylases and glucosidase are the enzymes that help to digest complex carbohydrates present in foodstuff into simple, soluble forms like glucose. Inhibitors of these enzymes present in food can be used as hypoglycemic or antihyperglycemic agents for the management of diabetes mellitus. Since some amylase inhibitors are heat resistant and do not get degraded while cooking (Temesgen and Retta 2015), presence of such inhibitors in foodstuff contributes to anti-nutritional properties. The presence of very weak anti-glucosidase activity in extracts of aerial parts of most of the species indicates possibility of using them all as food. On the contrary, high anti-amylase activity in extracts of aerial parts does not support it. However, continuous use of species like *A. concinnum*, *A. consanguineum*, and *A. tortuusum* var. *curvatum* as vegetables by people in different parts of Nepal indicates that the amylase inhibitors present in these plants are probably unstable compounds that get degraded during processing and/or cooking such that the people do not have any problems associated with digestion after their consumption.

A comparison of various nutritional and anti-nutritional properties among selected taxa of *Arisaema* Mart. revealed differences in values of different parameters including total phenolic and flavonoids content, and antioxidant potential among the tested species. In terms of nutritional value, the highest amount of carbohydrates content was found in aerial parts of *A. echinatum*. The highest amount of protein and total phosphorus was found in rhizome of *A. tortuusum* var. *curvatum*. In terms of TPC, TFC, and antioxidant activity, aerial part of *A. tortuusum* var. *curvatum* was found to be the best among the tested species. All the tested species showed moderate inhibitory activity against α-amylase and weak activity against α-glucosidase. These findings highlight the nutritional potential of different species of *Arisaema* and show the possibility of using the remaining species as food supplements/vegetables or animal feed, however, further studies on allergenicity and toxicity are needed in order to conclude them as being safe for general consumption.

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