

COLOCASIA BASED CROPPING SYSTEMS AFFECTS THE ANTIOXIDANT PROPERTIES AND PRODUCTIVITY OF COLOCASIA [COLOCASIA ESCULENTA (L.) SCHOTT] TUBER

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ABSTRACT

Four colocasia based cropping system *viz* Colocasia-Onion-Frenchbean (C-O-F), Colocasia-Frenchbean-Potato (C-F-P), Colocasia-Cabbage-Frenchbean (C-Ca-F), Colocasia-Coriander-Tomato (C-Co-T) were taken to find out the effect of different crops under colocasia based cropping system on the antioxidant activities (AA) and productivity of colocasia tuber. The highest concentrations of phenolics (0.966 + 0.009 mg gallic acid equivalent/100 mg fresh weight) and condensed tannins (0.022 + 0.001 mg catechins equivalent/100 mg fresh weight) were found in 'C-O-F', while the anthocyanin (4.29 + 0.04 mg/100 mg fresh weight) were found highest for 'C-Co-T' cropping system. Colocasia samples from 'C-O-F' cropping system showed highest DPPH and ABTS radical scavenging activities, while reducing power activity was found highest in the colocasia samples from 'C-Co-T' cropping system. Principal component analysis (PCA) showed strong correlation between phenolics, tannins and AA, while anthocyanin was found positively correlated with reducing power. Results of the finding provides evidence that the colocasia samples for 'C-O-F' cropping system showed higher antioxidant activities than samples from other cropping system in most of the determinations, while the productivity in terms of colocasia equivalent yield (52.38 ton/ha) was recorded highest in the 'C-O-F' cropping system.

INTRODUCTION

Antioxidants may be defined as any substance that when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate in a chain reaction (Halliwell and Whitemann, 2004; Leong and Shui, 2002). Antioxidants have become a popular research topic because they cannot be generated by the human body and hence have to be consumed in the diet. A major benefit from the diets rich in fruit and vegetables may be increased consumption of antioxidant vitamins such as ascorbate (vitamin C) and tocopherol (vitamin E), vitamin like compound (glutathione), and pigments such as phenolics and carotenoids (Moyer *et al.*, 2002). They act as major cellular redox buffers that can effectively quench reactive oxygen species (ROS) by donating one or more electrons to ROS. Natural phytochemicals content in fruits and vegetables were greatly affected by growing condition and environment (Jeanelle and Rui, 2004).

Colocasia esculenta (L.) Schott. belongs to family araceae, commonly known as Taro (English). In India, Colocasia is traditionally used as an abortifacient, to treat tuberculous, ulcers, pulmonary congestion, crippled extremities, fungal abscesses in animals, and as an anthelmintic (Singh *et al.*, 2011; Kubde *et al.*, 2010; Tattiyakul *et al.*, 2006). Recent pharmacological studies reveals that leaves of *C. esculenta* reported to had antibacterial, antifungal (Singh *et al.*, 2011), anthelmintic (Kubde *et al.*, 2010), anti-inflammatory (Biren *et*

al., 2007) and antidiabetic Kumawat *et al.*, 2010) activities. Moreover, α -amylase inhibitory activity (McEwan *et al.*, 2010), antidiabetic (Prakasam *et al.*, 2003), *in vivo* antiperoxidative and antioxidant activity (Prakasam *et al.*, 2005) were also attributed to tuber or root of *C. esculenta*.

Depending on the resources and technology available, different types of cropping systems are adopted by farmers. It may be due different sizes of fields, different types of soil, and may be on a slope or on flat land. But it is well accepted that cropping systems are the intensification of cropping in time and space dimensions (Mandal *et al.*, 2014). Different phytochemicals in plant system acts synergistically to increase their antioxidant effects. Along with high antioxidative properties and yield of colocasia tuber as affected by different colocasia based cropping systems, the present study has been undertaken with the novel colocasia-based cropping systems.

The objective of this study was to assesses and compare the variations in antioxidative capacity and economic yield of colocasia in novel colocasia-based cropping systems in Indian sub- Himalaya

MATERIALS AND METHODS

Experimental details

The field experiments were carried out at the research farm of the Vivekananda Parvatiya Krishi Anusandhan Sansthan, Hawalbagh, Almora, India during year 2007-10. The site was located at 29°36' N latitude and 79°40' E longitude at an

elevation of 1250 m above mean sea level. The three cropping seasons at this site include a rainy or kharif season from June to October, a winter or *rabi* season from November to February, and a summer or dry season from March to May. The soil was clay loam. The four cropping systems were: Colocasia-Onion-Frenchbean (C-O-F), Colocasia-Frenchbean-Potato (C-F-P), Colocasia-Cabbage-Frenchbean (C-Ca-F), Colocasia-Coriander-Tomato (C-Co-T). These systems were arranged in a randomized block design (RBD) with three replications. The net plot size was 13.5m². The crop was raised on natural soil fertility and the nutritional requirements of the crop were met through application of mineral fertilizers and farmyard manure (FYM). At the end of third year colocasia samples were collected and antioxidant properties and economic yield were recorded.

Extraction for phenolic compounds, condensed tannins and total anthocyanins

Five (\pm 0.2) grams, of fresh edible part of colocasia tuber was homogenized in 25mL of extraction solvent (400mL of acetone/400 mL of methanol/200 mL of water/10 mL of acetic acid) as described by Rababah *et al.* (2005) with some modification. The homogenate was transferred into a 50 ml Oak Ridge centrifuge tube and incubated in a water bath at 60 $^{\circ}$ N for 1h followed by a 3 min sonication. Sonicated samples were clarified by centrifugation at 13,000 rpm for 15 min at 4 $^{\circ}$ N, then filtered with Whatman filter paper no 1 and diluted to a final volume of 50 mL. Samples were stored in a 4 $^{\circ}$ N until time of analysis.

Determination of total phenolics (TP)

Total phenolics (TP) content was determined spectrophotometrically by the Folin-Ciocalteu method (Singleton and Rossi, 1965). Extracts (200 μ L) or gallic acid standard solutions were mixed with 2.6 mL of double distilled water. Generation of a standard curve was achieved by constructing five different concentrations of gallic acid (20, 40, 60, 80 and 100 mg/L). A blank was prepared using double distilled water instead of a sample. Subsequently, 200 μ L of Folin-Ciocalteu Reagent (FCR-1:5 dilution with double distilled water) were mixed with the sample, standard or blank. The reaction mixture was allowed to stand at room temperature for 6 min to permit the FCR reagent to react completely with oxidizable substrates or phenolates. Following incubation, 2.0 mL of 7% Na₂CO₃ solution were added to each mixture and allowed to stand at room temperature for 90 min. The absorbance was measured at 750 nm. Results are expressed as mg gallic acid equivalent (GAE) per 100 g fresh weight based on three replications per sample or standard.

Determination of condensed tannins (CT)

Condensed tannins were estimated using the method of Sun *et al.* (1998) with some modifications. To the freshly prepared extract (0.1 mL), 0.9 mL methanol, 2.5 mL of 1% vanillin reagent and 2.5 mL of 9M HCl was added. The solution was mixed thoroughly and absorbance at 500 nm was recorded after 20 min of incubation at 30 $^{\circ}$ C. Condensed tannins content was calculated from the standard calibration curve based on catechins.

Determination of total anthocyanins (Anthro)

Total anthocyanins analysis was performed following the method of Giusti and Wrolstad (2005). Briefly, colocasia

extract (100 μ L) was diluted with two different solutions (900 μ L each): 0.025 M potassium chloride buffer, pH = 1.0 and 0.4 M sodium acetate buffer, pH = 4.5. The absorbance was measured at maximum (510–520 nm) and, 700 nm against a blank cell filled with distilled water. The absorbance difference between the pH 1.0 and pH 4.5 samples was calculated: $A = (A_{e\text{ Vis-max}} - A_{700\text{ nm}})_{\text{pH } 1.0} - (A_{e\text{ Vis-max}} - A_{700\text{ nm}})_{\text{pH } 4.5}$

The monomeric anthocyanin pigment concentration was calculated using the following equation: Monomeric anthocyanin pigment (mg/L) = $(A \times MW \times DF \times 1000) / (\epsilon \times \lambda)$

where MW = 449.2 and ϵ = 26900 are, respectively, the molecular weight and molar absorptivity of cyanidin 3-O-glucoside that was used as a standard and was one of the major anthocyanins; DF is the dilution factor and l is the path length (cm). The total monomeric anthocyanins were reported on the basis of mg/100 g FW colocasia tuber.

Extraction for antioxidant activity measurements

Methanolic extract of colocasia samples were taken to measure antioxidant activities. Tubers were weighed, peeled, fractionated into little pieces and dried at 40 $^{\circ}$ C in a hot air oven to constant weight (Eleazu *et al.*, 2011). The dried samples were ground to fine powder by using an electric grinder. Two (2.0) grams grinded samples were extracted by semiautomatic soxlet apparatus (pelican, socsplus, 2AS, Chennai) in methanol at 100 $^{\circ}$ C for 1h and 90% methanol was recovered during recovery phase at 130 $^{\circ}$ C for 30 min, The methanolic extract of each were then evaporated at 80 $^{\circ}$ C in to dryness, redissolved in methanol to a concentration of 10 mg/ml and stored at 4 $^{\circ}$ C for further use. The all assays were carried out in triplicate and the results are expressed as mean values \pm standard error.

Determination of scavenging effects on DPPH radicals

The DPPH assay was done by measuring the decrease in absorbance of methanolic DPPH solution at 515 nm in the presence of the extract (Brand-Williams *et al.*, 1995). The stock solution was prepared by dissolving 24 mg DPPH with 100mL methanol and then stored at -20 $^{\circ}$ C until needed. The working solution was obtained by mixing 10mL stock solution with 45mL methanol to obtain an absorbance of 1.17 ± 0.02 units at 515 nm. Methanolic extract (150 μ L) of different concentration (0.5, 1.0 1.5 and 2 mg mL⁻¹) were allowed to react with 2850 μ L of the working DPPH solution for 24 h in the dark. Then the absorbance was taken at 515 nm. Butylated hydroxytoluene (BHT) was employed as a reference and the radical scavenging activity was calculated as the percentage of DPPH discoloration using the equation: DPPH radical scavenging (%) = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$, Where A_{sample} is the absorbance of the solution when the extract/reference has been added at a particular level, and A_{control} is the absorbance of the DPPH solution without extract added.

Determination of scavenging effect on ABTS radicals

The ABTS assay was done by measuring the decrease in absorbance of methanolic ABTS solution at 745 nm in the presence of the extract (Arnao *et al.*, 2001). The stock solutions included 7.0mM ABTS solution and 2.3mM Ammonium persulfate solution. The working solution was then prepared by mixing the two stock solutions in equal quantities and

allowing them to react for 12 h at room temperature in the dark. The solution was then diluted by mixing 1mL ABTS solution with 3mL methanol to obtain an absorbance of 0.9 ± 0.02 units at 745 nm. Methanolic extract (200 μL) of different concentration (0.5, 1.0 1.5 and 2 mg/mL) were allowed to react with 2000 μL of the ABTS solution for 30 min in a dark condition. Then the absorbance was taken at 745nm by using the spectrophotometer. BHT was employed as a reference and the percentage inhibition was calculated using the equation: $\text{ABTS radical scavenging (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$, Where A_{sample} is the absorbance of the solution when the extract/reference has been added at a particular level, and A_{control} is the absorbance of the ABTS solution without extract added.

Reducing power assay

The reducing power was determined according to the method of Huda fujan *et al.* (2009). Various concentrations (0.5, 1.0, 1.5, and 2.0mg mL⁻¹) of methanolic extracts (200 μL) were taken and volume made upto 1 ml by adding distilled water, in these added 2.5 mL of (0.2 M) sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Afterward 2.5mL of 10% trichloroacetic acid (w/v) were added; the mixture was centrifuged at 3000 rpm for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL demonized water and 0.5 mL of 0.1% of ferric chloride and the absorbance was measured at 700 nm by spectrophotometer. Higher absorbance indicates higher reducing power. The extract concentration providing 0.5 of absorbance (EC_{50}) was calculated from the graph of absorbance at 700 nm against extract concentration.

Determination of ferric reducing antioxidant power (FRAP)

The FRAP assay was done according to Benzie and Strain (1996) with some modifications. The stock solutions included 300mM acetate buffer (3.1 g C₂H₃NaO₂.3H₂O and 16mL C₂H₄O₂), pH 3.6, 10mM TPTZ (2, 4, 6- tripyridyl-s-triazine) solution in 40mM HCl and 20mM FeCl₃.6H₂O solution. The fresh working solution was prepared by mixing 25mL acetate buffer, 2.5mL TPTZ solution and 2.5mL FeCl₃.6H₂O solution and then warmed at 37°C before using. Methanolic extracts

(150 μL) were allowed to react with 2850 μL of the FRAP solution for 30 min in the dark condition. Readings of the colored product were taken at 593nm. The FRAP value was determined by plotting in a standard curve produced by the addition of ferrous sulphate (Merck, Darmstadt, Germany) to the FRAP reagent.

Determination of total antioxidant activity

The total antioxidant activity of the methanolic extract of both the sample was measured by spectrophotometrically using a phosphomolybdenum method (Prieto *et al.*, 1999), based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of specific green phosphate / Mo (V) compounds. Sample extract (0.3mL) of different concentrations (0.5, 1.0, 1.5, and 2.0 mg/mL) were combined with 2.7 mL of the reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample was capped and incubated in a boiling water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance was measured at 695nm. The total antioxidant activity was expressed as equivalents of trolox ($\mu\text{M/g}$ of extract).

Yield measurements and Colocasia equivalent yield (CEY)

Yields of main and by-products of each crop under various cropping systems were measured by harvesting 13.5 m² area in each plot at physiological maturity of respective crops. The economic part of individual crops was separated manually and harvested. All crops were cut at about 15cm from the surface, except the potato and coriander (Biswas *et al.*, 2006) Colocasia equivalent yield (CEY) was calculated to compare performance of several cropping systems by converting the economic yield of each crop into equivalent colocasia yield on a price basis, using the formula: $\text{CEY (of crop x)} = Yx (Px / Pc)$

Where, Yx is the yield of crop x (tons economic harvest product ha⁻¹), Px is the price of crop x, and Pc is the price of colocasia.

Statistical analysis

The statistical analyses were performed using the statistical package SPSS (Statistical Package for Social Science, SPSS Inc.,

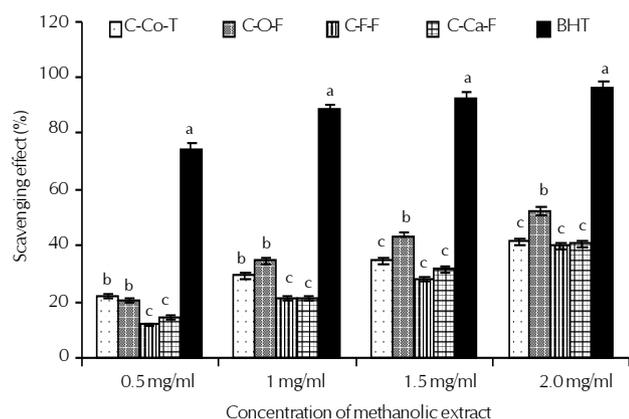


Figure 1: Scavenging effect of methanolic extracts from colocasia on DPPH radicals in different cropping systems. Each value is expressed as mean + standard error (n=3); C: Colocasia; O: Onion; F: Frenchbean; P: Potato; Ca: Cauliflower; T: Tomato

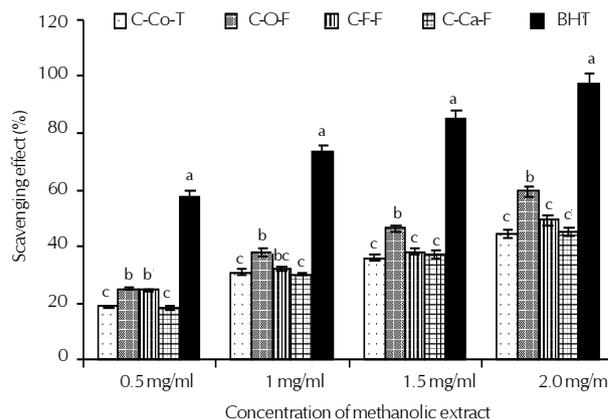


Figure 2: Scavenging effect of methanolic extracts from colocasia on ABTS radicals in different cropping systems. Each value is expressed as mean + standard error (n=3); C: Colocasia; O: Onion; F: Frenchbean; P: Potato; Ca: Cauliflower; T: Tomato

Chicago, IL). Analyses of variance were performed by ANOVA and significance of each group was verified with one-way analysis of variance followed by Duncan's multiple range test ($P < 0.05$). The 50% inhibitory concentration (EC_{50}) was calculated according to Concentration-Effect regression line. For multivariate comparison, principal component analyses (PCA) was used to display the correlations between the various antioxidant and related parameters and their relationship with different cropping systems. Multivariate analysis was carried out using the SAS JMP 9.0 version software.

RESULTS AND DISCUSSION

Total phenolics (TP), Condensed Tannins (CT) and Total Anthocyanin

The amount of total polyphenol (TP), condensed tannins (CT) and total anthocyanin in colocasia among all cropping system were tabulated in Table 1. The TP was determined from the regression equation of the calibration curve obtained from gallic acid ($y = 0.0033x$, $r > 0.99$). The TP was found maximum ($0.966 + 0.009$ mg GAE/100 mg FW) in colocasia samples from 'C-O-F' cropping system and found minimum ($0.85 + 0.029$ mg GAE/100 mg FW) in colocasia samples from 'C-Co-T' cropping system which was the non-significantly at par with the 'C-F-P' and 'C-Ca-F' cropping system (Table 1). The values of total polyphenols are comparable to those of the result found by Basu *et al.* (2012) from *Colocasia esculenta*. The key role of phenolic compounds as scavengers of free radicals is emphasized in several reports (Smirnoff and Cumbes, 1989; Komali *et al.*, 1999). The total CT content from the crops extract was assayed by vanillin-HCl colorimetric assay as described in materials and methods, determined from regression equation of calibration curve ($y = 0.0022$

$x + 0.013536$; $r = 0.997$) and expressed in catechins equivalents.

The result showed that The CT was found maximum ($0.022 + 0.001$ mg CE/100 mg FW) in colocasia samples from 'C-O-F' cropping system followed by the sample from 'C-Co-T' cropping system ($0.016 + 0.001$ CE/100 mg FW). Content of condensed tannins was found non-significantly at par between 'C-Ca-F' and 'C-F-P' cropping system (Table 1). Condensed tannins are very important plant constituents because of having active hydroxide ions (OH^-) and show antioxidant activity (Eric *et al.*, 2011).

The table 1 showed that the highest levels of total anthocyanin ($4.29 + 0.04$ mg/100 mg FW) was found in colocasia samples from 'C-Co-T' cropping system, followed by 'C-Ca-F' and the lowest ($4.04 + 0.09$ mg/100 mg FW) was in 'C-O-F' cropping system. The result were comparable with the as reported by Prajapati *et al.*, 2011.

Free radical scavenging Activities

The abilities for each concentration of the extract samples to scavenge DPPH and ABTS radicals are shown in figure 1 and 2 respectively. Scavenging effect (% inhibition) of the methanolic extracts from the sample of all the cropping systems on DPPH radicals increased with increase in concentration. At 0.5 to 2.0 mg/mL, the scavenging activities of methanolic extracts of colocasia samples from 'C-Co-T', 'C-O-F', 'C-F-P', 'C-Ca-F' cropping system on DPPH radical ranged from 21.95 to 41.09, 20.73 to 52.59, 11.63 to 39.96, and 14.46 to 40.46 %, respectively (Fig. 1).

However, BHT at same concentration showed excellent DPPH scavenging activities (74.28 to 95.89 % inhibition). Colocasia from 'C-O-F' cropping system showed highest, while 'C-F-P' cropping system showed lowest DPPH scavenging activities

Table 1: Total polyphenols, condensed tannins and total anthocyanin content in colocasia samples from four different colocasia based cropping system

Cropping system	Total Phenolics ^A (mg GAE/100 mg FW)	Condensed Tannins ^B (mg CE/100 mg FW)	Total anthocyanin ^C (mg/100 mg FW)
C-Co-T	0.85 + 0.029 ^b	0.016 + 0.001 ^b	4.29 + 0.04 ^a
C-O-F	0.966 + 0.009 ^a	0.022 + 0.001 ^a	4.04 + 0.09 ^{ab}
C-F-P	0.868 + 0.011 ^b	0.010 + 0.001 ^c	4.09 + 0.06 ^{ab}
C-Ca-F	0.864 + 0.014 ^b	0.011 + 0.001 ^c	3.95 + 0.03 ^b

Different letters in the same column indicate significant difference using LSD ($p < 0.05$); Values are the mean of three determinations; C: Colocasia, O: Onion, F: Frenchbean, P: Potato, Ca: Cabbage, Co: Coriander; GAE: Gallic acid Equivalent, CE: Catechins Equivalent, FW: Fresh Weight

Table 2: EC_{50} values (mg/mL) of colocasia extract from four different Cropping systems in the antioxidant activity evaluation assays

Cropping system	EC_{50} values of each free radical scavenging assay (mg/mL)		
	DPPH ^a	ABTS ^b	RPA ^c
C-Co-T	2.630	2.360	4.410
C-O-F	1.390	1.720	4.530
C-F-P	2.890	2.080	5.190
C-Ca-F	2.680	2.310	4.670
BHT	0.320	0.470	0.780

^a EC_{50} (mg/mL): effective concentration at which 50% of DPPH* radicals are scavenged; ^b EC_{50} (mg/mL): effective concentration at which 50% of ABTS* radicals are scavenged; ^c EC_{50} (mg/mL): effective concentration at which 0.50 absorbance got (Reducing power); C: Colocasia, O: Onion, F: Frenchbean, P: potato, Ca: Cabbage, Co: Coriander; The productivity in terms of colocasia equivalent yield (CEY) of all four cropping system was followed the order 'C-O-F' > 'C-Co-T' > 'C-F-P' > 'C-Ca-F' (Fig. 6).

at all the four concentrations.

At 0.5 to 2.0 mg/mL, the scavenging effect (% inhibition) of methanolic extracts of colocasia samples from 'C-Co-T', 'C-O-F', 'C-F-P', 'C-Ca-F' cropping system on ABTS radical ranged from 18.34 to 44.38, 25.17 to 59.48, 24.30.63 to 49.15 and 17.86 to 44.78 %, respectively (Fig. 2).

However, BHT at same concentration (0.5, 1.0, 1.5 and 2.0 mg/ml) showed excellent ABTS scavenging activities (58.03 to 97.85 % inhibition). Colocasia from 'C-O-F' cropping system showed highest ABTS scavenging effect at most of the concentrations. Free radical scavenging activities against DPPH free radical of colocasia methanolic extracts from 'C-Co-T', 'C-O-F', 'C-F-P', 'C-Ca-F' cropping system followed the order: BHT > 'C-O-F' > 'C-Co-T' > 'C-Ca-F' > 'C-F-P', While against

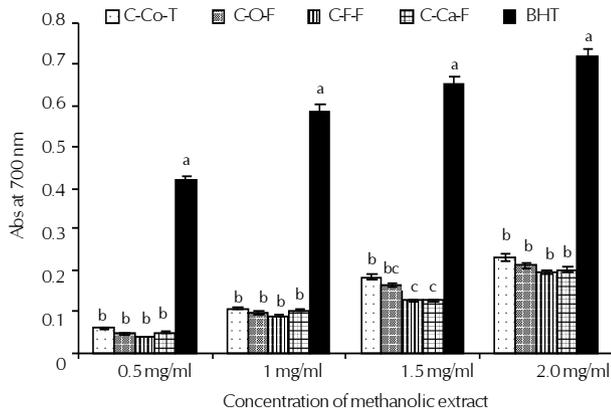


Figure 3: Reducing power of methanolic extracts from colocasia in different cropping systems. Each value is expressed as mean + standard error (n=3); C: Colocasia; O: Onion; F: Frenchbean; P: Potato; Ca: Cauliflower; T: Tomato

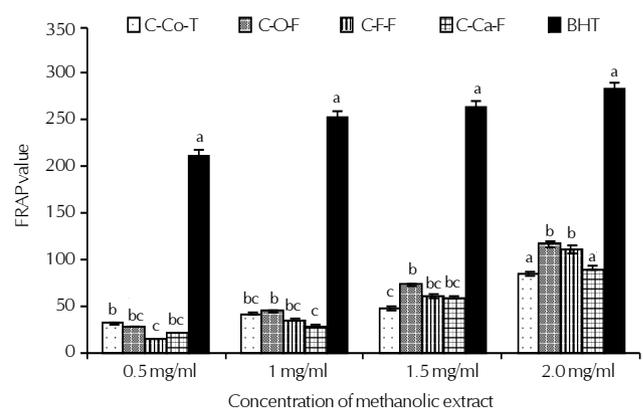


Figure 4: FRAP value of methanolic extracts from colocasia in different cropping systems. Each value is expressed as mean + standard error (n=3); C: Colocasia; O: Onion; F: Frenchbean; P: Potato; Ca: Cauliflower; T: Tomato

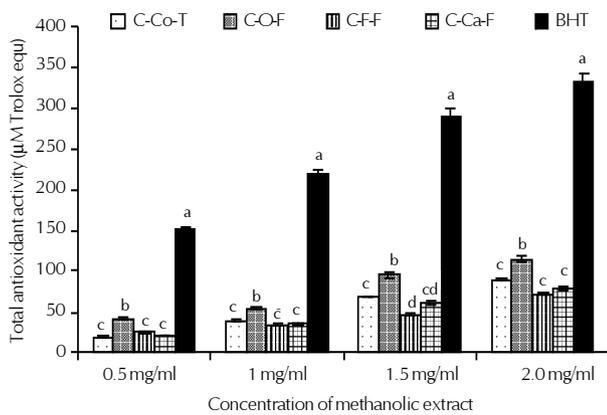


Figure 5: Total antioxidant activity of methanolic extracts from colocasia in different cropping systems. Each value is expressed as mean + standard error (n=3); C: Colocasia; O: Onion; F: Frenchbean; P: Potato; Ca: Cauliflower; T: Tomato

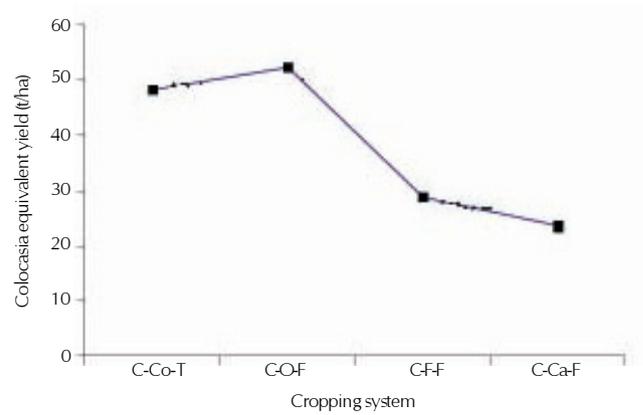


Figure 6: Colocasia equivalent yield (CEY) under different cropping systems
C: Colocasia; O: Onion; F: Frenchbean; P: Potato; Ca: Cauliflower; T: Tomato

ABTS it was BHT > 'C-O-F' > 'C-F-P' > 'C-Co-T' > 'C-Ca-F'. Reducing power serves as a significant indicator of potential as antioxidant. Colocasia samples from four different cropping systems were used to evaluate the reducing power at four different extract concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml) Reducing power significantly increased with the increase in extract concentration in a dose-dependent manner ($P < 0.05$). Colocasia sample from 'C-Co-T' showed highest reducing power followed by 'C-O-F' and lowest was by 'C-F-P' cropping system at all the four concentrations (Fig. 3).

It was reported that reducing power activities are associated with the presence of reducing agents, which shows antioxidant action by donating a hydrogen atom and breaking the free radical chain (Mathew and Abraham, 2006).

Ferric Reducing Antioxidant Power (FRAP)

FRAP assay is a colorimetric method based on the reduction of a ferric tripyridyltriazine (TPTZ) complex to its ferrous form. This reduction originates an intense blue complex with an absorption maximum at 593 nm (Benzie and Strain, 1996).

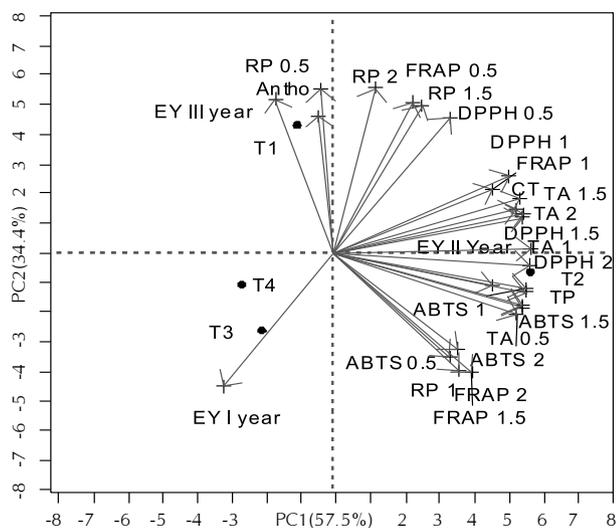
The antioxidant capacity of colocasia samples from all four cropping system systems were evaluated and expressed as FRAP value, are shown in Fig. 4.

Colocasia samples from 'C-O-F' showed highest FRAP value and FRAP value significantly increased with the increase in extract concentration in a dose-dependent manner.

Total Antioxidant Activity

The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics, α -tocopherol, and carotenoids (Sarikurkcu et al., 2008). Total antioxidant activity significantly increased with the increase in extract concentration in a dose-dependent manner ($P < 0.05$). At all four extract concentrations (0.5, 1.0, 1.5 and 2.0 mg/ml), colocasia samples from 'C-O-F' showed highest total antioxidant activity (41.31, 54.57, 95.09, 115.04 μ M Trolox equivalent) respectively, while minimum activity was recorded in 'C-F-P' cropping system at most of the concentrations (Figure 5).

The total antioxidant activity of plant seems to be due to the



T1- 'C-Co-T', T2- 'C-O-F', T3- 'C-F-P', T4- 'C-Ca-F'; C: Colocasia; O: Onion; F: Frenchbean; P: Potato; Ca: Cauliflower; T: Tomato

Figure 6: Multifactorial comparison methanolic extracts from colocasia in different cropping systems and various parameters using principal component analysis (PCA)

presence of phenolic compounds, flavonoids and anthocyanosides that may be acted by donating electrons and free radicals (Prieto *et al.*, 1999).

The EC_{50} values were calculated and tabulated in Table 2 to facilitate the comparison of the free radical scavenging activities (FRSA) of different samples. Lower EC_{50} implies a higher scavenging activity, as shown in Table 2. Colocasia samples from 'C-O-F' cropping system showed highest DPPH and ABTS radical scavenging activities, while reducing power activity was found highest in the colocasia samples from 'C-Co-T' cropping system.

Principal Component Analysis (PCA)

Principal component analysis clearly indicates correlation between various antioxidant activities and related parameters and their relationship in different cropping system (Parihar *et al.*, 2013). The principal component analysis (PCA) and their correlation are shown in Figure 6. The first principal component represents 57.5 per cent of variability, while the second principal component represents 34.4 per cent of variability among the data. Almost all parameters were occupied on the right side of the biplot.

This suggests that total polyphenols (TP) and condensed tannin (CT) were positive correlated with ABTS, DPPH and total antioxidant (TA) activities, while total anthocyanin (Antho) was positively correlated with reducing power (RP). Previously several studies reported (Goffman and Bergman, 2004; Itani *et al.*, 2002; Pal *et al.*, 2013) that antioxidant activity have positive correlation with the total phenol content and is especially associated with the content of tannic acid and catechins. The principal component analysis (PCA) and their correlation also showed that cropping system 'C-O-F' showed highest radical scavenging activities and total antioxidant activities.

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