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EVALUATION OF ANTIOXIDANT PROPERTIES OF DIFFERENT PARTS OF *AMORPHOPHALLUS COMMUTATUS*, AN ENDEMIC AROID OF WESTERN GHATS, SOUTH INDIA.

R. KAVITHA KRISHNA[1*] S.KRISHNAKUMARI[2] AND S.CHANDRAKALA[1]

[1] Research Department of Biotechnology, Dr.Mahalingam Centre for Research and Development, NGM College (Bharathiar University), Pollachi – 642001, Coimbatore, India.
[2] Department of Biochemistry, Kongunadu arts and science college, Coimbatore – 641029, India.

ABSTRACT

Amorphophallus commutatus (Araceae) a rare cormous herb endemic to western ghats has been used by the tribes of sitamata wild life sanctuary, Rajasthan, India and tribes living in the Western Ghats, India for various ailments. The current study was carried out with an objective to investigate the enzymatic and non enzymatic contents of the tuber and leaves of the plant adopting eight enzymatic methods (Superoxide dismutase, Catalase, Guaicol peroxidase,Ascorbic acid oxidase, Glutathione peroxidase, Glutathione reductase, glucose – 6- phosphate dehydrogenase and polyphenol oxidase) and three non enzymatic methods (Phenol, Ascorbic acid, Glutathine reduced). The tuber exhibited significant SOD (47.7±5.5 U/g tissue), AAO(0.38±0.12U/g tissue), PPO(0.8±0.45U/g tissue), Ascorbic acid (1.6±0.5mg/g tissue) and total phenol(0.2±0.22mg/g tissue). Young leaves contained significant CAT(64.3 ±6.02 U/g tissue), GR (1.3±0.7 U/g tissue) and Glutathione (6.21 ±0.6 mg/g tissue) content. The mature leaf exhibited significant G6PD activity (9.97±2.0 U/g tissue). Our results reveal the innate antioxidant potential of *Amorphophallus commutatus* and therefore can be utilised for supplementing the antioxidant needs in the diet.

KEYWORDS; Amorphophallus, Antioxidant enzymes, Araceae, Reactive oxygen species, Ascorbic acid

R. KAVITHA KRISHNA
Research Department of Biotechnology, Dr.Mahalingam Centre for Research and Development, NGM College (Bharathiar University), Pollachi – 642001, Coimbatore, India.

INTRODUCTION

Reactive oxygen intermediates (ROIs) like superoxide radicals (SO^{o-}), hydrogen peroxide (H_2O_2), or hydroxyl radicals (OH^o) are produced as the byproduct of normal cell metabolism and environmental stress (Dat, 2000). ROIs induce cell death by membrane lipid peroxidation, protein oxidation, inactivation of enzymes, alteration of intracellular redox state and damage to DNA. Thus, involved in the pathogenesis of number of disease and aging process[1].

Plants behold a defense mechanism to protect against these toxic ROIs. The defense mechanism involve antioxidant and intracellular enzymes such as superoxide dismutase (SOD), peroxidase (POD), glutathione peroxidase (GPX), catalase (CAT), and ascorbate peroxidase (APX)[2] . The non enzymatic antioxidants like glutathione, Ascorbic acid [3,4] and phenols[5]. These enzymatic and non enzymatic antioxidants can terminate or prevent the formation of free radicals by donating hydrogen or electrons to reactive radicals or species[6].

Plants of the genus *Amorphophallus* have a long history of use in tropical and subtropical Asia as a food source and as a traditional Chinese medicine (TCM)[7]. Westernghats in south India is one of the Biodiversity hotspots of the world. *Amorphophallus commutatus* (Schott) Engl (Araceae) a member of the, is a tuber depressed globose, is a rare cormous herb that is found in evergreen and semi-evergreen forest of south western India (endemic to Western Ghats). Tuberous corms of *A.commutatus* were used for treatment of piles, tumours and cysts[8]. Tubers of *Amorphophallus commutatus* has also been used as antidote for snake bite by tribal's living in fifty villages of Sitamata wildlife, sanctuary, Rajasthan, India[9]. There are no reports on the bioactivity of the plant *Amorphophallus commutatus*. Hence, this work is done with the aim of validating the enzymatic and non enzymatic antioxidant of different parts of the herb. As the presence of antioxidant activity is one of the primary indicator that the plant can be expected to possess bioactivity.

MATERIALS AND METHODS

2.1 Enzyme Extraction and assays

2.1.1. Plant Sample

Fresh tuber and leaves 1.0g were ground with 5 ml of 30% ethanol, in a pre-chilled mortar and pestle and the extracts were centrifuged at 10,000rpm at 4ºC for 10 minutes. The supernatant thus obtained were used with in four hours for various enzymatic antioxidants assays[10].

2.1.2 Protein Content

Protein content of tissue homogenates was determined by the method of Bradford (1976)[11], using Bovine serum albumin (BSA) as a standard. To 0.1 ml of sample 2ml of Bradford's dye solution was added and mixed gently. After 5 minutes, absorbance was read at 595 nm using spectrophotometer.

2.2 Antioxidant enzyme measurements

2.2.1. Superoxide dismutase (SOD)

The assay of superoxide dismutase was done according to the procedure of Das *et al*, (2000)[12]. In this method, 1.4ml aliquot of the reaction mixture (containing 1.11 ml of 50Mm phosphate buffer of pH 7.4, 0.075 ml of 20nM L-Methionine, 0.04ml of 10Mm hydroxylamine hydrochloride and 0.1ml of 50mM EDTA) was added to 100µl of the sample extract and incubated at 30ºc for 5 minutes. 80µl of 50µM riboflavin was then added and the tubes were exposed for 10 min to 200W- Philips fluorescent lamps. After the exposure time, 1ml of greiss reagent (mixture of equal volume of 1% sulphanilamide in 5% phosphoric acid) was added and absorbance of the colour formed was measured at 543nm. One unit of enzyme activity was measured as the amount of SOD capable of inhibiting 50% of nitrite formation

under assay conditions. The activity of SOD is expressed as units/g protein.

The SOD activity was calculated by the following formula,

$$\%\ inhibition\ of\ nitrite\ formation = 1 - \frac{A_S}{A_C}\ x100$$

Where A_S is the absorbance of the sample and A_C is the absorbance of the control.

2.2.2. Catalase (CAT)

The method of Luck (1974)[13], as mentioned in sadasivam and manikam (1991)[14] was adopted to measure the activity of catalase. The enzyme extract (0.1ml) was added to the reaction mixture containing 3ml of H_2O_2 and 0.01M phosphate buffer (pH 7.0) and the OD change was measured at 240nm, the time taken for decrease in the absorbance from 0.45 to 0.4 is noted as ΔT. The activity of the enzyme is expressed in the terms of µmole of H_2O_2 consumed/ min/ mg protein.

The activity of catalase was calculated by the following formula,

$$units\ in\ the\ assay\ mixture = \frac{17}{\Delta T}$$

$$\Delta T\ is\ time\ in\ seconds$$

2.2.3. Guaicol peroxidase (GPOD)

The assay was carried out by the method of Putter (1974)[15]. The reaction mixture consisted of 3ml of assay buffer (0.1M phosphate buffer, (pH 7.0), 20mM guaiacol, and 0.03 ml of 30% H_2O_2). To this 0.1ml of enzyme extract added and O.D change was measured at 436 nm. The peroxidase activity was calculated using an extinction coefficient of guaiacol dehydrogenase (liters /mol). The activity of were calculated by the formula.

$$Guaicol\ Peroxidase\ activity\ {}^{units}/_{litre} = \frac{3.18 \times 0.1 \times 1000}{6.39 \times 1 \times \Delta t \times 0.1}$$

Where 0.1=volume of sample; 6.39=extinction coefficient; Δt=time in minutes

2.2.4.Ascorbic acid oxidase (AAO)

Assay of ascorbic acid oxidase activity was carried out according to the procedure of oberbacher and vines (1963)[16]. The 3.0ml of the substrate solution (8.8mg of ascorbic acid in 300ml phosphate buffer, PH(5.6). 0.1ml of the enzyme extract was added .the absorbance change was measured at 265nm for every 30second for a period of 5minutes .one enzyme unit is equivalent to 0.01OD change per minutes.

2.2.5. Glucose- 6-phosphate dehydrogenase(G6PD)

The method of Balinsky and Bernstein (1963)[17] was adopted to assay glucose 6 – dehydrogenase. The reaction mixture containing 0.4 ml Tris- Hcl buffer, 0.2 ml of NADP, 0.2 ml of magnesium chloride, and 1.0 ml of water were to 0.2 ml of enzyme in a cuvette. The reaction was started by the addition of adding 0.2 ml of glucose - 6-phosphate and the increase in the absorbance was measured at 340 nm.

The activity of the enzyme is expressed in terms of units/g in which one unit is equal to the amount of enzyme that brought about a change in optical density of 0.01/minute.

2.2.6. Glutathione peroxidase(GSH-Px)

Glutathione peroxidase was assayed by the procedure of Wendel (1980)[18]. The 0.1 ml of

enzyme extract was added to the reaction mixture containing 50mM Sodium phosphate buffer with 40mM EDTA pH 7, 1mM Sodiumazide solution 1mg of β-NADPH, 1mM of DTT with sodium phosphate buffer, 200mM reduced glutathione, 0.042% of H_2O_2, and the decreased absorbance was recorded at 340nm for 5 minutes. The enzyme activity is expressed in terms of µg of glutathione utilized / min /mg protein. Glutathione activity was calculated by the formula,

$$U/ml = \frac{(\Delta A340/test-\Delta A340/blank)(2)(3.1)(DF)}{(6.22)(0.05)}$$

2=2µmoles of GSH produced per µmole of β-NADPH oxidized;
3.1=total volume (in millimeters) of assay
DF= Dilution Factor; 6.22=millimeter extinction co efficient of β-NADPH at 340 nm;
0.05 = volume (in millimeter) of enzyme used.

2.2.7. Glutathione reductase(GR)

The assay of glutathione reductase was done according to the procedure of David and Richard (1983)[19]. To 0.1 ml of sample, 1 ml of Potassium buffer (0.12M pH 7.2), 0.1ml of EDTA, 0.1ml of Sodium azide and 0.1 ml of oxidized glutathione were added and the volume was made up to 2 ml with water. The mixture was kept at room temperature for three minutes and 0.1ml of NADPH was added. The absorbance at 340nm was recorded at intervals of 15seconds for 2 to 3 minutes. One unit of GR is expressed as µM of NADPH oxidized/ minute/gram. The GR activity was calculated by the formula,

$$U/ml = \frac{\Delta A340nm/min \times 3 \times Df}{(6.22)(0.1)}$$

Where 6.22-millimeter extinction coefficient of β-NADPH; 0.1- volume of enzyme used for assay; 3-volume of reaction mixture.

2.2.8. Polyphenol oxidase(PPO)

Polyphenol oxidases activity was assayed by the procedure of Esterbauer *et al.,* (1997)[20]. Into a cuvette, 0.2 ml of the sample extract was added to the reaction mixture containing, 2.5 ml of phosphate buffer and 0.3 ml of catechol solution. The change in the absorbance was recorded every 30 sec up to 5 minutes. One unit of either catechol oxidase or laccase is defined as the amount of enzyme that transforms one µmole of dihydrophenol to one µmole of quinine/minute. The enzyme activity is expressed as u/g tissue. The activity of PPO can be Calculated using the formula,

Enzyme unit=k × (A/minute)
K for catechol oxidase=0.272

2.3. Non-enzymatic component measurement

Non- enzymic antioxidant contents such as ascorbic acid, glutathione reduced, total phenol were estimated in different parts of *Amorphophallus commutatus*.

2.3.1. Total phenols

The method proposed by Malick and Singh (1980)[21] was used to determine the total phenols in the different parts of the *Amorphophallus commutatus*.

Fresh tuber and the leaf sample were homogenized in 30% of ethanol and centrifuged at 10,000 rpm for 10 minutes. To 0.5 ml of supernatant added 0.5 ml of folin-ciocalteau reagent and after 5 minutes, 2.0 ml of 20% sodium carbonate was added. The tubes were placed in the boiling water bath for 1 minute. Then the blue colored complex was measured at 650 nm in a spectrophotometer. The values are expressed as mg phenols/g tissue.

2.3.2. Ascorbic acid

For the estimation of ascorbic acid, 1 g of the different parts of sample were homogenized by 4% of TCA after centrifugation a pinch of activated charcoal was added ,mixed vigorously using cyclo mixer and stand for 5 minutes. The tubes were centrifuged again to pellet the charcoal particles. Aliquots of supernatant were taken for the estimation and as adopted by Roe and Keuther (1943)[22].

To 0.5ml of charcoal treated supernatant 2.0 ml of 4% TCA, 0.5 ml of Di Nitro Phenyl Hydrazine was added followed by 2 drops of thiourea solution and mixed well. The tubes were incubated for 3 hours. Removed, placed in ice cold water and added 2.5ml of 85% H_2SO_4 drop by drop and the absorbance were recorded at 540nm. Concentration of ascorbic acid in the samples were calculated and expressed as mg/g tissue.

2.3.3. Reduced Glutathione (GSH)

The method of Moron *et al*., (1979)[23] was followed to determine the amount of reduced glutathione. For the estimation, 2.0 g of different part of the sample were homogenized with 5% TCA and centrifuged at 10,000 rpm for 10 minutes at 4ºc. Then supernatant was used for the estimation of GSH. To 0.1 ml of supernatant, 1.0 ml of phosphate buffer then 2.0 ml of freshly prepared DTNB (Ellman's reagent) solution was added and the intensity of the yellow color formed was read at 412 nm in a spectrophotometer after 10 minutes. The values are expressed as µmoles of GSH/g tissue.

3. RESULTS

Redox reactions that occur naturally are vital for controlling the metabolic process occurring in the living system. Free radicals or reactive oxygen species are introduced in to the living system as a product of normal metabolic function or from the environment. Plants have evolved a well regulated mechanism for scavenging ROS, generally through the production of various antioxidative enzymes such as Superoxide dismutase, Peroxidase, Glutathione peroxidase, Ascorbate oxidase, Glucose 6- Phospate-Dehydrogenase and Glutathione reductase. These enzymes are usually considered to be the most predominant ROS- scavenging in plant systems[24,25,26,27,4]. The result of this report reveals for the first time the enzymatic and non enzymatic antioxidant content present in different parts of *Amorphophallus commutatus*.

3.1 Protein content

Proteins are Macromolecules that act as alternate energy source when other energy sources are in short supply. They are the building block of any organism. The tuber, young leaves and mature leaves of *Amorphophallus commutatus* were analyzed for its protein content and the results obtained are represented in table .1. The tuber has been identified to contain significant quantity of protein corresponding to 80µg in one gram tissue followed by young leaf and mature leaf.

Table.1.
Protein content of different parts of Amorphophallus commutatus

Sample	*Concentration (µg/g of tissue*
Tuber	*80*
Young leaves	*60*
Mature leaves	*46*

3.2 Antioxidant enzyme measurements

3.2 .1 Superoxide dismutase (SOD)

The SOD activity of different part of extracts is represented in table-2 which shows that significant activity is observed in the tuber (47.7 U/g tissue) followed by matured leaves (21.1U/g tissue) and young leaves (17.3U/g tissue) respectively. The transition metal present in the enzyme reacts with O_2 that is superoxide taking its electron and superoxide is the only substrate for superoxide dismutase[28].

Table 2
Super Oxide Dismutase in different parts of Amorphophallus commutatus

Sample	*Enzyme activity*	
	U/g of tissue[a]	*U/g of protein*
Tuber	*47.7±5.5*	*11.67± 1.55*
Young leaf	*17.3± 3.05*	*5.9± 2.7*
Mature leaf	*21.1 ±4.2*	*5.7± 1.1*

Values are mean ± SD; n=3

[a]***1 unit=activity of enzyme that exhibits 50% inhibition of NBT reduction/minute***.

3.2.2 Catalase (CAT)

Catalase activity, of different parts of *Amorphophallus commutatus* are presented in table.3, which shows that significant activity is observed in young leaf (63.3u/g tissue) followed by mature leaves (54.3U/g tissue) and the tuber (2.7U/g tissue). Hydrogen peroxide is generated by the dismutation of superoxide radical by the enzyme superoxide dismutase. The H_2O_2 causes cell membrane damage leading to release of arachidonic acid is a long acting cell damaging molecule[28].

Table.3
Catalase in different parts of Amorphophallus commutatus

Sample	Enzyme activity	
	U/g tissue [a]	U/g protein
Tuber	2.7± 1.4	0.66± 1.55
Young leaf	64.3 ±6.02	19.8± 2.02
Mature leaf	57.3± 5.03	17.4± 2.8

Values are mean±SD;
n=3 [a] 1unit=µmoles of H2O2 utilized/ minute

3.2.3 Guaicol peroxidase (GPOD)

The activity of POD is represented in table-4, it shows that the significant higher activity observed in the mature leaves (1.9 U/g tissue) followed by young leaves (1.77U/g) and tuber (0.38 U/g tissue)

Table.4
Guaicol Peroxidase in different parts of Amorphophallus commutatus

SAMPLE	*Enzyme activity*	
	U/g tissue [a]	*U/g protein*
Tuber	*0.38±0.12*	*0.07±0.01*
Young leaf	*1.77±0.25*	*1.2± 0.971*
Mature leaf	*1.9±0.65*	*0.46±0.11*

Values are mean ±SD; n=3.
[a] 1unit= µmoles of guaicol oxidized/minute

3.2.4 Ascorbic acid oxidase (AAO)

The activity of ascorbate oxidase is represented in table-5 which shows that significant activity is observed in tuber(0.38 U/g tissue),followed by young leaves (0.010 U/g tissue) and matured leaves (0.005 u/g tissue) respectively.The predominant ascorbate activity in tuber is associated with high SOD activity and catalase, peroxidase and glutathione peroxidase, emphasizing the importance of ascorbate system in tuber part.

Table.5
Ascorbic acid oxidase Activity in Amorphophallus commutatus

	Enzyme activity	
SAMPLE	*U/g tissue* [a]	*U/g protein*
Tuber	*0.38± 0.12*	*0.443±0.056*
Young leaf	*0.01±0.004*	*0.01± 0.09*
Mature leaf	*0.005±0.004*	*0.003±0.004*

Values are mean±SD n=3
[a] I unit (AAO) = equivalent to 0.01change in OD /min

3.2.5 Activity of Glucose-6-phospate- dehydrogenase

The activity of G-6-P-d in different parts of *Amorphophallus commutatus* extracts is represented in table -6, shows significant activity in mature leaves (9.97 u/g tissue) followed by young leaves (2.5u/g tissue) and tuber (0.077 u/g tissue) respectively. The main function of the enzyme is to maintain GSH in reduced state[30].

Table.6
Glucose -6-phosphate dehydrogenase activity in Amorphophallus commutatus

	Enzyme activity	
SAMPLE	*U/g tissue* [a]	*U/g protein*
Tuber	*0.077± 0.025*	*0.057± 0.040*
Young leaf	*2.5±0.568*	*0.5± 0.3*
Mature leaf	*9.97± 2.0*	*3.74± 1.53*

Values are mean±SD n=3
[a] ***1unit=change in OD of 0.01/minute***

3.2.6 Activity of Glutothione peroxidase

The activity of GPx in different parts of plant extracts is represented in table -7 which shows the significant activity is observed in mature leaves (3.8 U/g tissue) followed by young leaves (2.3 u/g tissue) and tuber (0.46 u/ g tissue).

Table.7
Glutathione Peroxidase Activity in Amorphophallus commutatus

	Enzyme activity	
SAMPLE	*U/g tissue* [a]	*U/g protein*
Tuber	*0.46± 0.25*	*0.073± 0.020*
Young leaf	*2.3±0.66*	*0.35± 0.06*
Mature leaf	*3.8± 1.33*	*0.34± 0.27*

Values are mean±SD n=3
[a] ***1unit=µmoles of GSH H2O2 utilized/ minute***

3.2.7 Activity of Glutathione reductase

The activity of GR was assessed and the results obtained are shown in table-8. From the result young leaves (1.3 u/ g tissue) have a significant higher activity (1.3 u/ g tissue) compared to the tuber (0.65 u/ g tissue) and the mature leaves (0.323 u/g tissue). GR is a ubiquitious NADPH dependent enzyme and may be a rate limiting enzyme for defense against active oxygen toxicity[31].

Table - 8
Glutathione Reductase Activity in *Amorphophallus commutatus*

	Enzyme activity	
SAMPLE	*U/g tissue* [a]	*U/g protein*
Tuber	*0.65± 0.25*	*0.057± 0.004*
Young leaf	*1.3±0.7*	*0.3± 0.009*
Mature leaf	*0.323± 0.075*	*0.11± 0.045*

Values are mean±SD n=3
[a] ***1unit=µmoles of NADPH oxidized / minute***

3.2.8 Polyphenol oxidase

The activity of PPO was assessed and the results obtained are shown in table-9. From the result the tubers have a significant activity (0.8 u/g tissue) followed by young leaves (0.45u/g tissue) and mature leaves (0.23 u/g tissue).

Table-9
Poly Phenol Oxidase Activity in Amorphophallus commutatus

	Enzyme activity	
SAMPLE	U/g tissue [a]	*U/g protein*
Tuber	0.8± 0.45	0.057± 0.004
Young leaf	0.453±0.161	0.12± 0.063
Mature leaf	0.23± 0.11	0.15± 0.21

Values are mean±SD; n=3
[a] 1 unit=Activity of catechol oxidase/ laccase that transforms 1 unit of dihydrophenol to quinine/minute.

3.3. Determination of non- enzymatic antioxidant

The antioxidant belonging to second line of defense include glutathione, Ascorbic acid and Phenols. The commonly known non enzymatic antioxidants are Glutathione and ascorbic acid which are essential for redox buffering[32]. The concentration of different non- enzymatic antioxidants in *Amorphophallus commutatus* were also assesses and the results are represented in table-10.

Table -10
Non - enzymatic antioxidant content of Amorphophallus commutatus

Parts of plant	Reduced glutathione (mg/g tissue)	Vitamin C (mg/g tissue)	Total phenol (mg/g tissue)
Tuber	2.46±0.75	1.6± 0.5	0.2± 0.22
Young leaves	6.21 ±0.6	1.9± 0.9	0.02± 0.003
Matured leaves	3.83± 0.70	1.3± 0.7	0.019± 0.002

Values are mean±SD; n=3

3.3.1 Total phenol

The phenolics which act as reducing agents, hydrogen donors and singlet oxygen quenchers, in addition to having a metal chelting potential[33]. The level of total phenol content in different parts of *Amorphophallus commutatus* extracts of tuber showed a significant activity of 0.2 mg/g tissue than in the young leaves (0.02 mg/g tissue) and matured leaves (0.01 mg/g tissue).

3.3.2 Ascorbic acid

Ascorbic acid or Vitamin C is a natural water soluble antioxidant defense that protects cells against lipid peroxidation[34]. The vitamin C content in different parts of *Amorphophallus commutatus*, exhibited that significant quantity is identified in young leaves (1.9 mg/g tissue) followed by tuber (1.6 mg/g tissue) and matured leaves (1.3 mg/g tissue) respectively.

3.3.3 Reduced glutathione

It is an important antioxidant that is found to detoxify toxic substances by conjugation[35]. The level of reduced glutathione was estimated and the results were represented in table-10, which shows significant content were observed in

matured leaves (3.83 mg/g tissue) followed by young leaves (3 mg/g tissue) and tuber (2.26 mg/g tissue) respectively.

4. DISCUSSION

To elevate the damaging effects of ROS, plants have evolved intracellular enzymatic antioxidants that include superoxide dismutase (SOD), catalase (CAT), guiacol peroxidase (G-Px), ascorbic acid peroxidase (AAO)[36,2], glutathione peroxidsae (GSH-Px) and glutathione reductase (GR)[3]. They also posses non-enzymatic antioxidants such as Phenol, reduced glutathione (GSH) and ascorbate[4]. Plants are still a large source of natural antioxidants that might serve as leads for the development of novel drugs[37].

Therefore, the present investigation evaluate the enzymatic and non- enzymatic capacity of *Amorphophallus commutatus* tubers and leaves adapting eight enzymatic methods such as superoxide dismutase, catalase, peroxidase, ascorbate oxidase, glutathione peroxidase, glucose 6- phosphate dehydrogenase, glutathione reductase, polyphenol oxidase and three non- enzymatic methods such as reduced glutathione, total poly phenols and ascorbic acid.Analysis of the results indicated that the hydro alcoholic extract exhibited differential antioxidant profile.

The *Amorphophallus commutatus* tuber exhibited significantly greater activities of superoxide dismutase, ascorbic acid oxidase and polyphenol oxidase. It is also found to have higher non-enzymatic content of ascorbic acid and total phenol. The young leaves of *Amorphophallus commutatus* exhibited significant catalase and glutathione reductase activity and found to have significant glutathione content among the plant parts. The matured leaves of *Amorphophallus commutatus* exhibited significant activity of glucose-6-phospate-dehydrogenase and peroxidase.

The tuber with significant phenol content exhibits poly phenol oxidase whereas the leaves have no significant Poly phenol oxidase activity and Phenol content. In the young leaves the activity of glutathione reductase and Glutathione content are in significant quantity revealing the conversion of oxidized glutathione (G-S-S-G) to reduced (GSH) by Glutathione reductase. In mature leaves the activity of Glucose-6-Phosphate dehydrogenase activity might be high for it might require high amount of NADPH for other activities with its Hexose Monophosphate (HMP) shunt functioning effectively. Glucosr – 6 – phosphate dehydrogenase is the first enzyme in the HMP shunt.

Super oxide radicals are inactivated by the enzyme superoxide dismutase (SOD), the only enzyme known to use a free radical as a substrate. The radical scavenging activity of SOD is effective only when it is followed by increase in activity of catalase and other peroxidases[38]. SOD generates H_2O_2 as a product which is in turn more toxic to the cells and requires catalase or peroxidases to scavange. Thus a concomitant increase in catalase and or Peroxidase is essential for the beneficial effect from increase Superoxide dismutase activity. Catalase act in the microbody of cells, while Guaicol peroxidase exist in the apoplast, chloroplast and cytosol[39].

H_2O_2 can penetrate the cell membrane and then react with metal ions through the Fenton reaction to produce extremely highly toxic hydroxyl radicals, which cause DNA damage and cell injury. These harmful free radicals can be scavenged by intracellular antioxidant enzymes like Guaicol Peroxidase and Glutathione reductase that minimize or remove cellular reactive radical cascades and decrease cytotoxic oxidative damage in cells. Guaicol peroxidases are able to catalyze the reduction of lipid hydroperoxides to hydroxides during the oxidation of reduced Glutathione (GSH). Subsequently, Glutathione reductase regenerates GSH and provides reducing power for various coupled thiol transferase and peroxidase. Moreover, compounds inducing antioxidative enzymes or decreasing free radicals levels could decrease mutation

production and cancer initiation because they might reduce intracellular oxidative stress and DNA damage[40].

It is understood that defense against oxidative stress is primarily dependent upon orchestere synergism between exogenous and endogenous antioxidants. The exogenous antioxidants like Vitamin E and Vitamin C are recycled continuously by thiols like GSH and dihydrolipoate. Thus, vitamin C and Glutathione react cooperatively *in vivo* leading to greater protection against radical damage which could not be provided any single antioxidant[41].

Lee *et al*., 2011 reported the importance of active antioxidant enzyme system which will help in treating the chilling stress of cucumber. They have reported the enzymes superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and ascorbate oxidase. Chilling increased the activities of antioxidant enzyme such as CAT, GSH – Px and AAO elevated the contents of Ascorbic acid and GSH[4].

The reports of Suriyavathana and Indhu priya, 2011 are relevant to the current report. They have screened the antioxidant potential of Dioscorea bulbifera tuber, they have reported the presence of glutathione peroxidase, catalase, superoxide dismutase, glucose 6-phosphate dehydrogenase and glutathione s-transferase. The non-enzymatic antioxidants like vitamin-E, vitamin-C and reduced glutathione also been reported in Dioscorea bulbifera tuber[42].

Tubers tend to be starchy and typically rich in vitamins and minerals. The main nutritional value of roots and tubers lie in the potential ability to provide one of the cheapest source of dietary energy in the form of carbohydrate this make them an excellent addition to human diet. Antioxidant enzymes have the capacity to lower the free radical burden and neutralize the excess free radicals created by the stress and normal metabolic conditions. The present study has been initiated with the view and objective to explore the antioxidants store in *Amorphophallus commutatus*.

5. CONCLUSION

The present study reports for the first time the innate enzymatic and non enzymatic antioxidant potential of the plant Amorphophallus commutatus an endemic aroid of western ghats. The tuber exhibited significant SOD, AAO and PPO activity. The young leaves revealed the presence of significant CAT, GR and GSH content. The mature leaves exhibited significant G6PD activity. Tuber and young leaves harbour peroxidases revealing the scavenging of H2O2. Young contain signigicant glutathione content attributed by the presence of GR. Amorphophallus commutatus contain all the antioxidative enzymes which can regulate the free radical activity and can reduce the generation of free radicals and can prevent cellular and tissue damage in human body.

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