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Preliminary Phytochemical Screening, *in vitro* Antioxidant Activity, Total Phenolic and Total Flavonoid Contents of *Colocasia esculenta* Leaf Extract.

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Abstract : Medicinal plants are rich in antioxidants which are used to treat many human diseases. Antioxidants are the chemical substances which give their own electron to the free radicals thus check their damage causing potential in the body. The research is going on worldwide with main focus on natural antioxidants from plant origin. The aims of the present study were to check the presence of various phytochemicals, to explore the in vitro antioxidant activity, total phenolic and total flavonoid contents of Colocasia esculenta leaves. The results lead us to the conclusion that the extract of Colocasia esculenta leaves did possess the fair number of phytochemicals, showed the decent antioxidant activity and contained the good total phenolic and total flavonoid contents.

Keywords: Antioxidants, phytochemicals, *Colocasia esculenta,* total phenolic, total flavonoid contents.

Introduction

Reactive oxygen species (ROS) are produced continuously in the human body as a consequence of typical metabolic processes. If excessive free radicals including ROS are not inactivated, their chemical reactivity can smash up all types of cellular macromolecules, including proteins, carbohydrates, lipids and nucleic acids (Brieger et al., 2012). Although the human body has the capability of producing the copious quantities of antioxidant enzymes so as to neutralize the free radical, food rich in natural antioxidants is suggested so as to get rid of diet born free radicals (Rimbach *et al.,* 2005).

The therapeutic importance of drugs from medicinal plants have received the special attention of the researchers as the continuous use of the synthetic ones may culminate into many side effects and toxicity. Potentially active components from leaves, fruits, roots and herbs have been studied comprehensively in order to avoid oxidative cellular events. Phytochemicals are known to possess antioxidant activity (Wong et al., 2009). The results suggest that polyphenols, especially the flavonoids possess a high antioxidant power which can protect cells against the undesirable effects of ROS (Thielecke and Boschmann, 2009). Many plants have been documented which possess antioxidant properties like *Colocasia antiquoram* (Tuse et al., 2009), and *Solanum torvum* (Bhuvaneswari *et al.,* 2012). A decoction of leaves and those of wabula (*Merremia peltata*) is used for the treatment of cysts, while the sap of the leaf stack is used to treat conjunctivitis (Awasthi and Singh, 2004). *Colocasia esculenta,* commonly known as 'Taro' is a staple vegetable crop that has been used as food for over 9000 years, making it one of the world's oldest food crops. It is used as a source of starch, protein and vitamins. On the basis of traditional knowledge of medicinal system, the present study was undertaken to elucidate the *in vitro* antioxidant efficacy of ethanolic extract of *Colocasia esculenta* (EECE) leaves.

Materials and Methods

The fresh leaves of *Colocasia esculenta* were collected from Bhopal M.P during the month of August. The plant was identified and authenticated by Dr. Zia Ul Hassan (HOD Botany, Safia Science College, Bhopal. A voucher specimen bearing number 452/Bot/Safia/14 was submitted in the said department for future references. The leaves of *Colocasia esculenta* were washed thoroughly and shade dried for a week at room temperature. The dried leaves were reduced to moderately coarse powder by mechanical grinding. The powder thus obtained was extracted in 90% ethanol using Soxhlet apparatus. The ethanolic extract was dried under vacuum and the semi solid material thus obtained was stored in storage bottles which were kept at -4 ^{o}C for further use. The fresh stock solution of *Colocasia esculenta* 80 mg/ml was prepared in distilled water just before use. Phytochemical screening of the extracts was carried out according to the standard procedures (Trease and Evans, 1989 and Kokate *et al.,* 2006).

DPPH Assay (Gulcin *et al*., 2006; Jain and Jain 2011**)**

4 mg of DPPH was dissolved in 100 ml of the methanol in order to get the 0.1 mM DPPH solution. The test samples with different concentrations were prepared in methanol. 1 ml of DPPH solution was added to the 2 ml of test samples. The resulting mixtures were subjected to incubation for 10 minutes at room temperature. The absorbance of the mixtures was noted at 515 nm using methanol as blank. The percentage inhibition of DPPH molecules by the extract was calculated using the formula:

% Inhibition = [(AC 515 nm- AS 515 nm/ AC 515 nm) x 100].

Reducing Power Assay (Jain and Jain, 2011)

0.5 ml of the different concentrations of test sample was added to 0.5 ml of phosphate buffer (0.2 M, pH 6.6) and 0.5 ml of potassium ferricyanide (0.5 ml, 1% W/V). The resulting mixtures were subjected to incubation at 50° C for 20 min. The reaction was terminated by adding 1.5 ml of trichloroacetic acid solution (10% W/V). Finally, 0.5 ml of ferric chloride (0.1% W/V) was added to the test samples and the absorbance of the mixtures with different concentrations was measured at 700 nm. A graph was plotted between absorbance and concentration.

Total Phenolic Content Estimation (Ainsworth and Gillespie, 2007**)**

In order to estimate the total phenolic content in the ethanolic extract of *Colocasia esculenta* leaves, gallic acid was dissolved in methanol and its different concentrations were prepared ranging from 10 µg/ml to 100 µg/ml. The sample (100 µg/ml) under investigation was prepared in methanol. 2 ml of Folin-Ciocalteu reagent (1:10 in de-ionized water) was added to 0.5 ml of test sample/ different concentrations of Gallic acid. Sodium carbonate solution (4 ml) was added to all solutions. The solutions were subjected to incubation at room temperature for 30 minutes with intermittent shaking. The absorbance of all the solutions was noticed at 765 nm when methanol was kept as blank. The standard curve for Gallic acid concentrations was prepared and the line of regression was found. The absorbance noticed for the test sample was put in the line of regression of standard curve obtained for gallic acid. Total phenolic content in the test sample was thus calculated and expressed as mg/gm or µg/mg gallic acid equivalent.

Total Flavonoid Content Estimation (Alhakmani *et al*., 2013**)**

In the methanol were prepared the different concentrations of rutin (10 to 100 µg/ml). The test sample having the concentration of 100 µg/ml was also prepared in methanol. 2 ml of distilled water and 0.15 ml of 5% $NaNO_2$ solution were mixed with the 0.5 ml of the test sample. 0.15 ml of a 10% $AlCl_3$ was added to the resulting solution after six minutes and allowed to stand as such for another six minutes. After the incubation period, 2 ml of 4% NaOH solution was added to the solution. The final volume was brought to 5 ml by the distilled water, mixed properly and allowed to stand for 15 minutes. The absorbance of the solution was recorded at 510 nm using distilled water as the blank.

The standard curve for different concentration of rutin was prepared and line of regression was drawn. The total flavonoid content was thus calculated and expressed as mg/gm or, µg/mg rutin equivalent.

Results

The results of various phytochemical investigations were as follows:

Loss of weight and Percentage yield of plant extract

After proper shade drying and grinding the loss of weight of *Colocasia esculenta* was 85.05%. After subjecting the plant material to soxhlation, the extracts obtained was stored in air tight glass bottles at room temperature. The percentage yield of the extract was calculated and it was 8.6%. The organoleptic evaluation of the extracts was done.

Solubility

The solubility of the extract was checked in different solvents for further studies like DPPH assay, reducing power, total phenolic content and total flavonoid content. It was found that *Colocasia esculenta* leaf extract was soluble in methanol, pet ether, chloroform, ethanol, acetone, ethyl acetate and DMSO but sparingly soluble in water.

Observation of Phytochemical Screening

The qualitative phytochemical analysis was performed using standard procedures (Kokate *et al.,* 2006). The phytochemical analysis of ethanolic extract of *Colocasia esculenta* leaf extract gave the positive results for saponins, alkaloids, terpenoids, flavonoids, carbohydrates, glycosides, amino acids, tannins and phenolic compounds.

Antioxidant activity of *Colocasia esculenta* leaf extract

The DPPH radical reacts with suitable reducing agents losing colour stoichometrically with the number of electrons consumed, which is measured spectrophotometrically at 517 nm. The standard curve has been plotted by using various concentrations of ascorbic acid and its percentage inhibition by DPPH was calculated (Fig.1). The obtained result of ethanolic extract of *Colocasia esculenta (*EECE) was 0.89 mg/ml (Table 1). The scavenging effect was compared to that of the standard ascorbic acid showing IC_{50} value 18.53 µg/ml. The results, thus obtained suggest that EECE has the proton donating ability and can serve as free radical inhibitor or scavenger or exhibit significant DPPH radical inhibition (Fig. 2).

Reducing Power Assay

For determining antioxidant activity of *Colocasia esculenta* another method used was reducing power assay. In case of reducing power assay the higher the absorbance of the reaction mixture, the higher would be the reducing power, Table 2 represents the absorbance of ethanolic extracts of leaves of *Colocasia esculenta* at different concentrations and clearly indicates that absorbance increases with increasing concentration of EECE.

Presence of Total Phenolic Contents (TPC)

Different concentrations of gallic acid were made and absorbance taken at 765 nm (due to developed blue colour) using methanol as blank so as to draw a standard curve (Fig. 3). The total phenolic content in the ethanolic extract of *Colocasia esculenta* was 10.95 ± 0.505 µg/100 µg gallic

acid equivalent (Table 3).

Presence of Total Flavonoid Content

Different concentrations of Rutin were made and absorbance taken at 510 nm with water as blank. Standard cure was plotted thereafter (Fig. 4). The total flavonoid content of ethanolic extract of *Colocasia esculenta* was 11 ± 0.5 µg/100 µg rutin equivalent (Table 4).

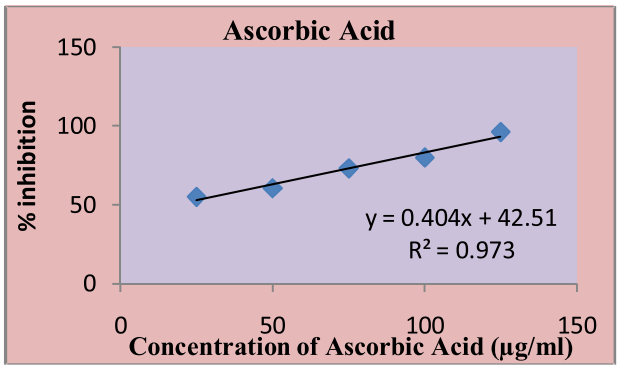

Fig. 1- Represents the regression curve of ascorbic acid by DPPH assay method.

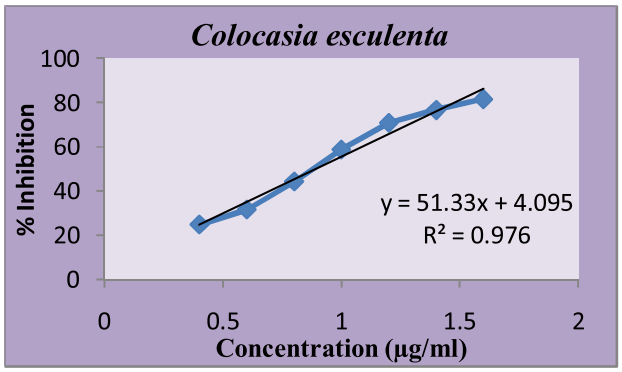

Fig. 2- Represents the inhibition of DPPH by *Colocasia esculenta*.

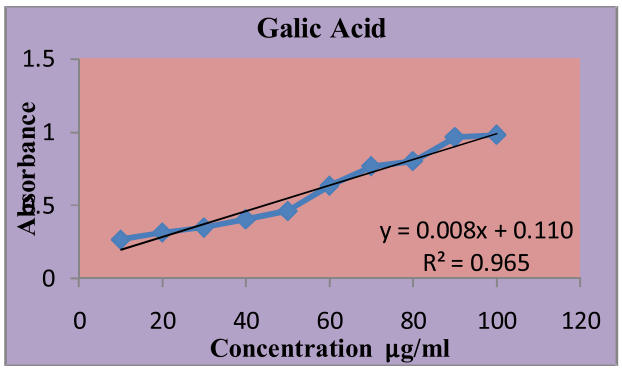

Fig. 3 - Represents the standard curve of Gallic Acid.

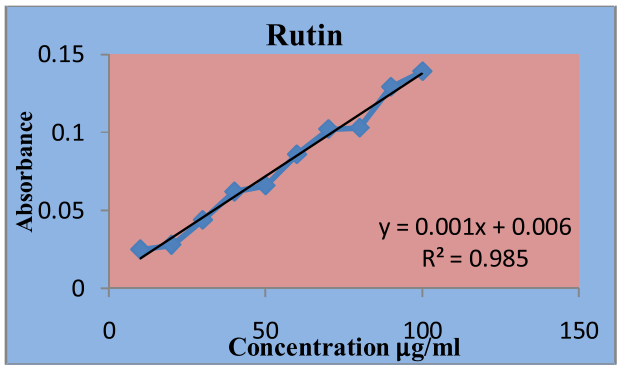

Fig. 4 - Represents the Standard curve of Rutin.

Table: 1 - Showing % inhibition of DPPH by ethanolic extract of *Colocasia esculenta*.

S. No.	Conc. (mg/ml)	% Inhibition	IC_{50} (mg/ml)
1	0.4	24.67	0.89 mg/ml
2	0.6	31.52	
3	0.8	44.22	
4	1	58.63	
5	1.2	70.75	
6	1.4	76.6	
7	1.6	81.59	

Table: 2 – Showing the Reducing Power of ethanolic extract of *Colocasia esculenta*.

S. No	Concentration µg/ml	Absorbance
1	100	0.473
2	200	0.670
3	300	0.820
4	400	0.925
5	500	1.145

Discussion

In present investigation, to substantiate the antioxidant potential of leaves of *Colocasia esculenta,* the preliminary phytochemical analysis of its ethanol extract was conducted. The DPPH test provides information on the reactivity of the test compounds with a stable free radical. DPPH gives a strong absorption band at 517nm in visible region. When the odd electron becomes paired off in the presence of a free radical scavenger, the absorption reduces and the DPPH solution is decolourised as the colour changes from deep violet to light yellow. The degree of reduction in absorbance measurement is indicative of the radical scavenging (antioxidant) power of the extract. Ethanolic extract of *Colocasia esculenta* appeared to be as potent as Vitamin C having IC_{50} value 18.53 µg/ml. It was found that the extract contained fair amount of

Table: 3 - Showing Total Phenolic Content in ethanolic extract of *Colocasia esculenta* leaves.

S. No.	Absorbance	Concentration	Total Phenolic content in µg/100 µg Gallic acid equivalent
1	0.197	100 µg/ml	10.87
2	0.194	100 µg/ml	10.50
3	0.202	100 µg/ml	11.50
Mean	0.197667		10.95
S. D.	0.00503		0.505

Table: 4 - Showing Total Flavonoid content in ethanolic extract of *Colocasia esculenta* leaves.

S. No.	Absorbance	Concentration	Total Flavonoid content in µg/100 µg Rutin equivalent
1	0.0170	100 µg/ml	11
2	0.0165	100 µg/ml	10.50
3	0.0175	100 µg/ml	11.50
MEAN±SD			11 ± 0.5

phytochemicals such as saponins, alkaloids, terpenoids, flavonoids, carbohydrates, glycosides, tannins, saponins and phenolic compounds. Such type of work on medicinal plants was conducted from time to time by many other researchers (Rosalki and Mcintyre, 1999; Abuelgasim, 2008). Antioxidant efficacy, phenolic contents and flavonoid contents were found in appreciable amount in leaves of *Rosemarinus officinalie* Felicia muricata (Ashafa *et al*., 2010), leaves and roots of Hypochaeris radicata (Jamuna, *et. al*., 2012)..

As for as the reducing power is concerned, it has been observed that the substances which have reduction potential, react with potassium ferricyanide (Fe^{3+}) leading to the formation of potassium ferrocyanide (Fe^{2+}), which finally reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm. Such type of work was carried by other researchers (Siju *et al.,* 2010). The reducing power increased with the increase in the extract concentrations leaf and root parts of *H. radicata* may have high amount of reductones and hence the antioxidant property (Senguttuvan, et. al., 2014). Ethanolic extract of *Colocasia esculenta* leaves also shows high level of reducing power. This may be served as significant indicator of its potential antioxidant activity of *Colocasia esculenta* leaves. The antioxidative efficacy of plants has been attributed to the presence of phenolic and flavonoid compounds as ingredients (Ilahi, et al., 2013). The higher concentrations of more bioactive flavonoids compounds were detected with 70% ethanol due to its higher polarity than pure ethanol (Joseph et. al., 2013). During the present study, a good amount of phenolic and flavonoid contents were observed in the ethanolic extract of *Colocasia esculenta* leaves. Thus, the present findings lend a strong support to the earlier reports that the metabolites of the plants viz. phenolic and flavonoids contents show fair amount of antioxidant activity (Rice-Evans, et al., 1995).

Conclusion

The present study clearly showed that the extract of *Colocasia esculenta* leaves possess the fair amount of antioxidative potential which may be attributed to the presence of various important phytochemicals viz. alkaloids, terpenoids, flavonoids, tannins, glycosides etc. The result shows the values of IC_{50} of ethanol extract of *Colocasia esculenta* leaves and ascorbic acid as a pattern, and points to a higher antioxidant activity of this plant extract when compared to the standard used ascorbic acid, showing the effectiveness of antioxidant activity. Therefore, we suggest the possibility that leaf extract of *Colocasia esculenta* can control the action of free radical and thus preventing cellular aging, becoming an alternative in the fight against diseases.

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