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Original Research Article

Extraction, identification of phytoconstituent's from tubers of *Colocasia esculata* and *Borassus flabellifer* and its invitro determination of antioxidant and oxidative DNA damage

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ABSTRACT

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Antioxidants have reactive oxygen species scavenging ability with great relevance in the prevention of oxidative stress. This study was undertaken to evaluate the in vitro antioxidant potential of leaves of tubers of *Colocasia esculata* and *Borassus flabellifer*. Methanolic extracts of the plants parts were subjected to *in vitro* screening models such as antioxidant activity and protective effect on DNA damage. 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) and phospho molybdenum reduction assays. The selected plants extracts showed the higher antioxidant properties and demonstrated their ability to protect against DNA damage. The results thus obtained suggested that *Colocasia esculata* and *Borassus flabellifer* possess the high antioxidant activity and protection against DNA damage.

Keywords: Antioxidants, DNA damage, *Colocasia esculata*, *Borassus flabellifer.*

1. INTRODUCTION

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidizing agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate

these chain reactions by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves (Sies, 1997). Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, causes oxidative stress and may damage or kill cells. As oxidative stress might be an important part of many human diseases, the use of antioxidants in pharmacology is intensively studied, particularly as treatments for stroke and neurodegenerative diseases (Bjelakovic et al., 2007).

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction. Their chief danger comes from the damage they can do when they react with important cellular components such as DNA, or the cell membrane. Cells may function poorly or die if this occurs. To prevent free radical damage the body has a defence system of antioxidants. Taro (*Colocasia esculenta* L.) is an herbaceous perennial plant widely cultivated in West and Central Africa. It is the third most important staple root/tuber crop after yam and cassava in Nigeria and second after cassava in Cameroon and first in Ghana (Echebiri, 2004). The main nutrient supplied by taro is carbohydrate (Jirarat et al., 2006), and it also contains proteins, vitamins and minerals.

Borassus flabellifer is a tall tree attaining a height of about 30 m, with a black stem and crown of leaves at the top; leaves are 0.9-1.5 m in diameter, palmately fan shaped, petiole edges with hard horny *spinescent serratures*; flowers are unisexual; fruits are large. This plant is widely distributed and cultivated in tropical Asian countries such as Thailand, Bangladesh, India, Myanmar, Sri Lanka, Malaysia, etc. (Janz, *et al*., 1994, Ariyasena *et al*., 2001). In India, it has been cultivated chiefly in the dry or sandy localities of Andhra Pradesh, Bihar, Karnataka, Kerala, Madhya Pradesh, Orissa, Tamil Nadu and West Bengal. Antioxidant principles from natural resources possess multi facetedness in their multitude and magnitude of activities and provide enormous scope in correcting the imbalance between pro-oxidant and antioxidant. Hence, there is no doubt that phytochemicals deserve a proper position in the therapeutic armamentarium. The present study aims to evaluate the antioxidant potential of tubers in *Colocasia esculenta* and *Borassus flabellifer*.

2. MATERIALS AND METHODS

2.1. COLLECTION OF SAMPLE

Fresh Palmyra sprouts and *Colocasia corms* were purchased from uzhavar sandhai in Villupuram. It was stored at room temperature.

2.2. PROCESSING OF SAMPLE

The collected samples were peeled to remove the outer covering and it was cut into small pieces. The weight of both the samples were weighed separately and noted down. The dried tubers and corms slices were then ground separately using an electric blender to obtain a fine powder. The powder was further passed through a sieve to obtain fine particles. The powdered samples were then stored in airtight polythene bags and labeled and further used for analysis.

2.3. SOLVENT EXTRACTION

Methanol is used as the solvent for extraction process using Soxhlet extractor. The prepared extract was concentrated in the rotator vacuum evaporator till the whole methanol was evaporated and collected at the other end- receiving flask of the rotary vacuum evaporator. After the separation it was kept in a water bath for about 2 hours to get the concentrated extract. The extract was weighed and collected.

2.4. PHYTOCHEMICAL ANALYSIS:

The Plant extract were subjected to phytochemical analysis by standard method (Harborne, Baxter, 1995)

2.5. DOT BLOT ASSAY:

A dot blot is a technique used to detecting, analyzing and identifying biomolecules. In a dot blot the biomolecules to be detected are not first separated by electrophoresis. Instead, a mixture containing the molecule to be detected is applied directly on a membrane as a dot, and then is spotted through circular templates directly onto the membrane or paper substrate.

TLC Silica gel 60 F_{254} strip is used for the antioxidant assay. The sample extracts are placed in the plates as dots using microcapillaries and to this added DPPH. The DPPH radical has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction.

The stable DPPH 2, 2-diphenyl-1-picrylhydrazyl.radical was used for determination of free radical-scavenging activity of the extracts.

2.6. FREE RADICAL SCAVENGING ACTIVITY

2.6.1. METAL CHEALATING ACTIVITY

Ferrozine can quantitatively chelate with Fe^{2+} and form a complex with a red color. This reaction is limited in the presence of other chelating agents and results in a decrease of the red color of the ferrozine-Fe^{2+} complexes. Measurement of the color reduction estimates the chelating activity to compete with ferrozine for the ferrous ions (Soler-Rivas et al., 2000). Chelation of ferrous ions is estimated using the method of Dinis et al. (1994). This method is based on the competition between ferrozine and the bio reactive components of the plant extracts in trapping ferrous ions. This is translated by a reduction in the absorbance at 562 nm of the ferrozine (Fe^{2+}) complex. To 0.5 ml of plant extract was made to 2.5 ml with water and 25 ul of 2 mM $FeCl_2$ and 100 µl of ferrozine (5 mM) was added. The

mixture was vigorously shaken and left to stand at room temperature for 10 min. A control tube was prepared similarly except that the extract was replaced with methanol. The absorbance was measured spectrophotometrically at 562 nm.

$$\% \text{ of chealating activity} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100$$

2.7. EVALUATION OF TOTAL ANTIOXIDANT CAPACITY (TAC)

Antioxidant capacity was evaluated by phospho molybdenum method Farhana et al, 2012 and Prieto et al, 2012).

2.8. OXIDATIVE DNA DAMAGE ASSAY USING YEAST AS INVITRO MODEL

Yeast was cultured in the YPD broth in an incubator. The cultured YPD broth was distributed in six eppendrof tubes and centrifuged for 10 minutes at 10,000 rpm to collect the cell pellet for extraction of DNA for the oxidative stress determination.

2.8.1. Isolation of the Yeast DNA from the Yeast Cells:

300 µl of CTAB buffer was added and vortexed. This was then made into a suspension and kept in a dry bath for 1 hour. Every twenty minutes it was tilted upside down. Added 100 µl of Chloroform and freeze for 5 minutes and again vortex. This results in the formation of three layers. The topmost layer contains the DNA and the proteins, the middle layer contains carbohydrates and lipids and the third layer constitute chloroform. The topmost layer is pipette out using a micropipette and transferred into another set of six eppendrof tubes without disturbing the pellet or middle layer, which is later discarded (Haley, L.D., 1971; Namjin Chung, 1996)

Added 100 µl of Isopropanol and refrigerated for 5-10 minutes. Isoproponal solubilizes the proteins and lipids if any and centrifugation for 10 minutes at 10,000rpm. Decanted the supernatant and to the pellet added 50 µl of 100% alcohol and vortexed. The contents in the six eppendrof tubes were emptied into a single eppendrof tube and centrifuged again for 8000rpm for 10 minutes. Washed with 1ml 70% Ethanol. Air dried the pellet and suspended in 50 µl TE. 50 µl of the sample extract was air dried. To this 50 µl of 1 X TE buffer was added and vortex. The sample extracts for both were prepared in the same manner.

2.8.2. Effect of Sample Extract on Oxidative Damage:

On a slide marked four points for Control, Negative control, S1, S2, 5 µl of gel loading dye was added to the four points. Mixed 10 µl of yeast DNA to all the four wells. 10 µl of Hydrogen Peroxide (H_2O_2) was added to Negative Control, S1 and S2. 10 µl samples were mixed to S1 and S2. From this reaction mixture 10 µl from each was loaded into wells made in 1% agarose gel and run at 100V for 15 minutes in a submarine gel electrophoretic apparatus. The DNA was visualized and photographed using UVITEC FIRE READER digital gel documentation system

3. RESULTS AND DICUSIONS

Borassus flabellifer a native to South and south East Asia is considered as a rich source of phtyoconstituents such as gums, saponins, glycosides and carbohydrates.*Colocasis esculents* grown in tropical and subtropical regions are known since ancient times for its curative properties such as analgesic, anti-inflammatory, anticancer and hypolipidemic effects.

In the present study methanolic extracts of the roots of Borassus flabellifer and colocasis esculenta was assayed for antioxidant activity using DPPH, Metal Chelating Assay, Phosphomolybdenum assay and oxidative DNA damage. Initially phytochemical screening was done to detect the presence or absence of the bio active compounds. From the results, the presence of alkaloids, tannins, saponins, carbohydrates, terpenoids etc was confirmed (Table.1).

The antioxidant activity protect cells against the damaging effects of Reactive Oxygen Species which result in oxidative stress leading to cellular damage.The radical scavenging activity was performed with DPPH qualitatively by Dot Blot assay which indicates good level of antioxidant activity for both the extracts(Figure.1). Metal chelating assay allows the estimation of the chelating activity of the extracts .On a comparison the extract of B.flabellifer shows 17.08 % metal chelating activity than *C.esculenta* possessing 6.83 % which is lower than the *B.flabellifer*. The metal chelating effects of the extracts samples were dependent on concentration and linearly increased with the sample concentration increase (Table.2, Figure.2).. Phosphomolybdenum assay and oxidative DNA damage again indicates high antioxidant activity. On the total antioxidant concentration *B.flabellifer* exhibits 494.14µg than *C.esculenta* possessing 333.72 µg of total antioxidant capacity.

The application of the extract for the prevention of oxidative DNA damage by protecting the DNA which inferred on agarose gel electrophoresis. A positive control was loaded and the sample DNA along with the toxin and extract was use for the assay (Figure.3). As discussed earlier high levels of ROS is a mutagenic factor for DNA damage. Antioxidants are the components of cellular defense mechanism against the reactive oxygen molecules.

Almost every part of *Borassus flabellifer* and *Colocasis esculenta* is known for its medicinal value since ancient times. But apart from its consumption as staple food, these are not taken for its pharmacological value. As this invitro study confirms appreciable levels of antioxidant which posses radical quenching activity and protects DNA damage, these may be graded and processed as health promoting foods or medicines.

Table-1: Inference for Phtyoconstituents

Phytochemical Test	***Borassus flabelifer***	***Colocasia esculenta***
Carbohydrates	+	+
Tanin	+	+
Flavonoids	-	+
Alkaloids	+	+
Phenols	+	+
Gum and mucilage	-	+
Terpenoids	+	+
Quinone	-	-
Anthocyanin	-	-
Fixed oil	-	+
Saponin	+	+
Glycosides	+	+
Anthoquinone	+	-
Resin	+	+
Volatile oils	-	-
Coumarins	-	+

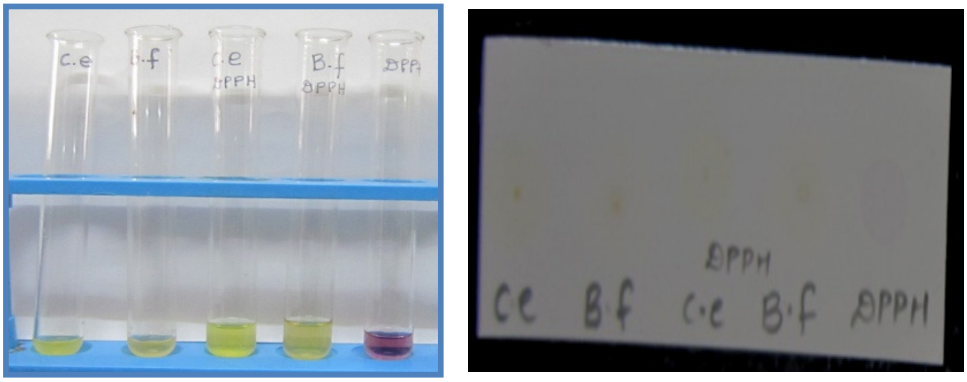

Figure-1: DPPH free radical activity

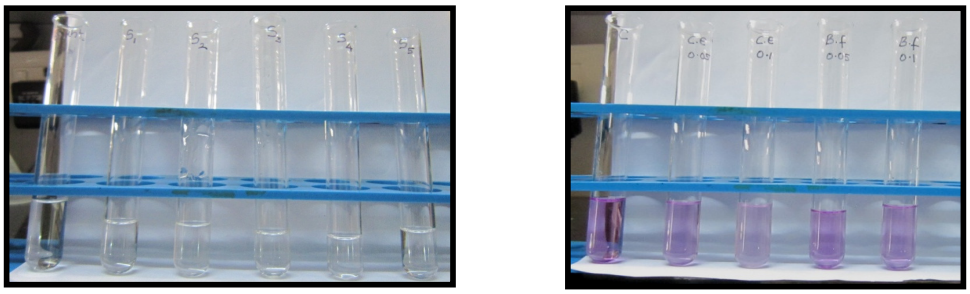

Figure-2: Total metal chelating activity

Table -2: Activity of metal removal

DESCRIPTION	**Absorbance**	**% of chelating activity**
CONTROL	0.322	
CE	0.301	6.83
BF	0.267	17.08

Table-3: Total antioxidant capacity depicting the extract concentration

Standard	Concentration	Absorbance
S1	100	0.047
S2	200	0.097
S3	300	0.144
S4	400	0.200
S5	500	0.225

Description	Absorbance	Total Antioxidant quantity(mg)
CE	0.158	333.72
BF	0.234	494.14

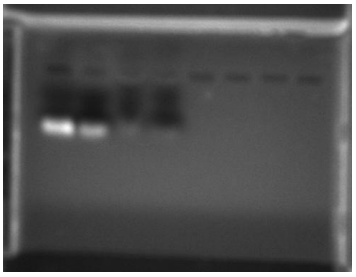

Figure-3: Agarose gel electrophoresis for oxidative DNA protection
Keys: Lane 1: Control DNA,Lane 2, DNA with extract and toxin (Sample 1),Lane 3: DNA with extract and toxin (Sample 2),Lane 4: DNA with toxin, Lane 5, 6, 7, 8: Unloaded

4. CONCLUSION

Free radicals have been implicated in the etiology of large number of major diseases. They can adversely alter many crucial biological molecules leading to loss of form and function. Such undesirable changes in the body can lead to diseased conditions. Antioxidants can protect against the damage induced by free radicals acting at various levels. Dietary and other components of plants form major sources of antioxidants. The relation between free radicals, antioxidants and functioning of various organs and organ systems is highly complex and the discovery of 'redox signaling' is a milestone in this crucial relationship. Recent research centers around various strategies to protect crucial tissues and organs against oxidative damage induced by free radicals. Many novel approaches are made and significant findings have come to light in the last few years. The traditional Indian diet, spices and medicinal plants are rich sources of natural antioxidants. Higher intake of foods with functional attributes including high level of antioxidants in functional foods is one strategy that is gaining importance in advanced countries and is making its appearance in our country. Coordinated research involving biomedical scientists, nutritionists and physicians can make significant difference to human health in the coming decades. Research on free radicals and antioxidants involving these is one such effort in the right direction.

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests regarding the publication of this paper.