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ANTHOCYANINS FROM INDIAN CASSAVA (*MANIHOT ESCULENTA* CRANTZ) AND ITS ANTIOXIDANT PROPERTIES

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Keywords:

Cassava *(Manihot esculenta),* Anthocyanin, quercetin, gallic acid, total phenol, Flavonoid content, Antioxidants, Free radicals scavenging effect

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ABSTRACT

Cassava ((*Manihot esculenta* Crantz) leaf stalk is a renewable raw material and it is a major industrial crop in Tamilnadu rich in antioxidant compounds. To utilize this source, an environmentally sustainable procedure has been developed for the extraction of anthocyanin and to analyze its antioxidant properties. So, the present research was focused on cassava for total phenol contents, flavanoids, anthocyanins and it's antioxidant activities were studied using leaf stalks. Three difficult solvents were tried to extract total phenol and anthocyanin from cassava leaf stalks. In the case of total phenol content acetone extract found to have maximum phenol content followed with acidified methanol and methanol. In the case of anthocyanin content, acidified methanol extract gave maximum yield followed with methanol and acetone extracts. The presence of phenolic compound and anthocyanin determine the free radical scavenging activity in cassava leaf stalk extract. The cassava leaf stalks, which was found to be a waste material after harvesting the tubers can be used as a valuable source for anthocyanin extraction, which will applicable in clinical and food industry.

INTRODUCTION: Cassava *(Manihot esculenta* Crantz) belongs to Euphorbiaceae family is a perennial crop native to tropical America with its center of origin in North-eastern and Central Brazil [1]. In the tropics, the roots of cassava are an important source of carbohydrates for human consumption. Cassava plants also produce green leaves that are consumed as vegetables in some parts of sub-Saharan Africa such as Democratic Republic of Congo (DRC), Uganda, Nigeria, and some Asian countries, the Philippines, Indonesia and Malaysia [2].

The dry powdered cassava leaf vegetable has reached commercial exploitation in the DRC, being exported to Belgium and France [3]. The leaves are known to be rich in vitamin A and proteins [3, 4, 5]. In addition to use as food, the crop has taken on more importance as a source of starch for industry and food processing and as an animal feed [1, 6, 7]. Cassava was introduced in Uganda sometime after 1862, and it rapidly spread to most parts of the country [8] where it provided a basic daily source of dietary energy. Roots are processed into a wide variety of granules, paste, flour, etc., or consumed freshly boiled or raw [9]. The leaves are also consumed as a green vegetable, which provides proteins and vitamins A and B.

Cassava has been recognised as one of the most important food crops in Uganda, second to bananas in terms of area cultivated, total production and per capita consumption [10]. Flavonoids are phenolic substances isolated from a wide range of vascular plants, and more than 8150 different flavonoids have been reported [11]. They act in plants as antioxidants, antimicrobials, photoreceptors, visual attractors, feeding repellents, and for light screening [12].

Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilating effects.

The leaves are used to treat hypertension, headache and pain. Cubans are commonly used cassava to treat irritable bowel syndrome. Cassava grown in drought are especially high in anthocyanin content [13, 14] identified 3-rutinosides of kaempferol and quercetin; the cyanogenic glycosides, lotaustralin and linamarin, from the fresh leaves of cassava. There has been no report, so far, on the anthocyanin content from the leaf stalks of cassava considering some natural edible components with confirmed antioxidant activity, special attention has been given to flavonoids, anthocyanins and chlorophyll, which are commonly found in fruits and vegetables.

Anthocyanins and other flavonoids of these groups of natural substances proved to have high degree of antioxidant activity and they are found to be wide spread in plant materials. They may be an important factor along with other flavonoids in the resistance of plants to insect attack. Among the plant materials fruits, vegetables and spices are reported to be rich in compounds with antioxidants. The pharmacological effect of flavonoids is due to their inhibition of certain enzymes and their antioxidant activity.

The searches for antioxidants form natural resources has received much attention and efforts have been put into identify compounds that can act as a suitable antioxidants to replace synthetic ones. However the use of these synthetic antioxidants has been questioned due to their potential health risks and toxicity. In addition these naturally occurring antioxidants can be formulated to give nuetraceuticals that can help to prevent oxidative damage from occurring in the body. Apart from food purposes, cassava leaf stalks as also suspected to have bioactive compounds that exhibit antioxidant and antiproliferative effects.

Anthocyanins and its antioxidant activity in plants: Anthocyanins are polyphenolic compounds responsible for cyanic colors ranging from salmon pink through red and violet to dark blue of most flowers, fruits, leaves and stems [15]. They comprise the largest group of the water-soluble pigments in the plant kingdom [16] and during the last years it has been an exponential increase in the report of new anthocyanin structures [17]. This can be explained by the use of improved analytical techniques, but the potential use of anthocyanins as health beneficial compounds is another reason for the increased scientific interest in these pigments. They play a definite role in attracting animals in pollination and seed dispersal.

Anthocyanins are involved in attraction of insects and animals for pollination and seed dispersal purposes as they constitute the chemical basis of flower color in angiosperms [18]. Their presence in young leaves, seedlings, roots and stems are not that obvious. There is increasing evidence that anthocyanins, particularly when they are located at the upper surface of the leaf or in the epidermal cells, also have a role in the physiological survival of plants. The functions of anthocyanins have in this context mainly been hypothesized as a compatible solute contributing to osmotic adjustment to drought and frost stress, as antioxidant and as UV and visible light protectant.

The anthocyanins constitute a major flavonoid group which are responsible for colours ranging from salmon pink through red and violet to dark blue of most flowers, fruits, stems, and leaves of angiosperms [15]. The major flavonoid classes are such as flavones, isoflavones, flavonones, anthocyanins and catechins which have strong antioxidant activity. They are probably the most important group of visible plant pigments besides chlorophyll. Recent studies have proven that anthocyanins mask the chlorophyll containing organelles and thereby protect chloroplasts against high light intensities to prevent photoinhibition.

To date, it is estimated that more than 400 anthocyanins have been found in nature [19]. Anthocyanins have been found to significantly suppress the growth of cultured tumour cells and have been shown to have greater inhibitory effect than other flavonoids. Antioxidative properties of anthocyanins arise from their high reactivity as hydrogen or electron donors, and from the ability of the polyphenol derived radicals to stabilize and delocalize the unpaired electrons, and from their ability to chelate transition metal ion. It has been

suggested that anthocyanins have the ability to stabilize DNA triple- helical complexes [20].

In addition to their potential as food colorants, anthocyanins are nowadays regarded as important nuetraceuticals mainly due to their possible antioxidant effects, and they have been given a potential therapeutic role related to some cardiovascular diseases, cancer treatment, inhibition of certain types of virus including Human Immunodeficiency Virus type 1 (HIV-1) and improvement of visual acuity.

They were effective in reversing age related deficits in several nerve disorders and behavioural parameters [21]. These compounds are more effective in decreasing capillary permeability and fragility and in their anti-inflammatory and antioedema activities [22]. Anthocyanins can be useful in controlling oxidative stress during pregnancies complicated by intrauterine growth retardation.

Antioxidants are compounds that significantly delays or inhibits oxidation of a substrate. It may be considered as the scavengers of free radicals. Fruits and vegetables contain different antioxidant compounds, such as ascorbic acid, tocopherol, glutathione and carotenoids. It contributes to protect against oxidative damages.

Antioxidant property of anthocyanin – high reactivity as hydrogen or electron donors, ability of the polyphenol derived radicals to stabilize and delocalize the unpaired electrons. Antioxidants are compounds with electron scavenging properties that may slow down or prevent the development of cancer. Quercetin**,** anthocyanins in grapes, red and apples are found to have good antioxidant property.

Objective: The present paper was focused on the extraction and analysis of biological activities of anthocyanins from leaf stalks of cassava, which was found to be a waste material after harvesting the tubers and leaves. The aim of the study is to determine the anthocyanin content in leaf stalk of cassava using different solvent system. To analyze and to evaluate the antioxidant properties of anthocyanin from cassava leaf stalk.

MATERIALS AND METHODS:

Sample collection: Cassava leaf stalk were collected from field and stored in sealed polyethylene bags at -20˚C.

Extraction: 0.5 gm of cassava leaf stalk were extracted with 10 ml of 3 different solvents (methanol, acidified methanol and acetone). The mixture was centrifuged at 10,000 rpm for 10 min and supernatant was taken for further analysis.

Analytical procedures:

Flavonoid Conformation Test:

A. $FeCl_3$: 1 ml of sample extraction was added with a small amount of $FeCl_3$**,** and results were observed.

B. $AlCl_3$: 1 ml of sample extraction was added with 5% of $AlCl_3$ solution, and results were observed.

Total Phenolic Assay: Total phenolic compounds in anthocyanin samples were quantified by using Folin-ciocalteu's method. A 50 µl of Folin-ciocalteu's reagent (50% v/v) were added to 10µl of sample extract and was incubated for 5 min at room temperature. After incubation 50µl of 20% (w/v) sodium carbonate and water was added to final volume of 400 µl. Blank was prepared by replacing the reagent by water to correct for interfering compounds. After 30 min of incubation, the absorbance was measured using spectrophotometer at 760 nm.

Stability at variable pH: The anthocyanin stability was tested by treating 1 ml of sample with 1 ml of P^H 1.0 and 4.5 solutions. The color change was observed.

Determination of Total Anthocyanin: The total amount of anthocyanin content was determined by using p^H differential method. A spectrophotometer was used for the spectral measurements at 210 nm and 750 nm. The absorbance of the samples (A) was calculated as follows:

A= (Absorbance λvis-max-A750) pH 1.0 – (Absorbance λvis-max-A750) pH 4.5 Anthocyanin pigment content (mg/liter) = (A X MW X DF X 1000)/(ε X 1).

Where, Molecular weight of anthocyanin (cyd-3-glu) = 449; Extraction coefficient (ε) = 29,600; DF=Diluted factor

Total Flavonoids Content: The flavonoid content was determined according as the aluminum chloride colorimetric method described[23]. Briefly, aliquots of 0.1g of cassava leaf extract were dissolved in 1 ml of deionized water. This solution (0.5 ml) was mixed with 1.5 ml of 95% alcohol, 0.1 ml of 10 % aluminium chloride hexahydrate ($AlCl_3$), 0.1 ml of 1 M potassium acetate (CH_3COOK), and 2.8 ml of deionized water. After incubation at room temperature for 40 min, the reaction mixture absorbance was measured at 415 nm against a deionized water blank on a spectrophotometer. Quercetin was used as a standard. Using a seven point standard curve (0-50mg/l), the levels of total flavonoid contents in cassava leaf stalk was determined in triplicate, respectively. The data was expressed as milligram quercetin equivalents (QE)/100 g fresh matter from fresh cassava leaf stalk extract.

Antioxidant Assays:

Quantification of Ascorbic Acid: This assay was carried out by the method [24] 0.1 ml of brominated sample extract was added with 2.9 ml of distilled water. Then 1 ml of 2 % DNPH reagent and 1-2 drops of thiourea was added with sample. After incubation at 37^0c for 3 hours, the range-red osazone crystals that were formed were dissolved by the addition of 7 ml of 80% sulphuric acid again incubated for 5 minutes. After incubation absorbance was measured at 450 nm. Vitamin C concentration was expressed in terms of mg/g of sample.

Scavenging activity of DPPH Radical: Scavenging activity of anthocycanins against DPPH radicals was assessed according to the method [25] with some modifications. Briefly, 0.1 mM DPPH-methanol solution was mixed with 1 ml of 0.1mM DPPH methanol solution. After the solution was incubated for 30 min at 25^oC in dark, the decrease in the absorbance at 517nm was measured. Control contained methanol instead of antioxidant solution while blanks contained methanol instead of DPPH solution in the experiment. Ascorbic acid and BHT were used as positive controls.

The inhibition of DPPH radicals by the samples was calculated according to the following equation:

DPPH-scavenging activity (%) = [1-(absorbance of the sample - absorbance of blank)/absorbance of the control] × 100

Hydroxyl Radical Scavenging Activity: The hydroxyl radical scavenging activity was determined according to the methods described [26]. 0.1 ml of the different extracts of anthocyanin samples extract was taken in different test tubes. 1.0 ml of iron-EDTA solution (0.1% ferrous ammonium sulfate and 0.26% EDTA), 0.5 ml of DMSO (0.85% v/v in 0.1 M Phosphate buffer, pH 7.4) were added to these tubes, and the reaction was initiated by adding 0.5 ml 0f 0.22% ascorbic acid. Test tubes were capped tightly and heated on a water bath at 80-90 ^{0}c for 15 min.

The reaction was terminated by the addition of 1 ml of ice cold TCA (17.5% w/v), 3 ml of Nash reagent (75 g of ammonium acetate, 3 ml of glacial acetic acid, and 2 ml of acetyl acetone were mixed and raised to 1 L with distilled water) was added to all of the tubes and left at room temperature for 15 min for the color development. The intensity of the yellow color formed was measured spectrophotometrically at 412 nm against the reagent blank. The percentage of hydroxyl radical scavenging activity is calculated by using the formula:

% of hydroxyl radical scavenging activity = 1-absorbance of sample/absorbance of blank × 100

Determination of Reducing Power: The reducing power was determined according to the method[27]. A 0.25ml aliquot of various concentrations of anthocyanins was mixed with 2.5 ml of 200mM sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide.

The mixture was then incubated at 50 º C for 20 min, after incubation 2.5 ml of 10% trichloroacetic acid (w/v) were added, the mixture was centrifuged at 650g for 10 min. A 5ml aliquot of the upper layer was mixed with 5ml of distilled water and 1ml of 0.1% ferric chloride at 700nm was measured. A higher absorbance indicates a higher reducing power.

Determination of Superoxide Radical-Scavenging Activity: Superoxide radicals were generated by the method[28], described [29], with some modifications all solutions were prepared in 0.05 M phosphate buffer (pH 7.8). The photo induced reactions were performed in aluminium foil-lined box with two 30W fluorescent lamps. The distance between the reaction solution and the lamp was adjusted until the intensity of illumination reached about 4000 lux. A 30µL aliquot of various concentrations of anthocyanins was mixed with 3ml of reaction buffer solution (1.3 mm riboflavin, 13 mM methionine, 63 µM nitro blue tetrazolium and 100µM EDTA, pH 7.8). The reaction solution was illuminated for 15 min at 25 º C. The reaction mixture, without sample, was used as a control. The scavenging activity was calculated as follows: scavenging activity (%) = (1-absorbance of the sample/absorbance) ×100

Metal Chelating Activity: The chelation of ferrous ions by the extract was estimated by the method [29] with slight modification and compared with that EDTA, BHT and that of ascorbic acid. The chelation test initially includes the addition of ferrous chloride. The antioxidants present in the sample chelates the ferrous ion from the ferrous chloride. The remaining ferrous combines with ferrozine to form ferrous-ferrozine complex. The intensity of the ferrous-ferrozine complex formation depends on the chelating capacity of the sample and the color formation was measured at 562 nm (Genesys5 UV-Vis Spectrophotometer).

Different concentrations of standard and extracts (100-500 µg/ml) were added to a solution of 100 µl $FeCl_2$ (1mM). The reaction was initiated by the addition of 250 µl ferrozine (1 mM). The mixture was finally quantified to 1.3 ml with methanol, shaken vigorously and left standing at room temperature for 10 min, after the mixture had reached equilibrium, the absorbance of the solution was measured spectrophotometrically. All the test and analysis were done in duplicate and average values were taken. The percentage inhibition of ferrous-ferrozine complex formation was calculated using the formula;

%= 1-As/Ac X 100;

Where, 'Ac' is the absorbance of the control, 'As' is the absorbance of the sample.

Molecular Assays:

Determination of inhibitory effect on deoxyribose degradation: Inhibitory effect of the anthocyanins on deoxyribose degradation was determined by measuring the reaction activity between either antioxidants and hydroxyl radicals (referred to as non-site-specific scavenging assay) or antioxidants and iron ions (referred to as site-specific scavenging assay), described [30]. For the non site specific scavenging activity assay, a 0.1 ml aliquot of different concentrations of anthocyanin was mixed with 1ml of reaction buffer (100 µM $FeCl_3$, 104µM EDTA, 1.5mM H_20_2, 2.5mM deoxyribose and 100µM L-Ascorbic acid pH 7.4) and incubated for 1 h at 37^{o}C. A 1ml of 0.5% 2-thiobarbituric acid in 0.025 M NaOH and 1 ml of 2.8% trichloroacetic acid were added to the mixture and it was heated for 30 min at 80^{o}C. The mixture was cooled on ice and the absorbance was measured at 532nm. Site- specific scavenging activity, which represented the ability of anthocyanins to chelate iron ions and interfere with hydroxyl radical generation, was measured using the same reaction buffer without EDTA. % inhibition of deoxyribose degradation was calculated as %=(1- absorbance of sample/absorbance of control) ×100.

RESULTS AND DISCUSSION:

Anthocyanin Extraction: The anthocyanin was extracted by using methanol, acidified methanol and acetone from cassava leaf stalk was done in methanol, acidified methanol and acetone. Absorbance of extract confirmed the presence of anthocyanin in cassava leaf stalk and further procedures were done for antioxidant analysis **(Plate 1).**

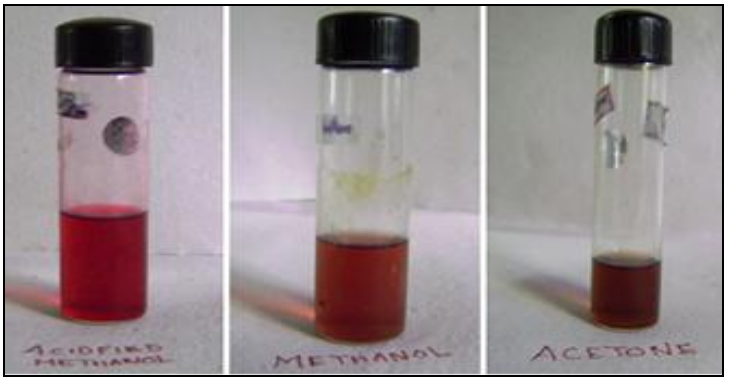

PLATE 1: ANTHOCYANIN EXTRACTED FROM CASSAVA LEAF STALKS BY USING ACIDIFIED METHANOL, METHANOL AND ACETONE

Flavonoid Confirmation Test: In the presence of $FeCl_3$ the methanol, acidified methanol and acetone extracts of cassava leaf stalk showed brown color which confirms the presence of Flavanoids. In the presence of **$AlCl_3$**, dark color was observed in methanol; acidified methanol and acetone extract which showed the presence of anthocyanin **(Plate 2).**

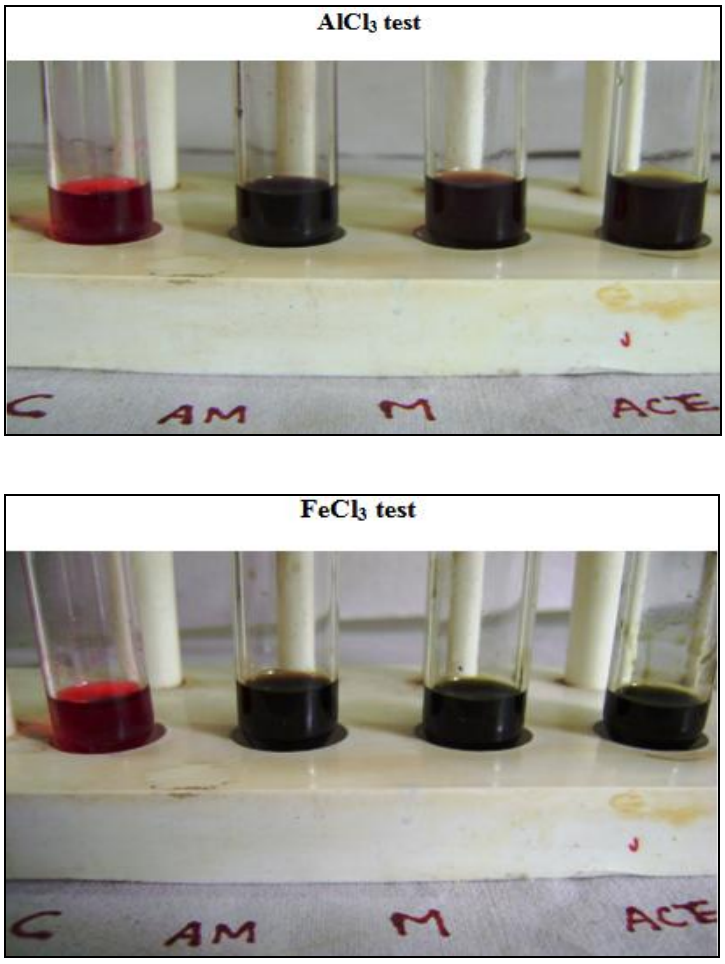

PLATE 2: CONFIRMATORY TESTS FOR FLAVONOIDS

Total Phenolic Assay: Total phenolic content of methanol, acidified methanol and acetone extracts varied from 64 to 164 mg/ g. The lowest content was found in methanol extract (64 mg/g) and while highest content was observed in acetone extract (164 mg/g) and acidified methanol extract (136mg/g) of cassava leaf stalk in **Table 1**. Gallic acid were used as standard for determine total phenol content. Results from this preliminary study will provide a better understanding of the antioxidant properties of this plant and allow the identification of plant with high antioxidant activity for further investigation and development into value added nuetraceuticals.

Total Flavonoid Content: This study shows that total flavonoid content in acidified methanol extract (114 mg/g), methanol extract (124mg/g), acetone (238mg/g) of cassava leaf stalk **(Table-1).** Our results suggest that the total phenolic and flavonoid content in different extracts of cassava leaf stalk varies considerably. In our results correlation was observed between total phenolic and flavonoid content.

TABLE 1: TOTAL ANTHOCYANIN, FLAVONOID AND PHENOL CONTENT IN CASSAVA (*MANIHOT ESCULENTA* CRANTZ)

Cassava leaf stalk extracts			
Assays	**1% methanol HCl**	**Methanol**	**Acetone**
Total anthocyanin (mg/g)	44±0.01	44±0.04	15±0.02
Total flavonoid content (mg/g)	116±0.01	124±0.03	238±0.03
Total phenol (mg/g)	136±0.01	64±0.01	164±0.02

Determination of Total Anthocyanin: The total content of anthocyanin in acidified methanol extracts (44mg/g) and methanol extract shows (44mg/g) and lowest content was observed in acetone extract (15mg/g) of cassava leaf stalk **(Figure 1).** Acidified methanol, methanol extracts resulted significantly higher values for total anthocyanin than acetone extract. The total anthocyanins extracted by acidified methanol extract and methanol extract were an average of 20 % higher than the acetone extract. Several authors reported that acidified methanol preserves the extracted anthocyanin in their original form better. It should be the solvent of choice for quantification and analysis of anthocyanins.

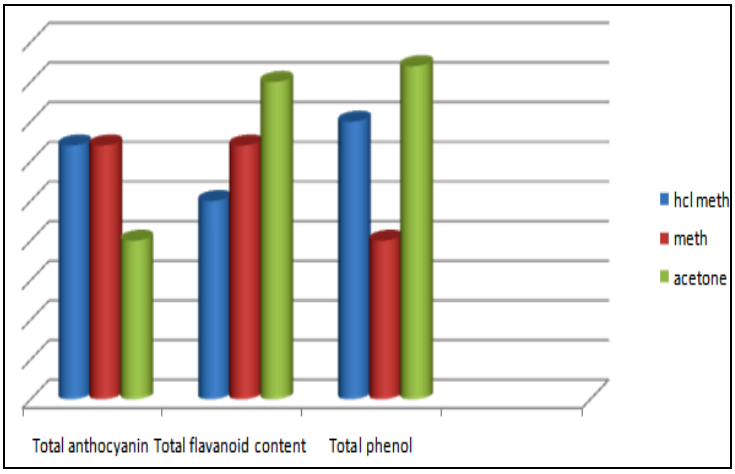

FIG. 1: COMPARISON OF TOTAL ANTHOCYANIN, FLAVONOID AND PHENOL CONTENT IN CASSAVA (*MANIHOT ESCULENTA* CRANTZ)

Stability at variable pH: The sample appears red color at pH 1 and color disappeared at pH 4.5. The results were found to be same in Acetone, methanol and acidified methanol extract. Giusti reported that, the anthocyanins are stable in low pH.

Antioxidant Analysis:

Ascorbic Acid Assay: Vitamin C content was very low in acetone extract (0.26 mg/g) where as in methanol and acidified methanol extracts of cassava leaf stalk concentration ranges from 0.46-0.55 mg/g. **(Table-2).** Ascorbate has been found chloroplast, vacuole and extracellular compartments of plant cell and shown to function as a reductant for many free radicals.

Scavenging activity of DPPH Radical: The ability of phenolic compounds to quench reactive species by hydrogen (H^+ ions) donation was measured through DPPH radical scavenging activity assay. Activity is measured as relative decrease absorbance at 517nm as reaction between DPPH and antioxidant progresses [26]. Antioxidant activity was evaluated with % inhibition at which the radical scavenging activity is listed in **table 2**. The results indicate % inhibition in cassava leaf stalk extract ranging from 10% to 68%.

The antioxidant activity of cassava leaf stalk extracted in acidified methanol, methanol and acetone were compared (**Table 2**). From our result it was observed that samples extracted in acidified methanol has significantly higher DPPH radical scavenging activity than those extracted in methanol and acetone. Shih suggested that polyphenol in the extracts were responsible for the antioxidant activity assayed by DPPH scavenging method in aqueous leaf extracts of cassava plant

Hydroxyl Radical Scavenging Activity: The Hydroxy radicals is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every living cells. This species is considered to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids. **Table 2** shows that hydroxyl radical scavenging activity of the anthocyanin extracted from the leaf stalks of cassava increases with increasing concentration of the different extracts, acetone exhibited a highest activity of 93.3%, followed by acidified methanol of 63% and methanol extract showed 54% activity. This is similar to observation of several others who have reported a dose dependent in other sample extracts [31]. The ability of acetone extracts of anthocyanins to quench hydroxyl radicals seems to directly relate to prevention of propagation of the process of lipid peroxidation and acetone extract seems to been an good scavenger of active species, thus reducing rate of chain reaction. A high positive correlation was observed between the polyphenol content and hydroxyl radical scavenging activity.

TABLE 2: ANTIOXIDANT ANALYSIS OF DIFFERENT SOLVENT EXTRACT IN CASSAVA (*MANIHOT ESCULENTA* CRANTZ)

Cassava leaf stalk extracts			
Antioxidant Assay's	1% methanol HCl	methanol	acetone
Ascorbic acid (mg/g)	0.55	0.46	0.26
DPPH radicals (%)	68	15.2	9.7
Hydroxyl radical scavenging activity (%)	63.1	54	93.3
Reducing power (%)	0.497	0.535	0.259
Superoxide radical-Scavenging activity (%)	49.3	56.8	26.8
Metal chelating activity (%)	79.5	80.1	43.2
Inhibitory effect on deoxyribose degradation (%)	15	27	36

Reducing Power: The potassium ferric cyanide reduction method was used to measure the ability of phenolic compounds to quench radicals through electron donation. Activity is monitored by measuring the absorbance, which increases as antioxidants reduce the ferric ion/ferric cyanide complex to the ferrous form. **Fig 2** shows reducing power of the different cassava leaf stalk extract as function of mg/ml concentration.

An increase in reducing power was observed in methanol extract followed by acidified methanol and acetone extracts of cassava leaf stalks shown in Table 2. The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking free radical chain by donating a hydrogen atom [32].

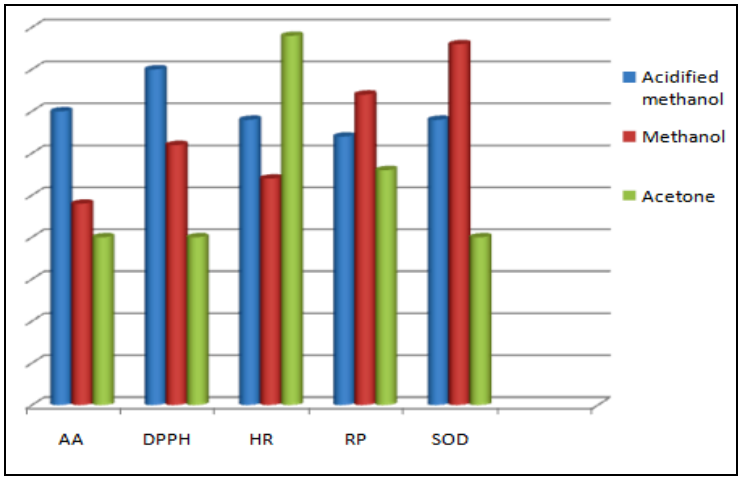

FIG. 2: COMPARISON OF ANTIOXIDANT ANALYSIS OF DIFFERENT SOLVENT EXTRACT IN CASSAVA (*MANIHOT ESCULENTA* CRANTZ)

Superoxide Radical-Scavenging Activity: Superoxide anion radicals are produced by a number of cellular reactions, including various enzyme systems such as lipooxygenase, peroxidase, NADPH oxidase and xanthine oxidase. Superoxide anion plays an important role in plant tissue and these involved in other cell damaging free radicals [33]. In the presence study superoxide radicals was generated by illuminating a solution containing riboflavin. Based on the results obtained as represented in the **Fig 2**, it is clear that methanol extract has better superoxide radical scavenging activity as compared to other extracts, which may be again due to higher amount of total phenol content as with the reducing power were shown in **Table-2**.

Anthocyanins exhibited an excellent superoxide anion scavenging activity, which was much higher than these of ascorbic acid and BHT. Further superoxide radicals also known to indirectly to initiate lipid peroxidation as result of H_2O_2 formation, creating precursors of hydroxyl radicals [34]. These results clearly suggest that the antioxidant activity of anthocyanins extracted from cassava leaf stalks is also related to its ability to scavenge superoxide radical scavenging activity.

Metal Chelating Activity: The ability of antioxidants to form insoluble metal complexes with ferrous ion (or) generate hindrance that prevent interaction between metal and lipid is evaluated using the ion chelating assay [35]. The activity is measured by monitoring the decrease in absorbance of red ferric (Fe^{2+}) - ferroin complex as antioxidants compete with ferrozine complex in chelating ferrous ion [36]. **Fig. 3** shows the metal chelating power of different solvent extracts of cassava leaf stalks. From the table it's clear that methanol extract was higher as compared with other extracts, while acetone extract showed lowest metal chelating activity in **Table 2**.

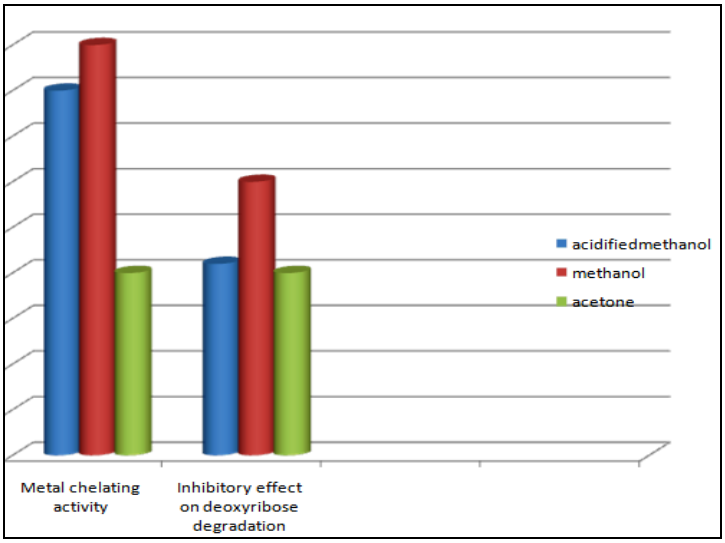

FIG. 3: COMPARISON OF ANTIOXIDANT ANALYSIS OF DIFFERENT SOLVENT EXTRACT IN CASSAVA (*MANIHOT ESCULENTA* CRANTZ)

No correlation found between ion chelating capacity and phenolic content for cassava leaf stalks. This may indicate the presence of other antioxidants responsible for metal chelation like non-phenolic metal chelators include phosphoric acid, ascorbic acid, carnosine, some amino acids, peptides and proteins such as transferring and ovotransferrin [37]. From the ascorbic acid analysis it was found that cassava leaf stalk extracts contain ascorbic acid in the higher concentration, so the metal chelating activity may be due to ascorbic acid. [38] reported that potato contains ascorbic acid and reduced tripeptides, which are responsible for metal chelating effect.

Molecular Assays:

Inhibitory Effects on Deoxyridose Degradation: Hydroxy radical can be formed by Fenton's reaction in the presence of reduced transition metals such as Fe_2^+ and H_2O_2, which is known to be the most reactive of all the reduced forms dioxygen and is taught to initiate cell damage in *in- vivo* [38]. To determine whether anthocyanins reduce hydroxyl radical generation by chelating metal ions or by directly hydroxyl radical scavenging, hydroxyl radicals, the effects of the anthocyanins on hydroxy radical generated by Fe^{3+}

ions were analyzed by determining the degree of deoxyribose degradation. Fig. 3 shows the inhibition of hydroxyl radical induce the deoxyribose degradation by anthocyanins in both site specific and non-site specific assays. Using the concentrations, relatively greater antioxidant activity was observed the site specific assay than in the non-site specific assay (Table 2), implying that the anthocyanin inhibited deoxyribose degradation mainly by chelating metal ions rather than by scavenging hydroxyl radical directly. In the present study acetone extract of cassava leaf stalks showed 36% of by site specific inhibition rather than the other extracts. Similar results were reported for extracts of *Opuntia ficus-Indica varsaboten* [39] and *Hypericum perforatum* [40].

CONCLUSION: This study represents the first report of anthocyanin extraction and antioxidant analysis in Indian cassava (*Manihot esculenta* Crantz). The data represented in the study demonstrate that the amount of anthocyanin content and phenolic compounds in different extracts of cassava leaf stalks. In antioxidant assays shows high reductants for many free radicals and methanol extracts shows excellent SOD activity due to high polyphenol content with reducing power. No correlation found between ion chelating capacity and phenolic content for cassava leaf stalks. This may indicate the presence of other antioxidants responsible for metal chelation. From the ascorbic acid analysis it was found that cassava leaf stalk extracts contain ascorbic acid in the higher concentration. The study on antiproliferative effect and anthocyanin specific compounds in Indian cassava leaf stalks are presently in progress.

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