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Assessment of Free Radical Scavenging Activity in Some Wild Edible Fruits of Odisha, India

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ABSTRACT

Our body tissue is constituted of innumerable cells that produce nitrogen free radicals during the process of day to day metabolism. These free radicals oxidize the neighboring cells and damage them. Thereby decay and degeneration of cells appear. Hence to neutralize their damaging activities, antioxidants, in our food are very essential. The study of antioxidant activities by DPPH, FRAP, Peroxidase, Catalase, Superoxide dismutase, Phenol and Carotenoid content of 5 wild edible fruits of Odisha here reveals encouraging results. FRAP value was found highest in *Citrus medica* (320.33±4.94 µM AEAC /g dry wt.), whereas lowest recorded in *Annona squamosa* (169.11±1.70 µM AEAC /g dry wt.). The highest DPPH scavenging activity was found in *Annona squamosa* (114.16±2.21 mg AEAC /100g dry wt.), where lowest was found in *Citrus medica* (10.45±4.29 mg AEAC /100g dry wt.). The Phenolic content was highest in *Diospyros* melanoxylon (1.6±0.05 g/100g) and lowest in *Annona squamosa* (0.18±0.01g/100g). Likewise, *Annona squamosa* showed highest peroxidase content (0.0098±0.0017Δ O.D / min / g fwt.) where *Syzygium cerasoides* showed lowest phenolic content (0.0017±0.0003Δ O.D / min / g fwt). It was found that catalase was found maximum activity in *Citrus medica* (0.0137±0.0017U/ml) and minimum in *Flacourtia indica* (0.001±0.0001U/ml). Correspondingly for superoxide dismutase (SOD), the highest value was exhibited in *Diospyros melanoxylon* (7.88±0.76Δ OD/min/g tissue wt.) and lowest in *Annona squamosa* (0.28±0.0028Δ OD/min/g tissue wt). The carotenoid content was highest recorded in *Syzygium cerasoides* (18.51±0.35mg/100g) and lowest in *Citrus medica* (0.50±0.45mg/100g). Thus, it was conclusive finding that these wild fruits may be utilized by large scale cultivation to obtain adequate antioxidants.

KEYWORDS: DPPH, FRAP, Peroxidase, Catalase, SOD, Phenol and Carotenoid.

INTRODUCTION

The science of interest has been remarkably diverted in the modern days to prevent aging process by preventing oxidation of cells that at times becomes very fast. Oxidation of cells is normal process of our body but fast aging, degeneration and death of cells are not normal. Untimely degeneration and death of cells can only be prevented by antioxidants like vitamin A, C, E, Carotenoids, Flavonoids and lycopene etc [1,2,3]. These items have been found to prevent oxidative process of free radicals, peroxidase activities, fat, protein, part of cells and DNA [4]. Our food, particularly fruits and vegetables contain these items and ultimately prevent oxidation, peroxidase activity etc. Certain wild edible fruits have been assayed for their antioxidant properties and have exhibited remarkably substantial quantity in their pulp. Hence these items not only prevent aging by taking as food but also are used in Ayurvedic medicine, Unani, Sidha and occasionally in allopathic medicines for their medicinal and nutritional properties.

The economically poor people of India, tribals in particular fail to have purchase capacity to eat appropriate nutritious food. In order to survive, they take some wild roots, fruits and leaves etc to get energy. Some of these wild fruits which give them nutrition are unknown to the modern civilization. There are certain nutritious fruits which are excellent providers of energy, vitamins and trace elements and they hardly have substantial amount of anti-nutritional property. The plant extracts and products provide not only energy through carbohydrate, protein, fat but also vitamins, minerals, trace elements and antioxidants. Many free radicals have been tie up with in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes, ageing and Alzheimer's disease [5,6].

Since many diseases like Obesity, Diabetic, Arthrosclerosis and Alcoholic cirrhosis are found mostly in sufficient percentage in upper and middle class people of Odisha and nutritional cirrhosis, malnutrition and Alzheimer's disease are found in almost no other state of India such high incidence has been tribal poor people of Odisha. It is high time to explore the potentiality of nutritious fruits untapped so far for domestic use in large scale. In almost no other state of India such high incidence has been reported so far. The present research paper taps 5

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ethno-medicinally important wild edible fruits namely *Annona squamosa* (Atta), *Citrus medica* (Bada Limbu), *Diospyros melanoxylon* (Kendu), *Flacourtia indica* (Bhaincha koli), *Syzygium cerasoides* (Kathajamu) for their in vitro free radical scavenging activity of Odisha.

MATERIALS

Sample collection

The present study was conducted to assess the antioxidant activities in selected tropical wild fruits (e.g. *Annona squamosa* (Atta), *Citrus medica* (Bada Limbu), *Diospyros melanoxylon* (Kendu), *Dillenia indica* (Oau), *Flacourtia indica* (Bhaincha koli), *Syzygium cerasoides* (Kathajamu)) of Odisha collected from different forest regions. Fruits were botanically identified with the help of Ref. Books e.g. The Flora of Odisha [7] and Wild Edible Fruit Plants of Eastern Ghats [8] and also compared with authentic herbarium sheets belonging to herbarium of Regional Plant Resource Centre, Bhubaneswar.

Preservation

Wild edible fruits were washed in running water and dried with tissue paper. Fruit samples were oven dried at 50 ºC and grinded into powdered form for analysis of DPPH and FRAP analysis. Fruit samples used for Peroxidase, Catalase and SOD enzyme assays were kept at - 20ºC, until required for further analysis.

Table1. Medicinal Properties of 5 wild edible fruits

Sl. No.	Name of fruit species	Plant parts used	Medicinal properties
1	 *Annona squamosa*	Leaves, fruits, bark	Cures Dysentery, cardiac problems, fainting, worm infections, constipation, hemorrhage, dysuria, fever, thirst, malignant tumors, and ulcers, used as abortifacient [9],[10]
2	 *Citrus medica*	Fruits, seeds, peels, leaves	Used as Antioxidant, antimutagenic properties, positive associations with bone, cardiovascular, and immune system of health. [11]
3	 *Diospyros melanoxylon*	Fruits, flowers, leaves, bark	Cures mental disorders, nervous breakdowns and palpitations of the heart, urinary, skin and blood diseases, diarrhoea [12]

4	 *Flacourtia indica*	Fruits, bark, root, gum	Appetizing, digestive, diuretic, intermittent fever, renal colic and cholera [13]
5	 *Syzygium cerasoides*	Stem, leaf, fruits, bark, seed	Cures Dysentery, anti diabatic properties [10,14].

METHODOLOGY

Extraction of fruit samples for Total antioxidant assay

The fruits were cleaned and dried in hot oven air after taking the initial fresh weight in moisture balance. Then the dried fruits were reweighed for final weight. Dried fruits were grounded separately into fine powder using motor and pestle. 2 gm of dried fruit powder was weighed and shift into a beaker. 50 ml of absolute methanol was added in to the beaker and the mixture was oscillating using magnetic stirrer for 24 hours at 37ºC. Each extract was filtered using Whatman No.1 filter paper. The supernatant was collected and the remnant was re-extracted twice. The solvents of two extracts were removed using hot plate. Then the extracts were filled in bottles and stored in the 4ºC for further uses.

Determination of Carotenoid

Carotenoid content was estimated following the method of [15]. Test fruit sample was prepared in 80% acetone and centrifuged at 5,000 rpm at 4oC for 20 minutes (Eppendorf cooling Centrifuge, 5430 R). The supernatant was used for Carotenoid analysis by taking absorbance at 400 nm, 645 nm and 663 nm using UV-Vis spectrophotometer (Spekol 2000, Analytik Jena, Germany). The quantity of pigments was calculated by the formula:

Carotenoids = [OD480 + 0.11 (OD663) – 0.638(OD645)] × 400

Determination of Total Phenol

Phenol content was estimated following the method of [16] modified by [17]**.** Sample was prepared in 60% methanol and centrifuged at 5,000 rpm at 4°C for 30 minutes (Eppendorf cooling Centrifuge, 5430 R). The supernatant was collected for phenol analysis. 0.1ml and 0.2 ml of sample extraction was added to 1ml of 0.1 N HCL and allowed to stand for few minutes.1ml of sodium nitrite molybdate mixture was added and shaken well and allowed to stand for few minutes. 5ml of distilled water was added to test tube. After that 2ml of 1N NaOH was added and allowed to stand for 15-20 minutes. Methanol was taken as blank reference and optical density (OD) was measured in UV-Vis spectrophotometer (Spekol 2000, Analytik Jena, Germany) at the wavelength 515 nm. A standard calibration curve was plotted using gallic acid (100-1000 µg/ml). The results were expressed as grams of gallic acid equivalents (GAE)/100 g.

Free radical scavenging activity (DPPH)

The total antioxidant activity of the fruit extracts was estimated on the basis of the radical scavenging effect of the stable DPPH free radical as per the modified protocol of [18]. DPPH solution (0.006% w/v) was prepared in 95% methanol. Methanol fruit extracts (1 ml) were mixed with DPPH solution, so that the final volume was 2 ml and discoloration was measured at 517 nm (Spekol 2000 UV-Vis Spectrophotometer) after incubation for 30 min in dark. In case of control, methanol was taken instead of the fruit sample. Ascorbic acid was used as a reference standard. Percentage scavenging of the DPPH free radical was measured using the following equation:

(%) Scavenging activity = $[(A_O-A_T)/A_O] \times 100$

Where, A_O is the absorbance of the control and A_T the absorbance of the sample.

Ferric reducing antioxidant power assay (FRAP)

The ferric reducing power of the fruit extracts was estimated according to the method of [19]. The stock solutions included 300 mM acetate buffer (3.1 g sodium acetate hydrate and 16 ml glacial acetic acid), pH 3.6, 10 mM TPTZ (2, 4, 6-tri-(2-pyridyl)-5-triazine) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. The temperature of the FRAP solution was raised to 37°C before use in water bath. Methanolic fruit extracts (100 µl) were allowed to react with 3ml of the FRAP solution for 10 min at 37°C in water bath. Readings of the coloured product (ferrous tri pyridyl triazine complex) were taken at 593 nm at 0 min and 10 min. The standard curve of Ascorbic acid was prepared with concentration ranging (100 µM to 1000 µM) at 593 nm. Results are expressed in µM Fe (II)/g dry mass and compared with that of ascorbic acid.

FRAP Value of Sample (µM) =

$$\frac{\text{Change in absorbance of sample from 0 min to 10 min}}{\text{Change in absorbance of Standard Ascorbic acid from 0 min to 10 min}} \times 100$$

Enzymatic assay for antioxidant activity in some wild edible fruits of Odisha

Peroxidase Enzyme Assay

Peroxidase enzyme activity was determined by the method of [20]. For enzyme extraction, 0.5 gm. of fresh wild edible fruit sample was grinded with 5ml of phosphate buffer (pH 6.5) in pre-cooled mortar and pestle and centrifuged at 7500 rpm at 4°C for 30 minutes (Eppendorf cooling Centrifuge, 5430 R). The clear supernatant was collected and stored at 4°C. For measuring the peroxidase activity, the reaction mixture containing 0.5 ml sample extract, 3.5 ml 0.1 M phosphate buffer (pH 6.5) and 0.2 ml of 0.1% Methanolic solutions of O-dianisidine were incubated in a water bath at 28 °C for 10 min. Then to the reaction mixture, 0.2 ml of 0.2 M hydrogen peroxide was added and the optical density (OD) was recorded at 530 nm in 1 min intervals up to 10 min with UV-Vis spectrophotometer (Spekol 2000, Analytik Jena, Germany). The enzyme activities were expressed in terms of an average increment in absorbance per minute per gram fresh weight (Δ O.D / min / g fwt).

Catalase Enzyme Assay

The enzyme extract was prepared by grinding 0.5 gm. of fresh wild edible fruit sample in 5ml of phosphate buffer (pH 7) in pre-cooled mortar and pestle and centrifuged at 4000 rpm at 4°C for 15 minutes (Eppendorf cooling Centrifuge, 5430 R). The supernatant was collected in eppendorf and stored at 4°C for Catalase enzyme assay which was done following the method of [21]. 3 ml of 50m M phosphate buffer (pH 7) was taken in clean test tube.0.1 ml enzyme extract and 0.4 ml of 30% H_2O_2 was added to the test tube. The optical density (OD) was measured at 240 nm in 1 min interval up to 4min in UV-Vis spectrophotometer (Spekol 2000, Analytik Jena, Germany). The average decrease per min of the sample was calculated and activity can be calculated by using the following formula:

Volume activity (U/ ml) = $(\Delta As - \Delta Ao) \times 3$ ml × dilution factor/0.0436×2 ml = $\Delta A \times 3.4 \times$ dilution factor.

Superoxide Dismutase Enzyme Assay

The enzyme extract was prepared by grinding 0.2 g of plant fruit sample with the help of pre chilled mortar and pestle by adding 5 ml of phosphate buffer (pH 7.8). The crushed material was then centrifuged for 30 min at 7500 rpm at 4°C (Eppendorf cold Centrifuge, 5430 R). Supernatant was collected for SOD assay. The SOD activity was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) according to the method [22] with some modification by [23]. The reaction mixture (3 mL) contains 0.1M potassium phosphate buffer (pH 7.8)13 mM methionine, 75 µM NBT, 0.1 mM EDTA, 100µM riboflavin and 0.1 mL of enzyme extract. Samples were illuminated using 40W fluorescent lamps for 10 min. The absorbance of reaction mixture was recorded at 560 nm using UV-Vis spectrophotometer (Spekol 2000, Analytik Jena, Germany). A non-irradiated reaction mixture was served as control. Identical solutions that were not illuminated served as blanks.

RESULTS AND DISCUSSION

Non-enzymatic assay for antioxidant activity

Carotenoid content

Carotenoids are precursors of Vitamin-A from vegetable source. The compound present in fruits is mainly β carotene and its trans and cis isomeric forms. We have studied the total carotenoid content of 5 wild edible fruits which has been noted in table 3. Of the 5 frruits, *Syzygium cerasoides* contains highest level of total carotenoids i.e 18.51±0.35 mg/100g and next higher content was noted in *Facourtia indica* i.e 9.18±0.18 mg/100g, *Diospyros melanoxylon* contained 6.33±0.16 mg/100g.The other 2 fruits studied here exhibited lower level of total carotenoid content i.e *Annona squamosa* 1.74±0.23 mg/100g and *Citrus medica* 0.50±0.45 mg/100g which appears to be

negligible. Another study of total carotenoid content was done by [24] on *Syzygium cumini* belonging to the same species as *Syzygium cerasoides* of our study. He found 89.2±5.4µg/100g and our finding on *Syzygium cerasoides* is18.51±0.35mg/100g.In our study on total carotenoid level in *Diospyros melanoxylon* we found 6.33±0.16mg/100g. In a study reported by [25] β-carotein was found to be 22.0±1.0. Our study on *Citrus medica* in fresh pulp stage as regards its carotenoid content exhibited 0.50±0.45mg/100g. A study of mandarin (*Citrus reticulata*) by [26] exhibited β carotene level to be 75.14±0.79mg/100g dw. It appears from analysis and findings on carotenoid content that the content % varies according to their occurrence in different geographical regions, average temperature, climatic conditions, rain, sunshine exposure and stages of maturation in addition to use of quality manures. It was also found that the content varies depending upon the part and stage of study of the fruit. The fresh pulp appears to contain higher level of carotenoid as against its dried wt. stage.

Table2. Total Carotenoid and Phenol content content of 5 ethno medicinally important wild edible fruits.

Name of fruit sample	Carotenoid (mg/100g)	Phenol (% fwt.)
Annona squamosa	1.74±0.23	0.18±0.01
Citrus medica	0.50±0.45	0.31±0.02
Diospyros melanoxylon	6.33±0.16	1.6±0.05
Facourtia indica	9.18±0.18	0.40±0.02
Syzygium cerasoides	18.51±0.35	0.46±0.06

Values expressed as mean±standard deviation (from 3 determinants)

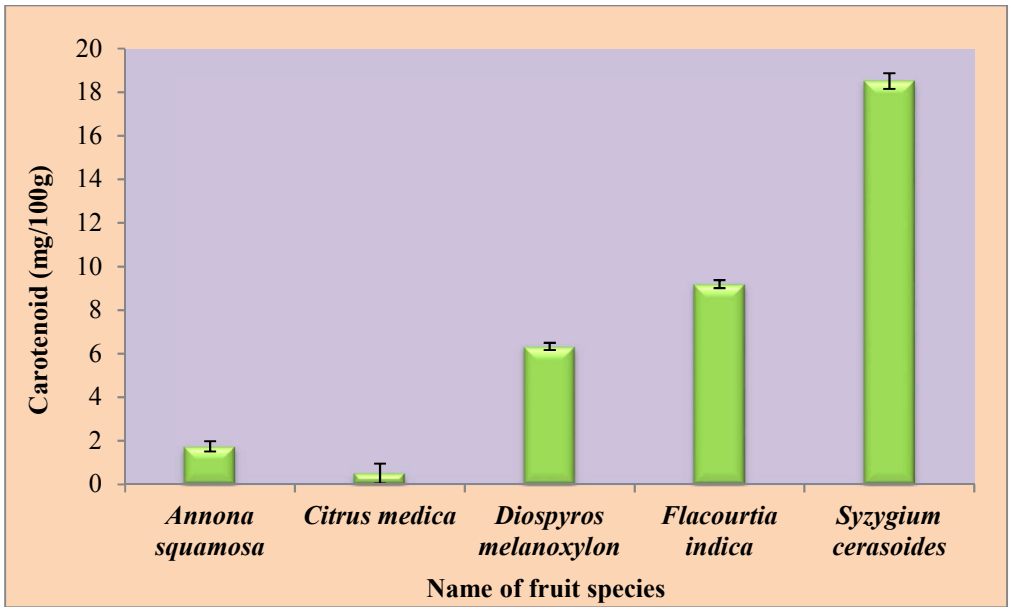

Figure 1: Carotenoid assay of 5 ethno medicinally important wild edible fruits of Odisha.

Total Phenol content

The phenol content of *Annona squamosa, Citrus medica, Diospyros melanoxylon, Facourtia indica* and *Syzygium cerasoides* were studied in fresh pulp stage and findings noted. It was found that *Diospyros melanoxylon* contained the maximum phenolic compound 1.6±0.05g/100g, *Annona squamosa* found to have minimum phenolic content i.e 0.18±0.01g/100g. Another study of phenol content by [27] shows phenolic content i.e. 183.01mg GAE/100g fresh mass. Phenol content of *Diospyros melanoxylon* to be 1.72±0.64g/100g. This finding is almost close to our findings as regards to phenol content. The phenol content in *Annona squamosa* as per our findings is 0.18±0.01g/100g. The phenol content of *Syzygium cerasoides* is found to be 0.46±0.06g/100g as against the parallel finding of [28] is 0.8. The phenol content of *Facourtia indica* was found to be 0.40±0.02g/100g as against findings by [29] to be 3.87±0.28mg/GAE/gm. The *Citrus medica* exhibited its phenol content to be 0.31±0.02g/100g as against similar species study by [30] in *Citrus lemon* to be 600µg/GAE/ml. except the unit of expression, the level of phenol content appears to be close to each other findings.

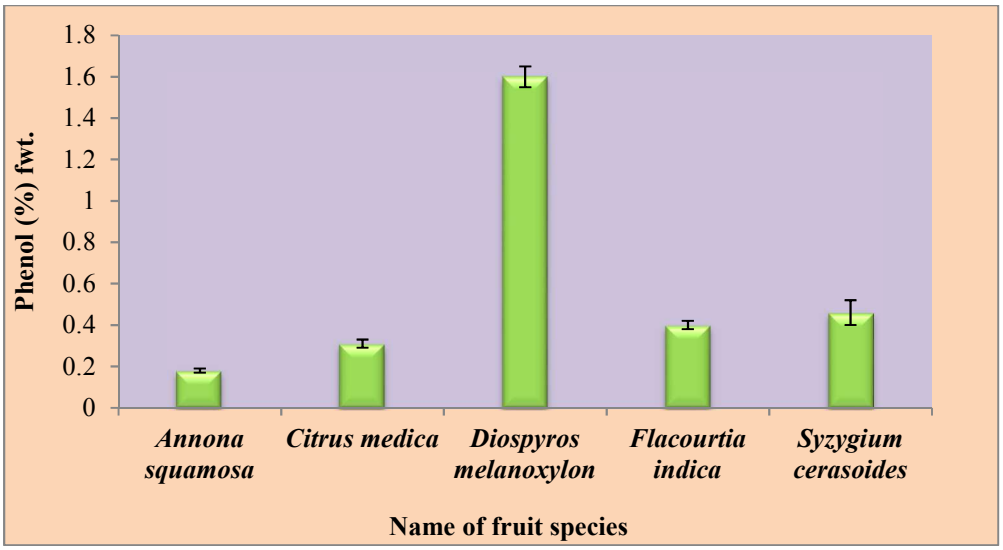

Figure 2: Phenol content in 5 ethno medicinally important wild edible fruits of Odisha.

DPPH radical scavenging assay

On study and analysis it was observed that *Annona squamosa* exhibited maximum percentage of scavenging activity i.e 114.16±2.21 mg AEAC /100g dry wt. and *Citrus medica* exhibited minimum DPPH activity i.e 10.45±4.29 mg AEAC /100g dry wt. *Diospyros melanoxylon*, *Flacourtia indica* and *Syzygium cerasoides* exhibited DPPH activity ranging from 62.71±2.71 mg AEAC /100g dry wt. to 86.76±0.06 mg AEAC /100g dry wt. shown in table 2. According to [31] the *Annona squamosa* fruit exhibited DPPH activity to be 97.77% compared to our findings 114.16±2.21 mg AEAC /100g dry wt. The difference may be due to climatic variation, sunlight exposure, method of harvest and storage in addition to use of quality manures. The DPPH activity of *Diospyros melanoxylon* must was studied by [32] and found 72.5±2.80% compared to the DPPH activity of *Diospyros melanoxylon* pulp studied by us to have 86.76±0.06 mg AEAC /100g dry wt. The status of must and pulp of the studied fruits has marginal difference both being the stage before addition of fermenting organisms for preparation of wine. The difference of DPPH activity appears to be very close to each other depending upon the climate of cultivation, rain, sunshine and manures used. The fruit *Flacourtia indica* and *Syzygium cerasoides* exhibited DPPH activity studied by the same method to be 80.94±0.41 mg AEAC /100g dry wt. and 62.71±2.71 mg AEAC /100g dry wt. compared to the findings of [29] in respect to *Flacourtia indica* 59.78% inhibition and *Syzygium cumini i* 92.06% inhibition.

Table3. Estimation of DPPH and FRAP assay of 5 ethno medicinally important wild edible fruits

Name of fruit sample	DPPH (mg AEAC/100g dry wt.)	FRAP (µM AEAC /g dry wt.)
Annona squamosa	114.16±2.21	169.11±1.70
Citrus medica	10.45±4.29	320.33±4.94
Diospyros melanoxylon	86.76±0.06	263.25±4.12
Facourtia indica	80.94±0.41	280.77±4.18
Syzygium cerasoides	62.71±2.71	172.56±7.59

Values expressed as mean±standard deviation (from 3 determinants)

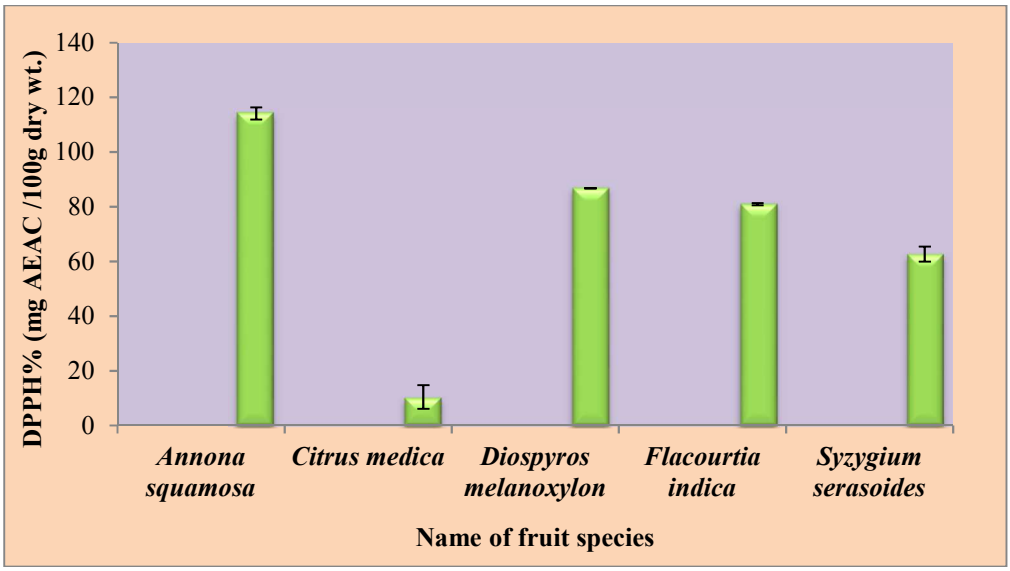

Figure 3: DPPH radical scavenging activity of 5wild edible fruits.

Ferric reducing/anti-oxidant power (FRAP) assay

Study of antioxidant scavenging activity by FRAP method exhibited the % of antiscavenging activity to be maximum in *Citrus medica* 320.33±4.94 µM AEAC /g dry wt. and followed by *Facourtia indica, Diospyros melanoxylon* , *Syzygium cerasoides* and *Annona squamosa* 169.11±1.70 µM AEAC /g dry wt. In a parallel study of dried pulp extract of *Annona squamosa* exhibited anti scavenging activity by FRAP method to be 45.58 µg BHT 100 mg^{-1} [33].This infers that the pulp has more antioxidant potential than its dried form. *Facourtia indica* and *Syzygium cumini* were studied by FRAP method for anti scavenging activity and we found 280.77±4.18 µM AEAC /g dry wt., 172.56±7.59 µM AEAC /g dry wt. respectively. In a study by [29] exhibited *Facourtia indica* 0.64±0.01 mmol $FeSO_4$/g and *Syzygium cumini* 1.91± 0.01mmol $FeSO_4$/g. *Citrus lemon* having same species as *citrus medica* was parallelly studied by [30] and her finding was 0.58 compared to our study on *citrus medica* of the same species as *Citrus lemon* by FRAP method and our findings exhibited 320.33±4.94 µM AEAC /g dry wt.

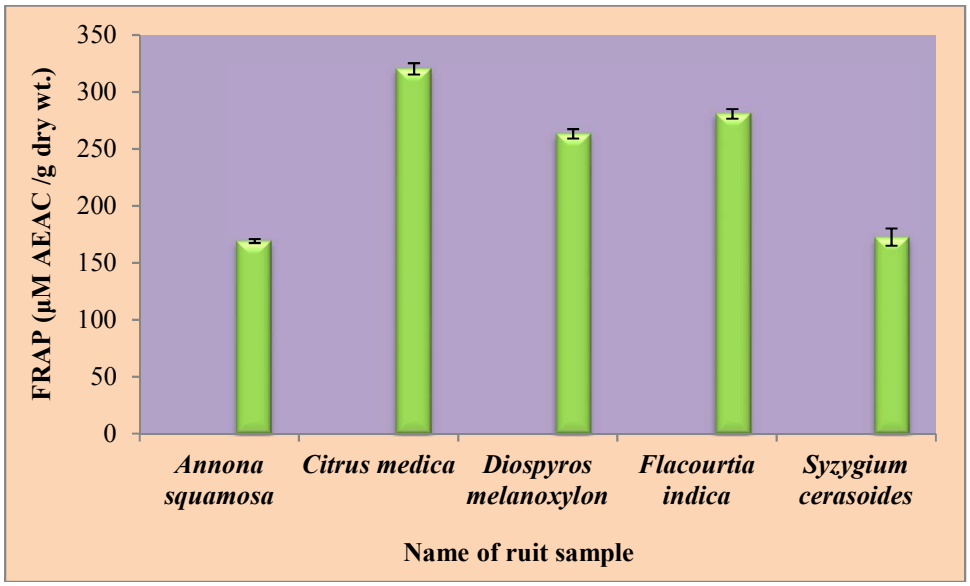

Figure 4: Ferric reducing anti-oxidant power of 5 wild fruits

Enzymatic assay for antioxidant activity in some wild edible fruits of Odisha

Peroxidase assay

Peroxidase activity of 5 wild edible fruits were analyzed to study the potential of prevention of oxidation of neighboring tissue by nitrogen free radicals those ultimate damage the cells. It was found that *Annona squamosa* exhibited maximum peroxidase activity against N free radicals i.e. 0.0098±0.0017 Δ O.D / min / g fwt. and *Syzygium cerasoides* exhibited minimum peroxidase activity out of 5 fruits i.e. 0.0017±0.0003Δ O.D / min / g fwt. In a reported that the same species named *Syzygium cumini* revealed peroxidase activity level $0.0081OD^{-1}min^{-1}gm^{-1}$tissue wt.[33].The peroxidase activity of *Citrus medica*, *Diospyros melanoxylon*, *Facourtia indica* showed 0.003±0.0001Δ O.D / min / g fwt., 0.0062±0.001 Δ O.D / min / g fwt., 0.0038±0.0011Δ O.D / min / g fwt respectively. Among 5 wild edible fruits, the peroxidase activity that acts ultimately as an antioxidant was sufficiently present in *Annona squamosa*.

Table4. Antioxidant analysis of 5 wild edible fruits

Name of fruit species	POX ΔO.D/min/g fwt.	CAT (U/ml)	SOD ΔO.D/min/g tissue wt
Annona squamosa	0.0098±0.0017	0.0236±0.0021	0.28±0.0028
Citrus medica	0.0030±0.0001	0.0137±0.0017	1.24±0.0092
Diospyros melanoxylon	0.0062±0.0011	0.0013±0.0007	7.88±0.7600
Facourtia indica	0.0038±0.0011	0.0010±0.0001	0.30±0.1800
Syzygium cerasoides	0.0017±0.0003	0.0124±0.0023	0.45±0.2900

Values expressed as mean±standard deviation (from 3 determinants)

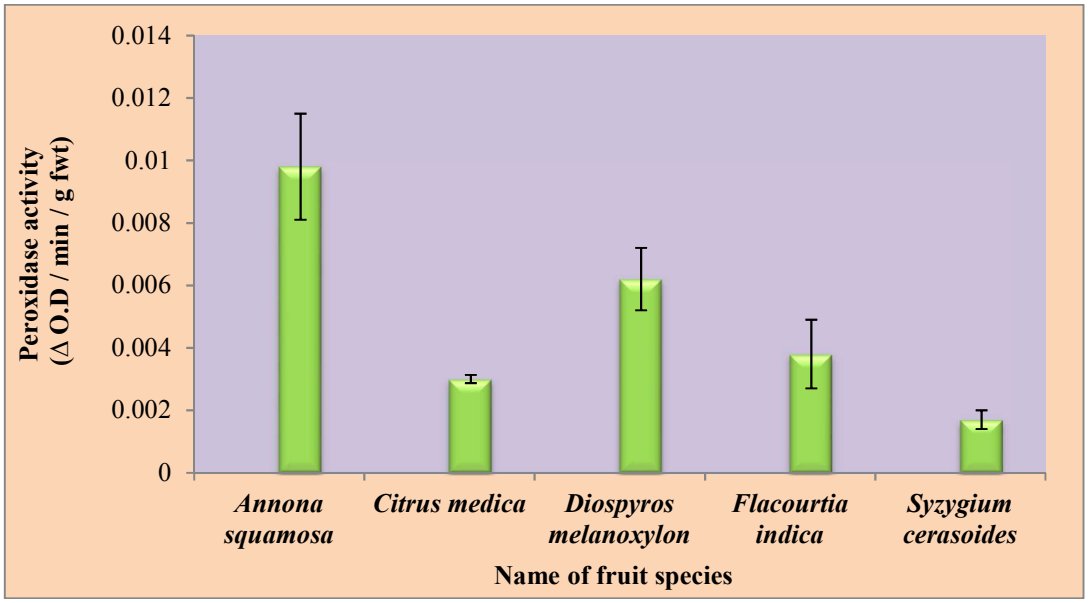

Figure 5: Peroxidase activity of 5 ethno medicinally important wild edible fruits of Odisha.

Catalase assay

Catalase is an enzyme present in all living organisms (plant, bacteria and animals.).This is a miraculous enzyme that catalyses the breakdown of hydrogen peroxide produced in the degenerating and decomposed cells during the process of our metabolism. The deleterious effect of H_2O_2 oxidise the neighboring cells and cause irrecoverable damage. The catalase breaks down H_2O_2 to H_2O and oxygen both being harmless and non damaging to our tissues. There by the catalase enzyme protects our cell and is an excellent antioxidant. On analysis and study of catalase activity of wild edible fruits, we found encouraging quantity of catalase activity. It was found that *Annona squamosa* exhibited maximum activity potential i.e 0.0236±0.002U/ml and *Citrus medica* 0.0137±0.0017 U/ml, *Syzygium cerasoides* 0.0124±0.0023 U/ml, *Diospyros melanoxylon* 0.0013±0.0007 U/ml showed catalase activity in descending order. *Facourtia indica* 0.001±0.0001 U/ml exhibited minimum catalase activity out of 5 studied fruit species. An another study was done by [33] reported that the same species named *Syzygium cumini* obtained catalase activity level 3.67×10^4 (I.E.U.) in 1gm fresh wt. tissue**.**

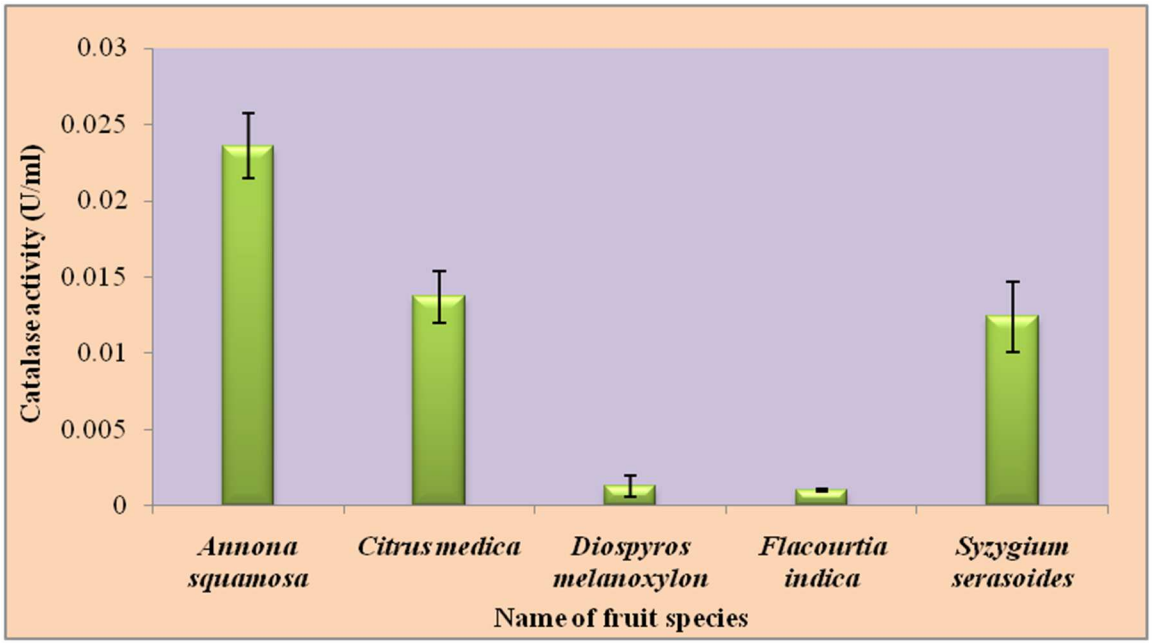

Figure 6: Catalase assay of 5 ethno medicinally important wild edible fruits of Odisha.

Superoxide Dismutase assay

SOD activity of wild fruits was studied and the result has been noted. On analysis it was found that these fruits exhibited various levels of SOD activity out of which *Diospyros melanoxylon* exhibited maximum SOD activity i.e 7.88±0.70 Δ O.D / min / g tissue wt. and other exhibited less SOD activity noted in descending order *Annona squamosa* 0.28±0.0028 Δ O.D / min / g tissue wt., *Citrus medica*1.24±0.0092 Δ O.D / min / g tissue wt., *Syzygium cerasoides* 0.45±0.29Δ O.D / min / g tissue wt., *Facourtia indica* 0.30±0.18Δ O.D / min / g tissue wt. Superoxidese are produced in our cells during the process of metabolism. The same species of *Syzygium cerasoides* named *Syzygium cumini* the SOD activity was found to be 0.0047 Δ O.D / min / g tissue wt [33]. These superoxides oxidise and damage our cells. Certain fruits provide enzyme named SOD that prevents the superoxide to damage tissue. Either they convert it to free O_2 or to H_2O_2 which is less harmful and further reacted by peroxidase and catalase to be converted to harmless form i.e H_2O and O_2. Hence SOD enzymes available from fruits protect our cells as an anti-oxidant. Further studies of other fruits may provide additional information to protect our health from being damaged untimely.

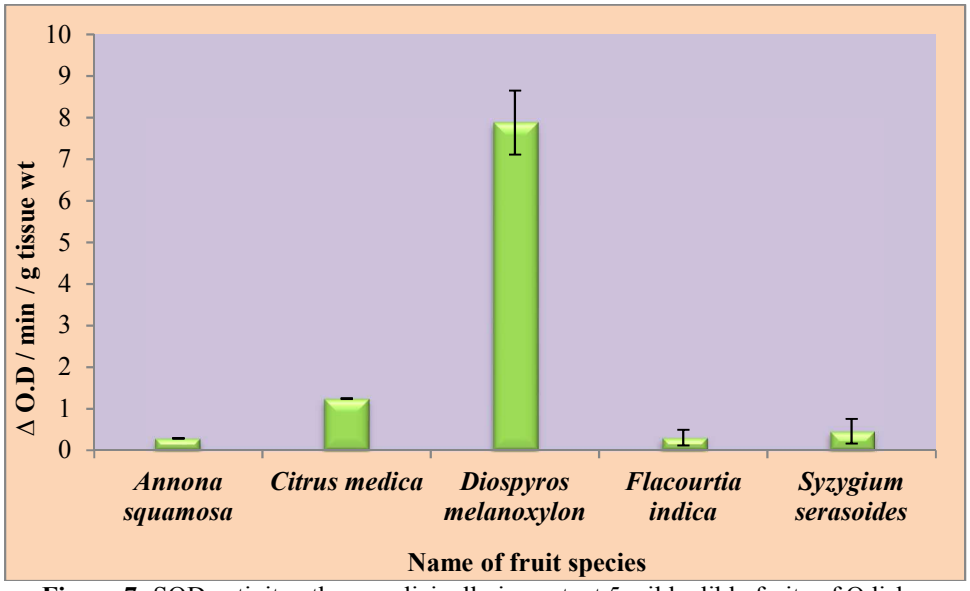

Figure 7: SOD activity ethno medicinally important 5 wild edible fruits of Odisha.

Conclusion

The study of antioxidant activity of 5 wild edible fruits showed that the biochemicals present in these fruits impart a significant effect on our health by protecting our cells from being damaged and destroyed. Out of 5 fruits studied, *Annona squamosa* and *Citrus medica* exhibited maximum antioxidant activity through DPPH free radical scavenging and FRAP activity. For asessment of antioxidant activity, phenol carotenoid content was evaluated. Maximum phenol content was found in *Diospyros melonoxylon* and carotenoid level in *Syzygium cerasoides*. In study of enzymatic assays for quantitization of antioxidant properties in the selected fruits, high peroxidase and catalase activity was detected in *Annona squamosa*. Superoxide dismutase enzyme activity (SOD) was pronouncibly seen in *Diospyros melonoxylon.* Amongst all the selected wild edible fruits, *Annona squamosa* and *Diospyros melanoxylon* exhibited higher potency in terms of their antioxidant property. From the research finally it could be opined that these two wild edible fruit plants should be mass multiplied for large scale cultivation, so that through utilization of these fruits one can provide excellent result as any other exotic marketed fruit, particularly for economically poor section of people of our society.

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