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DPPH Free Radical Scavenging Activity of Some Leafy Vegetables used by Tribals of Odisha, India

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The objective of the present investigation was to conduct phytochemical screening of phenolic content and antioxidant activity of six leafy vegetables used by tribals of Koraput district in odisha in different extracts. The results revealed that *Olax psittacorum* has the highest antioxidant activity which is very close to *leucas aspera*. Other four leafy vegetables like *Ipomoea Cairica*, *Commelina benghalensis*, *Cassia tora* and *Bauhinia purpurea* are also equally potent. The results show that the ethanolic extract possesses more antioxidant activity than methanol and hexane extract. The phytochemical analysis of leafy vegetables indicates the presence of phenolic contents in different degrees and the positive correlation between antioxidant activity and total polyphenols (r=0.993) suggests that phenolic compounds are the major antioxidant components in the leafy vegetables. The results indicate that rich phenolics of the leafy vegetables and its high antioxidant activity may be responsible for its wide use in the diet of tribals and may provide a source of dietary antioxidants.

Keyword: DPPH, leafy vegetables, $IC_{50,}$ total phenolics, tribals

1. Introduction

Tribals in odisha are generally residing in the hilly and remote areas surrounded by natural forest resources. Besides the crops grown under settled and shifting cultivations these tribals depend on the phytodiversity to supplement their diet. They mainly depend on the available wild fruits and the wild produces of forest since time immemorial as they are the conventional food items[1,2]. These wild fruits, leaves, tubers and other forest produces make them resistant to different diseases and also enable them to develop stamina to work hard in the field. Therefore the green leafy vegetables or the wild leaves constitute an important source of diet of the aboriginal tribals for sustaining their livelihood.

Green leafy vegetables (GLVs) are a good source of minerals and vitamins. Ethono-botanical reports offer information on medicinal properties of GlVs like anti-diabetic[3], anticarcinogenic[4] ,hypolipidemic[5] and antibacterial activity[6]. In most of the studies, crude extracts of GLVs were used to demonstrate their health beneficial potency. They are particularly rich in antioxidants and contain varying amount of phytochemicals like vitamin C, flavonoids and carotenoids. The leafy vegetables are chlorophyll rich has been proven to help build red blood cells and help to decrease the risk of heart disease, stroke, and certain cancers[7].

Antioxidants which are immensely present in GLVs work by significantly slowing or preventing the oxidative or damage from oxygen process caused by free radicals such as superoxide radical ($O_2^{\cdot}$), Hydroxyl radical (OH) and non-free radical species such as H_2O_2 and singlet oxygen ($^1O_2$) is associated with cellular and metabolic injury ,accelerating aging, cancer, cardio-vascular diseases, neurodegenerative diseases and Inflammation[8,9,10].

Studies to date have demonstrated that phytochemicals in common fruits and vegetables can have complementary and over lapping mechanisms of action, including scavenging of oxidative agents, stimulation of the immune system, hormone metabolism and antibacterial and antiviral effects[11]. Many research works have also been done for antioxidant activity of leafy vegetables[12], antioxidant activity of selected leafy vegetables of odisha[13], antioxidant activity of *Leucas aspera*[14], *Ipomoca cairica*[15], *Bauhinia purpurea*[16] , *Cassia tora*[17] but these studies are not done in the tribal locality of Odisha which differ widely in environmental factors and where these leafy vegetables constitute a major part of the daily diet of tribals and also have been a part of ethno-medicine used in the treatment of various diseases (Table -1). Therefore the objectives of the present study involves the free radical scavenging activity of some leafy vegetables widely used by tribals and also to determine the level of correlation between the phenolic contents and antioxidant activity by using statistical analysis.

2. Materials and Methods:

2.1 Plant Materials: Leafy vegetables were collected from Koraput district of Odisha. The plant specimens were further authenticated at the Regional plant Resource Center located at Bhubaneswar. The leaves were washed, shade dried and then milled into coarse powder by wind mill. Six plant materials, which were tested for their antioxidant activity were *Olax psittacorum* (oleaceae), *Bauhinia purpurea* (caesalpinaceae), *Ipomoea cairica* (convolvulaceae), *Cassia tora* (caesalpinaceae), *Commelina benghalensis* (commelinaceae), *Leucas aspera* (lamiaceae).

Table 1: Botanical Information, Traditional uses and References of the Studied Leafy Vegetables

Botanical Name with Family	Traditional Uses	References
Olax psittacorum(Oleaceae)	Used to cure Headache	K Jeyaprakash *et al*, 2011[18]
Bauhinia purpurea (caesalpinaceae)	Goiter, worm infection, Cuts & Wounds	R.K Sahu *et al*, 2011[2]
Cassia tora(Caesalpinaceae)	Ringworm, skin diseases, Indigestion & stomach complaints	Santosh Shukla *et al*, 2013[19]
Leucas aspera(Lamiaceae)	Ear ache, sore, snake bite and jaundice	R.K Sahu *et al*, 2011[2]
Ipomoea cairica (Convolvulaceae)	Treatment of rheumatism and inflammations	Ferreira A *et al*, 2006[20]
Commelina Benghalensis (Commelinaceae)	Suppurative sores, snake bite, swelling, burns	Ghani, 2003[21]

2.2 Preparation of the Plant Extract: The powdered plant material was weighed and extracted with solvents like Methanol, and Ethanol and Hexane using soxhlet apparatus for 48 hours. The solvent was then removed under reduced pressure by using rotary evaporator, which obtained a greenish-black coloured sticky residue .The remaining residue was stored in dessicators for further use.

2.3 DPPH Radical Scavenging Assay: The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams *et al*[22] with slight modifications. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of plant extract solution of varying concentrations (50, 100, 150, 200 and 250 µg/ml). Corresponding blank sample were prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard. Mixer of 1ml methanol and 1ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula.

$$\text{Inhibition \%} = \text{Ac-As/Ac} \times 100$$

Where Ac is the absorbance of the control
As is the absorbance of the sample

2.4 Determination of Total Phenol Content: Phenol was determined by folin-Ciocalteau reagent in alkaline medium and was expressed in terms of catechol used as standard[23] in µg/ml.

3. Results and Discussions:
DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extract. The Table 2, 3, and 4 shows the antioxidant activities of the ethanol, methanol and hexane extracts of six green leafy vegetables (GLVs) assessed using the DPPH radical scavenging. 50- 250 µg/ml of methanol extracts produced moderate to high DPPH scavenging activity in all the six experimental plants. The highest DPPH scavenging activity was observed in *Olax psittacorum* (91.14 %) followed by *Ipomoea cairica* (83.49 %), *Leucas aspera* (83.25 %), *Commelina benghalensis* (81.4 %),*Bauhinia purpurea* (77.2 %), and *Cassia tora* (63.84%). Similarly a ethanolic extract produced moderate to high DPPH radical scavenging activity in six GLVs. The highest scavenging activity was observed in *Olax psittacorum* (97.81 %) followed by *Leucas aspera* (96.2 %), *Ipomoea cairica* (88.21 %), *Commelina benghalensis* (86.47 %), *Bauhinia purpurea*(79.25 %), and *Cassia tora* (83.76 %). However low to moderate DPPH scavenging activity was found in hexane extract and the highest scavenging was observed in *Commelina benghalensis* (78.62 %), *Olax psittacorum* (77.4 %), *Cassia tora* (76.82%), *Leucas aspera* (76.18 %), *Bauhinia purpurea*(71.97%), *Ipomoea cairica* (61.92%).

Generally, the antioxidant properties of these extracts were found to be concentration dependent. Based on the results obtained, the ethanol and methanol extracts which are more polar solvent extracts, were more effective antioxidants compared to the non polar hexane extract in DPPH assay[16].

Table 2: DPPH scavenging activities of leafy vegetables in Methanolic extracts (values represent means±.S.D,n=3)

Conc.of extracts (µg/ml)	AA	OP	BP	CT	IC	CB	LA
50	87.85±.05	84.82±.16	68.89± .28	41.72± .16	68.66±.13	75.93±.23	77.15± .23
100	89.72±.16*	87.43± .15*	69.97± .23**	48.42± .16*	70.62±.06*	77.72±.15*	79.0± .08*
150	91.24±.04*	89.13± .05*	71.7± .3*	58.9± .21	72.32±.24*	78.74±.09*	81.1± .15*
200	93.4±.12*	90.43± .05*	73.97± .46*	60.48± .16	75.09±.29*	80.13±.08*	82.28±.05*
250	96.43±.1*	91.14± .16*	77.2±.46*	63.84± .16	83.49±.4.5**	81.4±.15*	83.25±.15*

Table 3: DPPH scavenging activities of leafy vegetables in Ethanolic extracts(values represent means±SD,n=3)

Conc.of extracts (µg/ml)	AA	OP	BP	CT	IC	CB	LA
50	87.85±.05	90.68±.08	69.47±.24	73.25±.21	79.79±.23	77.36±.31	85.61±.1
100	89.72±.16*	92.95±.07*	73.2±.34*	76.29±.21*	82.75±.06*	78.59±.08**	88.93±.06*
150	91.24±.04*	95.07±.89*	75.04±.92*	78.33±.15*	85.09±.11*	79.92±.23**	91.56±.11*
200	93.4±.12*	96.84±.65*	77.55±.63*	81.19±.16*	87.02±.13*	84.53±.23**	93.65±.12
250	96.43±.1*	97.81±.07*	79.25±.24	83.76±.16*	88.21±.11*	86.47±.15**	96.2±.96*

Table 4: DPPH scavenging activities of leafy vegetables in Hexane extract(values represent means±SD,n=3)

Conc.of extracts (µg/ml)	AA	OP	BP	CT	IC	CB	LA
50	87.85±.05	72.12±.24	66.31±.35	56.36±.15	53.65±.06	70.18±.11	60.8± .16
100	89.72±.16*	73.52±.46**	67.7±.24**	57.35±.11*	54.96±.12*	72.28±.06**	65.07±.1
150	91.24±.04*	75.05±±.41*	69.35±.35*	59.53±.4*	57.07±.17*	73.83±.06**	70.68±.1
200	93.4±.12*	76.05±.17*	70.93±.35*	62.78±.12	59.04±.06*	77.35±.13*	74.74±.4*
250	96.43±.1*	77.4±.36*	71.97±.24*	76.82±.11	61.92±.06*	78.62±.12**	76.18±.45*

* Significant at 1% level ** Significant at 5% level

AA-Ascorbic Acid, OP-*Olax psittacorum*, BP-*Bauhinia purpurea*, CT-*Cassia tora*, IC-*Ipomoea cairica*, CB-*Commelina benghalensis*, LA-*Leucas aspera*.

Fig.-1 and Table-5 shows the comparative data of DPPH radical scavenging activity as determined by the IC_{50} values of the different leafy vegetables. An IC_{50} value is the concetration of the sample required to scavenge 50% of the free radicals present in the system. IC_{50} value is inversely related to the antioxidant activity of crude extracts. Lowest IC_{50} value and highest activity was found in etanolic extracts of *Olax psittacorum* followed by *Leucas aspera, Ipomoea cairica, Commelina benghalensis, Cassia tora* , *Bauhinia purpurea* as compared to ascorbic acid.

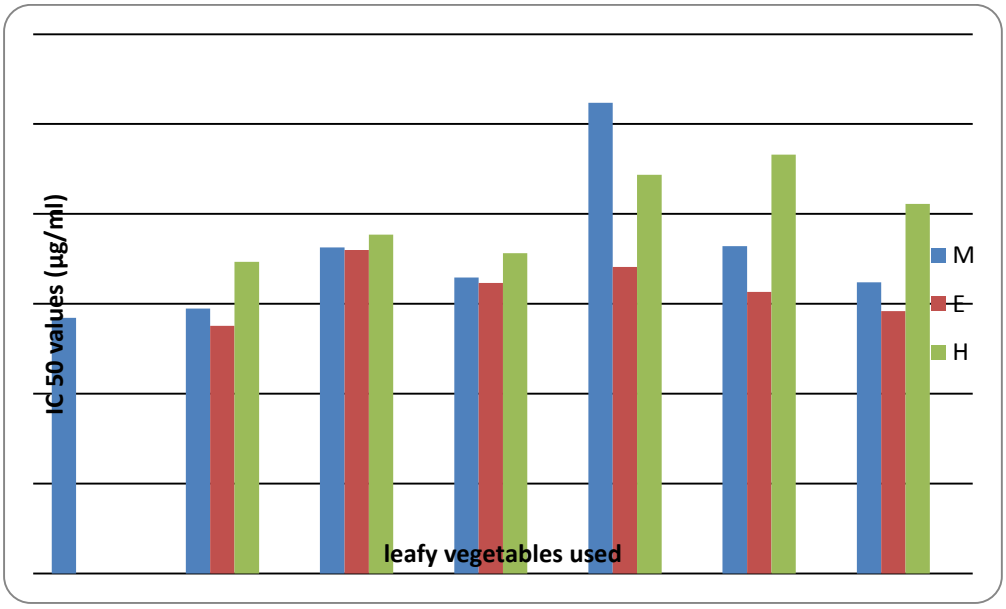

Fig 1: Shows IC_{50} values of six leafy vegetables in different extracts

The total phenolic content of the ethanol, methanol, and hexane extract were also determined. In all the six leafy vegetables ethanolic extract was found to content the highest phenolic content in comparison to methanol and hexane (Table-5).

Table 5: Phenol content and IC_{50}values of leafy vegetables in different solvents

Plants Name	Solvent Used	Phenol content (µg/ml)	IC_{50} Values (µg/ml)
Olax psittacorum	Methanol	175	29.47
	Ethanol	215.28	27.56
	Hexane	141.31	34.66
Bauhinia purpurea	Methanol	17.94	36.28
	Ethanol	122.82	35.98
	Hexane	27.6	37.70
Cassia tora	Methanol	136.62	52.38
	Ethanol	147.66	34.12
	Hexane	31.74	44.35
Ipomoea cairica	Methanol	144.9	36.41
	Ethanol	168.36	31.33
	Hexane	108.74	46.59
Commelina benghalensis	Methanol	123.09	32.92
	Ethanol	175.53	32.31
	Hexane	73.96	35.62
Leucas aspera	Methanol	167	32.40
	Ethanol	193.2	29.20
	Hexane	131.1	41.11

Studies on medicinal plants/herbs with high phenolic contents have gained importance over the past few years due to the high antioxidant[16], anti-inflammatory[24] and anti-carcinogenic activities [16] and are of great value in decreasing the risk of many human diseases.

The antioxidative activities observed can be attributed to either the different mechanisms exhibited by different polyphenolic compounds that is, tocopherols, flavonoids and other organic acids and to the synergistic effects of different compounds. Many studies have shown that many polyphenols contribute significantly to the antioxidant activity[25,26] and act as highly effective free radical scavengers which is mainly due to their redox properties, which can play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides[27].

There are no report on antioxidant activity of *Olax psittacorum* and phenolic content of *commelina benghalensis* earlier. Leucas aspera of Assam district in India exhibit antioxidant property with IC_{50} value of 54.78 µg/ml with a phenol content of 45.30 mg / g dry extract[28]. Iron chelating activity and total antioxidant activity has been investigated in *Bauhinia purpurea*[29] but the DPPH free radical scavenging has not been worked out. DPPH scavenging activity of *Cassia tora* was evaluated at 20-80 µg/ml and the highest as found to be 71.18 %[17]. The phytochemical and antioxidant activity of *Ipomoea cairica* was experimented with DPPH scavenging activity with 82.58 % at 500 µg/ml[15] .Several comprehensive works have been done on the effects of phenolic compounds and antioxidant activity of *leucas aspera, Ipomoea cairica, Bauhinia purpurea, Cassia tora* as discussed above. Same trend was obtained in our study also. A significant correlation was observed between phenolic content and the scavenging of DPPH radical in all the leafy vegetables (fig- 2) ($r = 0.993$, $p < 0.5$) indicate that the radical scavenging capacity of each extract might be related to their concentration of phenolic hydroxyl groups.

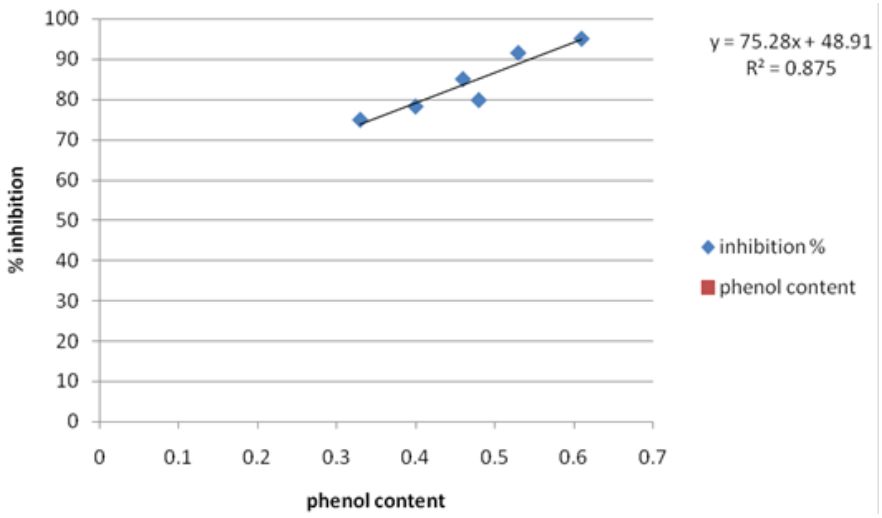

Fig 5: It Shows Correlation between antioxidant activity and total phenol content in leaves of six leafy vegetables, r=0.993

Our present work reveals that the leaves of four plants used by the tribals of Koraput district in Odisha possess good antioxidant potential in comparison to the plant from other places like Assam, Bangladesh, Tamilnadu and Andhra Pradesh presumably because of its phytochemical constituents. Based on the results of this study it can be concluded that the leafy vegetables are good source of food and medicine to treat and control many diseases. These findings could also be of commercial interest to both Pharmaceutical companies and research institutes in the production of new drugs.

4. Conclusion:

From the study it is concluded that the antioxidant capacities, total phonolic content of the six leafy vegetables which are widely used by the tribals of Odisha (India) are considered as good sources of antioxidants as observed in DPPH scavenging assay. Among all Olax *psittacorum* has the highest antioxidant activity which is very close to *leucas aspera*. Other four leafy vegetables like *Ipomoea Cairica*, *Commelina benghalensis*, *Cassia tora* and *Bauhinia purpurea* are also equally potent proves its wide use as food in the daily diet of tribals with nutritional and therapeutic value can be used as an accessible source of natural antioxidants with consequent health benefits particularly *Olax psittacorum* and *leucas aspera* can be consider as a model herbal drug for experimental studies including free radical induced disorders like cancer, diabetics aging and cardiovascular diseases.

The present finding partially validates the traditional knowledge of the tribals of Odisha about the goodness of consumption of these plants required more research works.

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