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ANTIOXIDANT ACTIVITIES OF LEAF EXTRACTS OF SOME COMMON BETEL VARIETIES (*PIPER BETLE* L.) AVAILABLE IN ODISHA

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Antioxidant activity, Betel, Betel variety of Odisha, Reactive oxygen, Scavenging activity

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ABSTRACT: Natural antioxidants play an important role in the prevention of many age-related diseases and promotion of health. Among natural antioxidants from plants, flavonoids and other phenolic compounds are potent antioxidants and chelating agents. Betel (*Piper betle*) is the leaf of a vine belonging to the family Piperaceae, which also includes pepper and kava. It is valued both as mild stimulant and appetiser. Betel leaf is mostly consumed in Asia, and elsewhere in the world by some Asian emigrants, with or without tobacco, the later is an addictive psychostimulating and euphoria-inducing formulation with adverse health effects. Betel leaf is said to have antioxidant properties. No reports are available on the antioxidant potential of the leaf extracts of common betel verities chewed in Odisha. Keeping this in view, the antioxidant activities of leaf extracts of five selected betel varieties (Vishnupuri, Desawari, Ghajipur, Desipaan and Jaleswar) were carried out. Results revealed that the leaves have sound ROS scavenging activity. Present papers highlights the antioxidant and medicinal importances of common betel varieties, the traditional edible leaf of Odisha.

INTRODUCTION: Antioxidants provide protection against degenerative diseases including cancer, coronary heart, and Alzheimer's diseases. Reactive Oxygen Species (ROS), contribute to cellular aging, mutagenesis, carcinogenesis, and coronary heart disease, likely through destabilization of membranes, DNA and protein damage and oxidation of low-density lipoprotein (LDL). Furthermore, antioxidants scavenge reactive species, and upregulate antioxidant defences. Plants are rich sources of natural antioxidants, the best known are tocopherols, carotenoids, Vitamin C, flavonoids, and different other phenolic compounds. Recently, among natural antioxidants, flavonoids have received increasing attention [1-2].

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As compared with Vitamin C and E, dietary flavonoids are considered to be more powerful antioxidants. Flavonoids are known to be highly effective antioxidants playing the role by scavenging oxygen radicals. They also posses interesting anti-cancer, hypolipidemic, anti-ageing, and anti-inflammatory activities. Moreover, the protective effects of flavonoids in biological systems are attributed to their capacity to scavenge free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce alpha-tocopherol radicals, and inhibit oxidases.

Furthermore, phenolic compounds have phenolic hydroxyl groups which can dissociate to negatively charged phenolates. Dissociated phenolics can form hydrogen and ionic bonds with various proteins, which lead to a disturbance of their 3D-structures and in consequence to a change in their bioactivity [3-4]. Several reports on antioxidant activity of plant species exist even in the cultivated climbers like betel [5-6]. To the best of our knowledge no effort has been made to evaluate antioxidant activity of leaves of common betel verities available in Odisha.

Therefore, in the present study, the antioxidant activities of the leaf extracts of five selected (Vishnupuri, Desawari, Ghajipur, Desipaan and Jaleswar) betel verities were evaluated.

Methodology:

Collection of plant materials: The five common betel leaf (**Fig. 1**) (Vishnupuri, Desawari, Ghajipur, Desipaan and Jaleswar) were collected from the different places of Odisha (**Fig. 2a**) from the local cultivars. The plant parts were collected from:

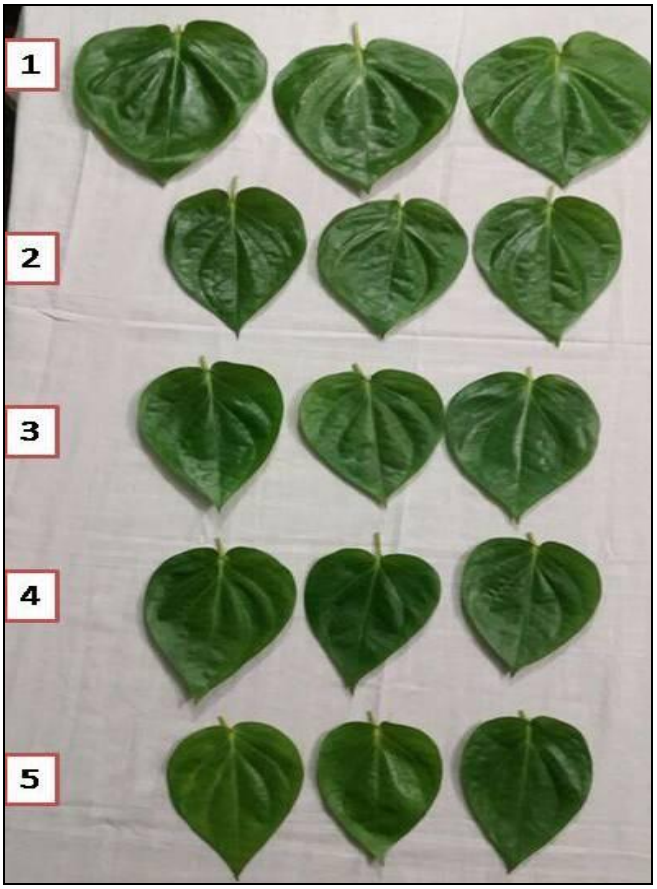
Vishnupuri: Nirgundi of Bhadrak district

Deswari: Niali of Cuttack district

Ghajipur: Hinjali of Ganjam district

Desipaan: Kujanga of Jagatsinghpur district

Jaleswar: Jaleswar of Balasore district



(1: Jaleswar; 2: Ghajipur; 3: Vishnupuri; 4: Desawari; 5: Desipan)

FIG. 1: EXPERIMENTAL LEAF OF BETEL VERITIES AVAILABLE IN ODISHA

Preparation of plant extracts: Soxhlet method was adopted to obtain the plant extract [7-8]. The plant parts of experimental plants were collected and dried at room temperature under shade and were powdered after drying using mechanical devices (**Fig. 2b**). The powdered material of the experimental plant was kept in thimble and extraction was carried out using the Soxhlet apparatus (**Fig. 2c**). The residues were collected and left for air drying. The extracts were stored in refrigerator for further experimental work.

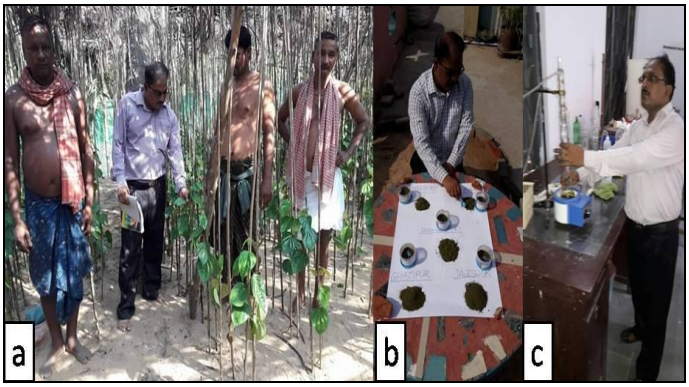


FIG. 2: (a) COLLECTION (b AND c) EXTRACT PREPARATION OF BETEL VERITIES OF ODISHA

Estimation of antioxidant activity: In order to study the antioxidant activity of experimental plant extracts, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, metal chelating (MC) activity, , ABTS (2,2'-azino-bis;3-ethylbenzothiazoline-6-sulphonic acid) scavenging activity and superoxide radical scavenging activity (SAS) were evaluated. The standard methods were adopted for the said scavenging activity. DPPH was carried out followed by Cao *et al.,* (1997) [9], metal chelating activity was done using Gouda *et al.,* (2013) [10], ABTS was performed followed by Re *et al.,* (1999) [11] and SAS was evaluated by Saeed *et al.,* (2012) [12]. The % inhibition was mathematically calculated and details were expressed in mean.

DPPH was carried out using 5.0 mL of dilutions (100µg/mL) of the experimental compounds and standard were mixed with 1 mL of a 0.001 % ethanolic solution of DPPH. DPPH solution was freshly prepared in each experiments and was stored in dark at 4± 2 °C. The compounds were incubated for 20-30 minutes in the dark at 30±2 °C. After incubation, Spectrophotometer readings were taken at 517 nm. All determination was performed in triplicate for better documentation.

Superoxide radical scavenging activity was evaluated using modified standard method. The

stock solution was prepared by 3.0 mL of tris-HCL buffer containing 0.5 mL of NBT, 0.5 mL of NADH solution. 1.0 mL of compounds of 100 µg/mL concentrations was mixed with 0.5 mL of stock solution. In the mixture 0.5 mL of PMS was added to start the reaction and incubated at 25±2 °C for 5±2 min. Readings were taken at 560 nm against ascorbic acid.

The Metal Chelating Activity of the plant extracts was determined using Gouda *et al.,* (2013) [10]. About 1ml of plant extract added to a solution of 0.5 mL ferrous chloride (0.2mM). Then about 0.2 ml. of Ferozin (5mM) was added to it and incubated at room temperature for 10 minute. The absorbency of the solution was then measured at 562 nm. The pre-formed radical monocation of 2,2'-azinobis - (3-ethylbenzothiazoline – 6 -sulfonic acid) (ABTS*+) was generated by oxidation of ABTS with potassium persulfate and was reduced in the presence of such hydrogen-donating antioxidants. The influences of both the concentration of antioxidant and duration of reaction on the inhibition of the radical cation absorption were taken into account while determining the antioxidant activity. ABTS was dissolved in deionised water and potassium sulphate was added and mixture was left overnight at room temperature. The readings were taken at 734 nm. The % inhibition was mathematically calculated and details were expressed as mean ± SD.

RESULTS AND DISCUSSION: The experiments revealed that the local betel verities of Odisha possess sound antioxidant activities. The results indicated that the leaves of the experimental plant are rich with phenolic compounds. The ethanol extract of the experimental plant parts showed highest antioxidant activities followed by the methanol, acetone and aqueous extract (**Table 1**). Such results are indicative of the fact that leaves contain polar compounds which are soluble more in ethanol as a solvent. Among the used verities of betel, Desipan showed highest activity (**Fig. 3**) followed by Vishnupuri, Desawari, Ghazipur and Jaleswar. It was also observed that ethanol extract of Desipan (leaf) showed highest scavenging activity using DPPH assay and Metal Chelating activity (**Table 1**). While experimenting both different extracts it was noticed that methanol extract of Desipan (leaf) had highest scavenging activity using SAS activity and ethanol extract of Desipan (leaf) showed highest scavenging activity using ABTS assay. Details are documented in **Table 1**.

TABLE 1: ANTIOXIDANT ACTIVITY OF FIVE SELECTED BETEL VARIETIES (MEAN±SD)

DHHP Assay					
Extract	Vishnupuri	Desawari	Ghazipur	Desipan	Jaleswar
Methanol	42±0.14	46±0.40	45±0.10	52±0.02	45±0.03
Ethanol	42±0.04	43±0.10	41±0.52	53±0.34	47±0.45
Acetone	43±0.14	38±0.11	40±0.34	50±0.71	40±0.40
Aqueous	40±0.38	39±0.12	38±0.12	48±0.14	43±0.34
Metal chelating					
Extract	Vishnupuri	Desawari	Ghazipur	Desipan	Jaleswar
Methanol	32±0.80	38±0.25	41±0.43	58±0.10	32±0.49
Ethanol	38±0.01	36±0.90	45±0.95	59±0.32	36±0.38
Acetone	39±0.23	36±0.40	48±0.92	53±0.71	38±0.03
Aqueous	35±0.52	30±0.50	42±0.16	50±0.40	39±0.52
SAS					
Extract	Vishnupuri	Desawari	Ghazipur	Desipan	Jaleswar
Methanol	67±0.06	68±0.25	45±0.04	90±0.10	46±0.88
Ethanol	70±0.08	46±0.33	42±0.90	68±0.35	49±0.34
Acetone	56±0.02	46±0.33	40±0.26	69±0.05	41±0.12
Aqueous	54±0.33	51±0.10	47±0.10	59±0.02	37±0.22
ABTS					
Extract	Vishnupuri	Desawari	Ghazipur	Desipan	Jaleswar
Methanol	45±0.40	32±0.80	33±0.25	67±0.06	29±0.02
Ethanol	47±0.50	35±0.52	33±0.71	70±0.10	28±0.10
Acetone	40±0.41	32±0.11	34±0.07	56±0.33	27±0.22
Aqueous	43±0.30	32±0.15	30±0.22	44±0.11	27±0.33

Hydroxyl Radical Scavenging Activity					
Extract	Vishnupuri	Desawari	Ghazipur	Desipan	Jaleswar
Methanol	48±0.30	42±0.17	41±0.30	71±0.24	51±0.15
Ethanol	46±0.20	43±0.18	45±0.71	78±0.22	49±0.33
Acetone	46±0.28	41±0.51	43±0.64	68±0.18	40±0.11
Aqueous	40±0.30	40±0.33	40±0.60	65±0.10	40±0.33

TABLE 2: ANTIOXIDANT ACTIVITY OF STANDARD (BUTYLATED HYDROXYTOLUENE,) BY DIFFERENT ASSAYS

Standard					
Standard	141.62 ± 0.26[a]	91.38 ± 0.32[b]	100.86 ± 0.08[c]	91.719 ± 0.03[d]	103.22 ± 0.12[e]

(a:DPPH, b:Metal Chelating, c:SAS, d: ABTS, e: HRS Activity)

Ethanol extract of Desipan (leaf) showed highest scavenging activity using hydroxyl radical scavenging activity as compared with Standard (**Table 1**, **Table 2**). There is no reports or less reports are available on the antioxidant activities of used local verities of betel of Odisha. However, Bhuvaneswari *et al.*, (2014) [13] had reported the antioxidant activity of six cultivars of *Piper betel* (Banarasi, Bangla, Calcutta, Kammar, Kumbhakonam and Vellai). Jaiswal *et al*., (2014) [14] reported the antioxidant activity of six different varieties (Banarasisafeda, Calcutta, Cuttack, Desibangla, Maharashtra and Sofia) of *Piper betel*.

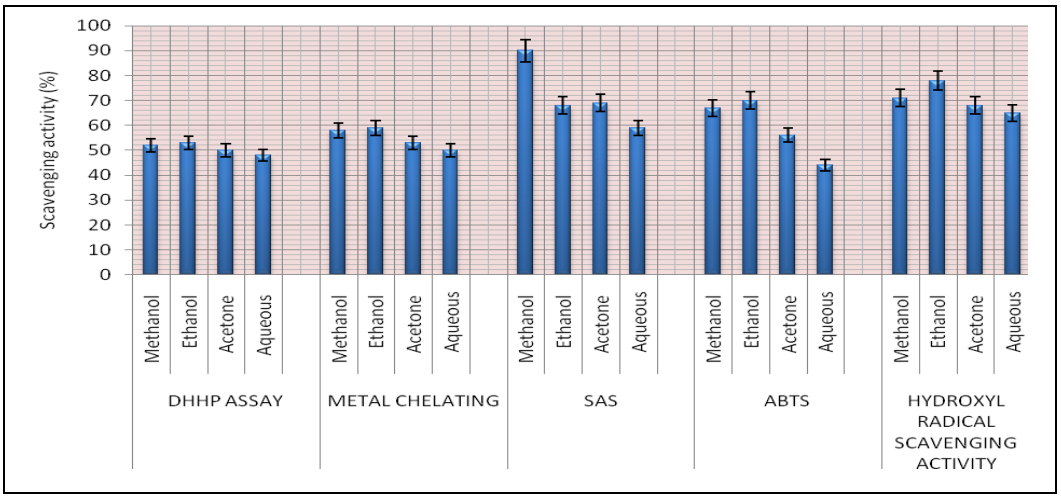


FIG. 3: ANTIOXIDANT ACTIVITY OF DESIPAN (LEAF) EXTRACTS, A COOMON BETEL VARIETY FOUND IN ODISHA

CONCLUSION: Present study revealed that Betel varieties found in the state of Odisha posses variable antioxidant properties. Hence, the leaf extract of betel may be utilised as a source for the production of antioxidant drugs. Further research is needed to evaluate their pharmacological as well as antimicrobial potential. So that the efficacy of the betel leaf can be assessed since these leaves are the common chewing material among many in a state like Odisha.

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