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EVALUATION OF PHYTOCHEMICAL CONTENT, NUTRITIONAL VALUE AND ANTIOXIDANT ACTIVITY OF *OLAXSCANDENS* (ROXB) LEAVES

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ABSTRACT

Leaves of *Olax scandens* (Roxb.) are consumed as vegetables in some parts of India. The present study was carried out to evaluate the phytochemical content, nutritional value and antioxidant activity of *Olaxscandens* (Roxb.) leaves following standard procedures. Qualitative analysis of the leaf powder reveals that carbohydrates, alkaloids, saponin, tannin and triterpenoids are present and glycosides, flavonoids, phenols and phytosterols are absent in leaves.The nutritional analysis of dried leaf powder of *O. scandens* shows the presence of macronutrients ie. Protein (12.89%w/w) carbohydrate (62.73%w/w) and fat (3.77%w/w) for each 100g dry sample.The leaf is also a good source for minerals like Calcium (2.52%), magnesium (0.77%) phosphorous (0.15 %) and Zinc (27.14mg/kg).Total antioxidant capacity of the leaf was 100.44±0.002 Mcg and IC50 values of the extract and Ascorbic acid were found to be >1000µg/ml and 11.67±0.58µg/ml respectively. Percentage scavenging of DPPH radical was found to rise with increasing concentration of the crude extract.

Key words: Antioxidants,Badru, DPPH, Leafy vegetables, Nutritional value,*Olax scandens* (Roxb.),

INTRODUCTION

Vegetables have been used as medicine since ancient times and playing a very important role in diet and nutrition. They are the most readily available sources of carbohydrates, fats, important proteins, vitamins, minerals, essential amino acids, and fibers[1]. Their bioactive substances have a wide range of biological functions, including antioxidant and antimicrobial activities[2] and can be helpful in the management of oxidative stress and age related human aliments[3]. Regular consumption of fruits and vegetables has always been associated with health benefits, but their mechanism has become clear only in the recent decades. Fruits and vegetables contain a wide variety of biologically active, non-nutritive compounds known as phytochemicals. Leafy vegetables are natural source of antioxidants and rich in phytochemicals[4,5]. These phytochemicals impart health benefits beyond basic nutrition[6]. Fruits and vegetables contain different antioxidant compounds such as Vitamin C, vitamin E, and carotenoids, whose activities have been established in recent years. Flavonoids, tannins and other phenolic constituents present in food of plant origin are also potential antioxidants. [7]

Olax scandens (Roxb.), an ethno-medicinal plant, has been reported for its use as food and medicinal purpose. Different parts of the plant are used in conditions like fever, constipation, cough etc. Its leaves are roasted and eaten as vegetable[8]. Fresh young leaves chewedin mouth ulcer[9]. Fomentation of boiled leaves is applied externally in headache[10].Further, its tender stem is also used as vegetable[11].Though

the plant *Olax scandens* is reported for many biological activities and used as vegetable, it is not evaluated for its chemical constituents and nutritional value. With the advent of modern systems of medicine need has been felt to investigate the active constituents present in these plants. Based on this, present study was undertaken to generate standardized data on various phyto and physico-chemical characteristics of leaves of the plant *Olax scandens* (Roxb.), along with nutritional composition and possible antioxidant activity.The outcome of the present study will be helpful to confirm its suitability as an edible vegetable.

MATERIALS AND METHODS:

Collection and preservation of the sample

Olax scandens (Roxb.), known as Badru,was identified from its natural habitat Balangir, Odisha, during September 2012, leaves were collected and authenticated by local taxonomist with the help of botanical flora.[12] A sample specimen was preserved in Pharmacognosy laboratory of IPGT & RA Jamnagar (SPECIMEN NO- PHM 6062/21/09/2012) and the sample was preserved in a solution prepared from 70% ethyl alcohol: glacial acetic acid: formalin (AAF) in the ratio of 90:5:5.[13]

Physico-chemical analysis

Physicochemical parameters and phytochemical screening were carried out as per the guidelines of Ayurvedic Pharmacopoeia of India.[14]

HPTLC study

Extraction of Alkaloids

Powdered plant materials were moistened with ammonium hydroxide and kept in a stoppered flask for about 1 hour. Then it was extracted with chloroform two-three times. About 5 ml chloroform extract was taken in a dish and chloroform evaporated. The dried substances were tested for the presence of alkaloid, and used for the chromatographic study. Chromatographic conditions are as follows.[15]

Chromatographic conditions

⇨ Application mode	:	CAMAG Linomate V
⇨ Development chamber	:	Camag Twin trough Chamber.
⇨ Plates	:	Pre-coated Silica Gel GF254 plates.
⇨ Chamber Saturation	:	30 min.
⇨ Development time	:	30 min.
⇨ Development distance	:	7 cm.
⇨ Scanner	:	Camag Scanner III.
⇨ Detection	:	Deuterium Lamp and Mercury Lamp.
⇨ Data system	:	Win cats software
⇨ Solvent system	:	Toluene: Ethyl acetate: formic acid
7	**:**	**2 : 0.5 v/v**

Nutritional evaluation

Estimation of energy value- The sample calorific value was estimated (in Kcal) by multiplying the percentage crude protein, crude lipid and carbohydrate by the recommended factor (2.44, 8.37 and 3.57 respectively) used in analysis. The caloric value was determined based on the Atwater factor.[16]

Carbohydrates were determined by using cupric tartrate, the precipitate formed was compared with dextrose of known concentration.[17] Estimation of crude fat was performed using n – Hexane as solvent by Soxhlet extraction method.[18]The crude protein was determined by the Kjeldahl method with slight modification and the absorbance at 470 nm.[19]Determination of moisture content was carried out by standard procedure mentioned in Ayurvedic Pharmacopeia of India.[20]All the minerals except phosphorus were analyzed from a triple

acid-digested sample by an atomic absorption spectrophotometer.[21]The phosphorus content in the triple acid digested extract was determined colorimetrically.[22]

Antioxidant assay

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity:

100 µM DPPH solution was obtained using methanol. 10.5 mg/ml, 10.5mg/ml and 21 mg/ml concentrationsof ascorbic acid, rutin, and extract was obtained by using Dimethyl sulfoxide(DMSO) which was serially diluted with DMSO to obtain lower concentrations. Various concentrations of sample were added to DPPH solution and the absorbance of DPPH reagent was determinedat 490 nmafter 30 min of incubation, using a micro plate reader.[23]

Total antioxidants assay

Weighed accurately 55 mg of the aqueous extract, standard, ascorbic acid and dissolved in 5 ml of DMSO. The lower dilutions were made serially with DMSO. An aliquot of 0.1 ml of the sample solution containing a reducing species in DMSO was combined with 1 ml of reagent solution (0.6 M Sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in water bath at 95 °C for 90 min and the absorbance was measured at 695 nm. The total antioxidant capacity was expressed as mM equivalent of ascorbic acid.[24]

RESULTS AND DISCUSSION

Powder of leaves was tested for various physico-chemical parameters such as loss on drying, ash value, water, methanol soluble extractive value. The observed results were depicted in table 1

Table 1: PreliminaryPhysico-chemical analysis of leaf powder of *Olax scandens* (Roxb.)

Sr. No.	Test	Results
1.	Loss on Drying	6.042 % w/w
2.	Ash Value	9.896 % w/w
3.	Acid Insoluble Ash	0.284 %w/w
4.	Water Soluble Extract	29.70% w/w
5.	Methanol Soluble Extract	22.40 % w/w
6.	pH	6.0

Preliminary qualitative chemical test:

Preliminary qualitative chemical test for leaves was done following standard procedure. The observed results are presented in a tabular form (Table 2)

Table 2: PreliminaryQualitative analysis of leaf powder of *Olax scandens.*

S.No	Test	Results
1	**Test for carbohydrates** a. Molisch's test	 +
2	**Test for Glycosides** a. Modified Borntrager's test b. Keller-Killiani test	 - -
3	**Test for Saponins** a. Foam test	 +
4	**Test for Alkaloids** a. Mayer's test b. Dragendorff's test	 + +

5	**Test for Flavonoids** a. Alkaline reagent test	 -
6	**Test for Phenolics and Tannins** a. Ferric chloride test b. Test for Tannins	 - +
	Test for Phytosterols and Triterpenoids a. Leiberman-Bucharat test b. Salkowaski test	 - +
8	**Test for fixed oils and fats** **a. Oily spot test**	 -

"+": Positive, "-": Negative

Carbohydrates, alkaloids, saponin, tannin and triterpenoidsarepresent and Glycosides, flavonoids, phenols and phytosterols are absent in leaves.

HPTLC

Results of the HPTLC study of the leaves scanned under 254 nm & 366 nm showed 4 spots at 254 nm and 1 spot at 366 nm.

Table 3: Showing HPTLC profile for *Olax scandens*

Solvent system	**Track No**	**Under UV light**			
Toluene: Ethyl acetate: Formic acid 7.5: 2: 0.5 V/V		254nm (Short UV)		366nm (Long UV)	
		Number of spots	Rf value	Number of spots	Rf value
	Track 1 (Leaves)	4	0.16, 0.6, 0.66, 0.68	1	0.01

Nutritionalanalysis

The results of nutritional analysis of *Olax scandens* leaf is presented in table 4

Table 4: Nutritional values of leaf powder of *Olax scandens* (Roxb.)

Sr. No	Parameters	Results
1.	Energy	336.41 K Cals/100g
2.	Carbohydrate	62.73 gm
3.	Fat	3.77 gm
4.	Protein	12.89 gm
5.	Calcium	2.52 mg/Kg
6.	Magnesium	0.77 mg/Kg
7.	Phosporous	0.15 mg/Kg
8.	Zinc	27.14 mg/Kg

The results obtained in the nutritional analysis of dried leaf powder of *O. scandens* shows the presence of macronutrients ie. protein(12.89%w/w) carbohydrate (62.73%w/w) and fat (3.77%w/w) for each 100g dry sample. Macronutrients are nutrients that provide calories or energy. These are substances needed for growth, metabolism, and for other body functions. According to the Dietary Reference Intakes published by the USDA, 45% - 65% of calories should come from carbohydrate, 10% - 35% of calories should come from protein and 20% -35% of caloriesshouldcome from fat.

The leaf is also a good source for minerals like Calcium (2.52%), magnesium (0.77%) phosphorous (0.15 %) and Zinc (27.14mg/kg).

Calcium is an essential mineral for bone formation, deficiency of which leads to reduced bone formation, osteoporosis and bone fracture with an overall restriction in bone formation (bone size) and growth. Magnesium plays a vital role in the activity of many enzymes and phosphorous is an important component of energy intermediates[25].

Leaves showed higher values of Zinc (27.14 mg/Kg) indicating them as a good source of this vital trace element. Zinc is a component of many metallo enzymes and also a membrane stabilizer and a stimulator of the immune response.[26,27] Its deficiency leads to loss of appetite, and impaired immune function. In more severe cases, zinc deficiency causes hair loss, diarrhea, delayed sexual maturation, impotence, hypogonadism in males, and eye and skin lesions.[28,29] The high zinc content may be useful in skin diseases and rheumatism.

The value of zinc is found to be higher than that of the previous study.[30] The variability in the content in the same species may be related to genetic origin, geographical source and the levels of soil fertility.The present result indicates the potentiality of plant *Olaxscandens* as source of unconventional food. Being wild, it is easily accessible and cheaper vegetable source.

Antioxidant activity

DPPH radical scavenging activity

Results of the DPPH radical scavenging assay is given in Table 5. The IC50 values of the extract and Ascorbic acid were found to be >1000µg/ml and 11.67±0.58µg/ml respectively. Percentage scavenging of DPPH radical was found to rise with increasing concentration of the crude extract (Figure 1).

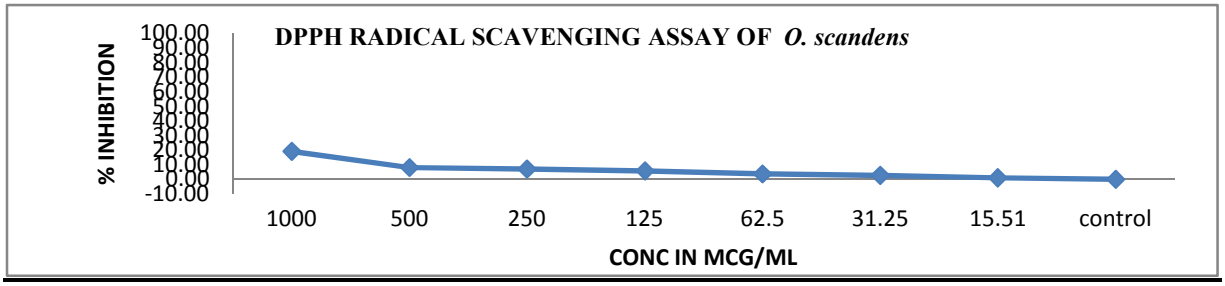
Total antioxidant capacity

Total antioxidant capacity of the extract is 100.44±0.002 Mcg (Table 5). Total antioxidant capacity is expressed as the number of equivalents of ascorbic acid (AAE).

Table 5: DPPH radical scavenging activity and total antioxidant capacity of *O. scandens*

Samples	**IC_{50} values µg/ml by methods**	**Mcg per equivalent of Ascorbic acid**
	DPPH	**Total antioxidant activity*[a]**
O.scandens	>1000	**100.44±0.002**
Standard	**Ascorbic acid**	
	11.67±0.58	

*[a]The total antioxidant capacity was expressed as mg equivalent of ascorbic acid per gram of dry weight.



CONCLUSION

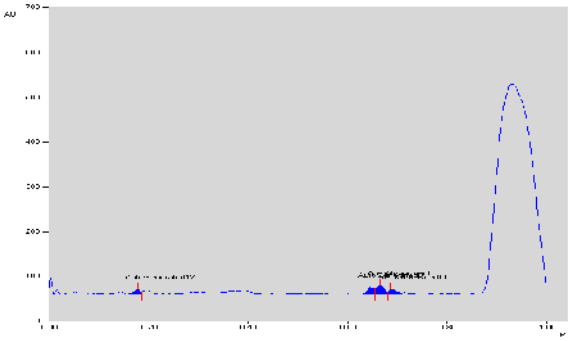
Observed physicochemical and phytochemical parameters can be considered as the standard for future references. Leaves of *O. scandens* area good source of macronutrients ie. Protein, carbohydrate and fat and minerals like

calcium, magnesium, phosphorous and zinc.Leaves of *O. scandens* also possess mild antioxidant activity when compared with the ascorbic acid. The results ofpresent studyindicates the potentiality of plant *Olaxscandens* as source of unconventional food. Being wild, it is easily accessible and cheaper vegetable source.

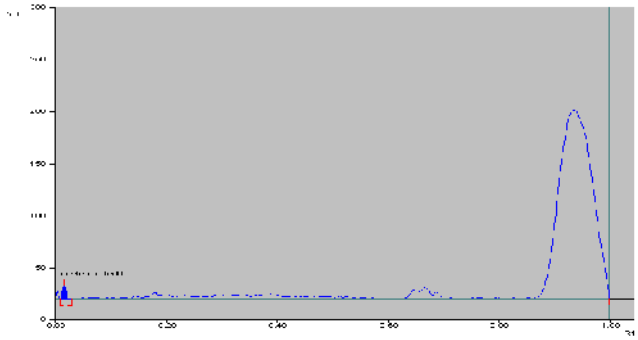
PHOTOGRAPHS PLATE A

TLC Plate at 254 nm

TLC Plate at 366 nm



Peak Display at 254 nm



Peak Display at 366nm

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