

Phytochemical Analysis and Antioxidant Property of Wild Edible Plant *Dioscorea alata*

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Abstract: - In this study investigation of qualitative and quantitative characters and Antioxidant activity of wild edible plant species. Analysis of qualitative character was performed for edible parts of the wild edible species *D. alata*. The plant was collected from the area in Amravati district and edible part analysed for dry matter, the qualitative nutritional and nutraceutical were performed as well as antioxidant activity performed by DPPH method. The percentage of free radical inhibition was 32.59% at 20µg/ml concentration of sample. Quantitative estimation represents the amount of nutrition present in plant. The purpose of present study is to explore qualitative research and provide information about nutritional content in selected wild edible plant.

Introduction:

Dioscorea alata L. (Fam: Dioscoreaceae) commonly known as, greater yam and locally (in Assam) known as kath aloo, are climbing perennial vines with heart-shaped leaves. Being well known for their edible tubers that serve as important staple foods and potential sources of ingredients for fabricated foods in South Asian countries, China, Africa and some parts of South America, they are rich in starch content (between 70% to 80% of dried matter) (Das *et. al.*, 2012; Huang *et. al.*, 2006). Among various yams, *D. alata* is also known for its high nutritional value, with a crude protein content of 7.4% and vitamin C content ranging from 13.0–24.7 mg per 100 grams (Osagie, 1992). The main constituents of *Dioscorea* spp. are dioscorin, saponins and dioscin having medicinal values which were well documented previously (Olayemi and Ajaiyeoba, 2007). Throughout decades much work has been done on various medicinal and clinical aspects of the *D. alata* tubers (Das *et. al.*, 2012; Chen *et. al.*, 2003), but no such substantial evidence exists on the leaves of the same plant.

Material and Methods:

Plant material:

Plant material and preparation of extracts Fresh leaves of *D. alata* were collected from Amravati district.

Physical examination

Colour	Observed dry powder sample, it is brownish to green in colour
Odour	Take a power sample and smell it, characteristic odour
Texture	It is amorphous in nature
Taste	Characteristic

Qualitative Nutritional Analysis

1. Test for Carbohydrate	
i. Molisch test	Take a powder sample of each extract in a two test tube add Molisch reagent in it and slowly add H ₂ SO ₄ from side of test tube. A purple red ring appear at the junction of liquid in both extracts, indicates presence of carbohydrate
ii. Fehling test	Take a powder sample of each extract in a two test tube add Fehling solution A and Fehling solution B then boil it for two minutes. A yellow red ppt observed in both extracts, indicates presence of carbohydrate.
iii. Benedict test	Take a powder sample of each extract in a two test tube add 2ml Benedict reagent and heat for 5 minutes. A green yellow colour appeared in both extracts, indicate presence of carbohydrate.
2. Test for Protein	
i. Biuret test	Take 3ml sample extract; add 4% NaOH and few drops of 1% CuSO ₄ solution. A pink colour appeared in water extract, indicates presence of protein in water extract.
ii. Millons test	Add 5ml of Millons reagent in each sample extract and warm it's for few minutes. A brick red ppt is formed in water extract, indicates presence of protein in water extract.
3. Test for Amino Acid	
i. Ninhydrin test	Take a 3ml of sample extract, add 3 drops of 5% ninhydrin solution then boil it for 10 min. A purple or bluish colour is appeared in water extract, indicates presence of Amino acid in water extract.

Qualitative Nutraceutical Analysis

1. Test for Alkaloids	
i. Dragendorff's test	Take a 3ml of sample extract, add 3 drops of dragendorff reagent. A orange brown ppt form in alcoholic extract, indicates presence of Alkaloid in alcoholic extract.
ii. Hager's test	Add few drops of Hager reagent in 3ml of plant extracts. A formation of yellow ppt in Alcoholic extracts indicates presence of Alkaloid in alcoholic extract.
2. Test for Flavonoid	
i. Shinoda test	Take a sample extract, add 5ml 95% ethanol and add few drops of conc. HCL in it then 0.5 gm magnesium ribbon add. a orange to pink colour appeared in both extract, indicates presence of flavonoid.
ii. Sulphuric acid test	Take a sample extract and add 80% sulphuric acid (H ₂ SO ₄), a Flavones dissolve into it and gives deep yellow colour in both extracts, indicates presence of flavonoid.
3. Test for Tannins	
i. Ferric chloride test	Take a sample extract in a test tube; add few drops of ferric chloride solution. A greenish black colour formed in both extract, indicates presence of tannins.
4. Test for Phenolics	
i. 5% Ferric chloride test	Take a sample extract in a test tube; add 5% ferric chloride

	solution. A deep blue black colour formed in both extracts, indicates presence of phenolics.
5. Test for glycosides	
i. General test for identification of glycone moieties	Take a sample extract, add 5ml dilute sulphuric acid then warm on water bath and neutralize with 5% sodium hydroxide after these add 0.1 ml of Fehling A & B solution until red ppt formed in both extracts, confirmed glycosides.
ii. Cardiac Glycoside Legal test	Take a sample extract in a test tube, add 1ml of pyridine and 1ml of sodium nitroprusside. No deep red blood colour in both extracts, confirmed Absence of cardiac glycosides.
iii. Anthraquinone glycosides (Borntranger test)	1ml sulphuric acid add in plant extract boil it for 5 min. then cool and shake filtrate with equal volume of chloroform then separate organic solvent layer and mix with half volume of dilute ammonia. No pink or red colour formed in both extracts, confirmed absence of Anthraquinone glycosides.
iv. Cyanogenic glycosides	Take a sample extract in a conical flask and moisten with few drops of water. Moistened picric acid paper with 5% aqueous sodium carbonate solution and suspended by cork, warm the flask gently at 37°C. Hydrogen cyanide liberated from cyanogenic glycosides in alcoholic extracts, confirmed cyanogenic glycosides.
v. Isothiocyanate glycosides	sample extract treated with 20% NaOH solution. No bright yellow colour formed, confirmed absence of isothiocyanate glycosides.
6. Test for Steroids	
i. Salkowski reaction	Take 2ml extract, add 2ml chloroform and 2ml H ₂ SO ₄ shake well. Formation of chloroform layer and green fluorescence in alcoholic extract, confirmed steroid.
7. Test for Vitamin	
i. Vitamin A (Antimony Tri-chloride test)	Reflux extract with 5ml chloroform and add 1ml antimony trichloride solution. Blue colour appeared in alcoholic extract, confirmed vit- A.
ii. Vitamin E	A fat soluble vitamin absent in both extracts.
iii. Vitamin C test by Iodophenol indicator	Take 5ml of extract in a test tube and add few drops of Iodophenol indicator. Solution turns blue in water extract, confirmed vitamin C present.

Quantitative Nutritional and nutraceuticals Analysis

1. Estimation of Carbohydrate

Principle: Carbohydrate commonly found compound in many plants. In hot acidic media glucose is dehydrated to hydroxymethyl furfural which forms a green colored product with phenol and has an absorption max at 490nm.

Reagent: Phenol 5%, sulphuric acid, standard glucose.

Procedure:

- Take 100mg of the glucose into test tube. Add 5ml of 2.5N HCL and boil in water bath for 3hr to hydrolyze the sugar. Cool to room temperature

- b) Add sufficient quantity of solid sodium carbonate until the effervescence ceases. This indicated complete neutralization. Make the volume up to 100ml
- c) Pipette out 0.2, 0.4, 0.6, 0.8 and 1 ml of working standard into series of test tube
- d) Similarly prepare sample to be studied. Pipetted out 0.1 and 0.2ml of this solution two separate test tube make the volume up to 1ml with water.
- e) Set the blank with all reagent without sample
- f) Add 1ml of phenol solution to each tube and keep in water bath at 25 to 30°C for 20min. Now measure the color at 490nm.
- g) Calculated the total amount of carbohydrate in the sample from glucose std graph.
- h) Absorbance correspond to 0.1ml of test = x mg of glucose.
- i) 100ml of sample solution contain = $x/0.1 \times 100$ mg of glucose = % of total carbohydrate present.

2. Estimation of protein:

Principle: The principle of Biuret assay is based on the fact that under alkaline condition compounds containing two or more peptide bond form a purple complex with copper salt in the reagent.

Reagents: 0.85%(w/v) sodium chloride solution, 0.5%(w/v) working protein std (WPS), Biuret reagent, Protein sample solution

Procedure:

- a) Pipette (in millimeter) the reagent into suitable container as given in the table
 - b) Mix thoroughly by vortexing and incubate for 30min at 25°C
 - c) Transfer to suitable cuvettes and record the absorbance at 550nm. for test standard and blank.
 - d) Prepare std calibration curve by plotting the absorbance of std vs mg of protein
- Determine the mg of protein present in sample by using std curve.

3. Estimation of total lipid/ fat content in sample

Procedure:

- a) Take a sample of 5gm.
- b) Homogenized it with 5ml of chloroform and 5ml of methanol. For 2 min.
- c) 5ml of chloroform is added and the mixture homogenized another 30s the mixture allows to separate.
- d) The lower solvent phase removed and passed through whatman #1 filter paper
- e) Residue allow to dry.
- f) The lipid are then gravimetrically determined. The sample to evaporate in overnight
- g) Recording the weight of sample.

4. Estimation of Total phenolic content by Folin Ciocalteu method [3].

Principle: The folin ciocalteu reagent (FCR) also called gallic acid equivalent method (GAE) uses a mixture of phosphor-molybdate and phosphor tungstate for the colorimetric assay of phenolic and polyphenolic antioxidant. It works by measuring the amount of the substance needed to inhibit the oxidation of reagent. However this reagent dose not only measure total phenol but react with any reducing substance. The reagent therefore measures the total reducing capacity of sample, not just the level of phenolic compound.

Reagent: dilute folin ciocalteu reagent with equal volume of distilled water, 20% sodium carbonate in water, Gallic acid

Procedure:

- Prepare calibration curve of std gallic acid (10-100ug/ml) in water
- 1mg per ml of extract solution
- Mix 1ml of each sample with 0.25ml of FCR and 1.25ml of 20% sodium carbonate solution.
- Allow the mixture to react for 40 min at room temp.
- After the reaction period, mix the content and measure the blue color at 725nm in comparison with std. calculated the amount of total phenol from calibration curve as gallic acid equivalent by following formula:

$$T = C.V./M$$

Where, T = total content of phenolic compound

C= concentration of gallic acid

V= volume of extract

M= gram weight of plant extract

5. Estimation of total alkaloids in sample [3]

Principle: Bromocresol green react with alkaloids having nitrogen atom with in ring and form yellow colored complex which can be easily measured colorimetrically. BCG dose not react with alkaloid having nitrogen in side chain so this method is not useful for determination of amine or amide alkaloids.

Reagent: bromocresol green solution, phosphate buffer solution pH4.7, standard atropine solution Dissolved (1mg atropine in 10ml of distilled water)

Procedure:

- Take 0.4,0.6,0.8,1.0 and 1.2 ml atropine solution in separate test tube.
- Add 5ml of phosphate buffer solution (pH4.7) and 5ml of BCG solution.
- Shake well and extract the yellow-colored complex with chloroform.
- Separate chloroform and make up volume to 10ml.
- Measure the absorbance at 470nm against blank.
- Now prepare the methanolic extract of plant material. Dry and dissolved in 2N HCl. filter and wash with chloroform. Adjust the pH neutral with 0.1N NaOH.
- Now add 5ml of phosphate buffer solution pH 4.7 and 5ml of BCG solution.
- Shake well and extract the yellow-colored complex with chloroform.
- Separated chloroform and make up the volume to 10ml and measure the absorbance at 470nm.
- Calculate concentration of total alkaloids from calibration curve of atropine std.

Biological Activity

DPPH Assay for antioxidant Activity

Procedure: 1. Stock solution of DPPH (2, 2-diphenyl-1-picrylhydrazyl) was prepared by dissolving 1.083 mg in 10 ml of ethanol

2. Stock solution of formulation (100 µg/ml) was prepared by dissolving 1 ml of formulation in 10 ml of ethanol. From this stock solution, further dilutions were prepared of concentrations 10, 20, 30, 40 and 50 µg/ml using ethanol
3. Similarly stock solution of standard ascorbic acid (1 mg/ml) was prepared by dissolving 10 mg ascorbic acid in 10 ml ethanol. From this stock solution further dilutions of concentrations 1, 2, 3, 4, and 5 µg/ml were prepared.
4. Absorbance of blank (5 ml ethanol + 1 ml DPPH solution) as a positive control was recorded using colorimeter at 420 nm
5. Similarly the absorbance of formulation and comparative standard ascorbic acid were taken at 420 nm and recorded.
6. Since the colour of formulation after addition of DPPH was interfering with the absorbance, absorbance of all concentrations of formulation were taken before addition of the DPPH and those subtracted from the final absorbance of all concentrations to remove the error.

Observations and Results:

The WEP *D. alata* analysed in the present work. Leaf extract prepared by using two solvent water and alcohol, according to solubility of compounds it shows presence or absence of compounds. Result reveals presence of carbohydrate in both water and alcoholic extract. Proteins found only in water extract and absent in alcoholic extract, similarly amino acid also found in water extract only.

Alkaloid present only in alcoholic extract, Flavonoids present in both extracts, Tannins & Phenolics present in both extracts, Aglycone moieties in glycosides present in both extracts. Cardiac glycosides absent in both extracts. Anthraquinone glycosides also absent in both extracts. The cyanogenic glycosides present only in alcoholic extract. Saponin glycosides and Isothiocyanate glycosides absent in both extracts. Steroids only present in alcoholic extracts. Vitamin A is present in alcoholic extract and Vitamin E absent in both extracts. Vitamin C present only in water extracts. The Antioxidant screening by DPPH radical scavenging shows dose dependent increase in antioxidant activity, highest free radical inhibition 70.16% at 50 µg/ml sample concentration.

The presence of high raw material used in the preparation of new drug, and high nutritional value may fulfil the nutrient diet of humans.

Qualitative Analysis :

Table 1- Phytochemical analysis of *D. alata* (Qualitative test).

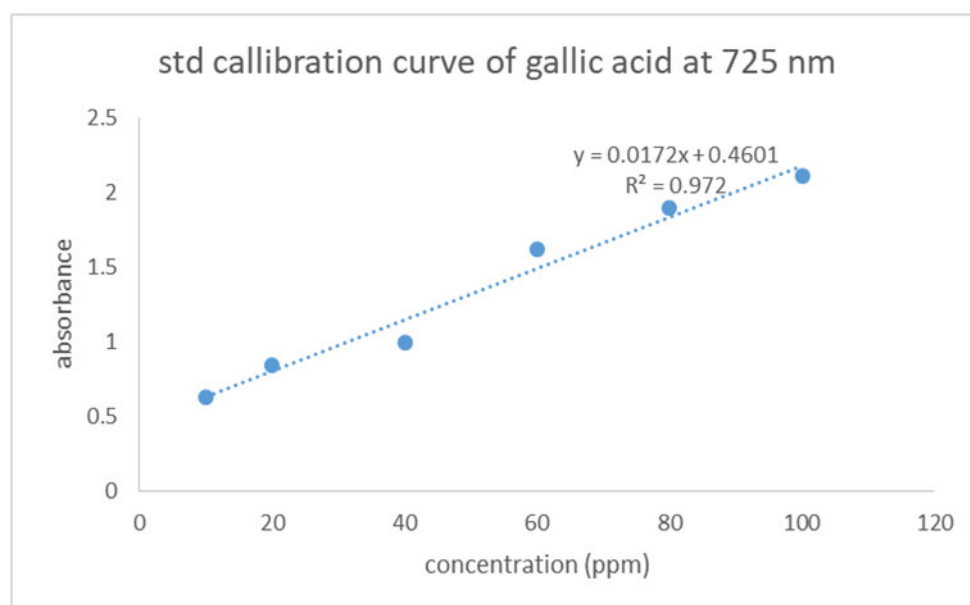
Test	Observation
Carbohydrate- molisch test	+ve
Amino acid	+ve
Protein- Biuret test	-ve
Gums- Benedicts test	+ve
Fehlings test	+ve
Phenol	+ve
Fat and lipid	+ve

Flavonoids	+ve
Phytosterol	+ve
Alkaloids	+ve

Quantitative Analysis :

1) Estimation of total phenolic content by folin ciocalteu method.

Concentration (ppm)	Absorbance
10	0.631
20	0.844
40	0.991
60	1.622
80	1.898
100	2.11



Concentration (ppm)	Absorbance
Aqueous extract 10 ppm	1.121
Alcoholic extract 10 ppm	1.958

The concentration of unknown sample of water extract (x) = $y - c/m$
 $= 1.121 - 0.4601/0.0172$
 $= 38.42 \mu\text{g/ml}$

Total phenolic content for aqueous test sample (T) = C.V/M

$$= 0.03842 \times 1 / 0.01 \text{ g}$$

$$= 3.84 \%$$

Total phenolic content for alcoholic test sample (T) = y-c/m

$$= 1.958 - 0.4601 / 0.0172$$

$$= 87.08 \mu\text{g/ml}$$

Total phenolic content for alcoholic test sample (T) = C.V/M

$$= 0.08708 \times 1 / 0.01 \text{ g}$$

$$= 8.708 \%$$

2) Estimation of total fat and lipid-

a) Initial weight of sample (x) = 10.00 gm

b) Final weight of the sample (y) = 9.80 gm

c) Difference in weight of the sample (x-y) = 0.2 gm in 10 gm of sample

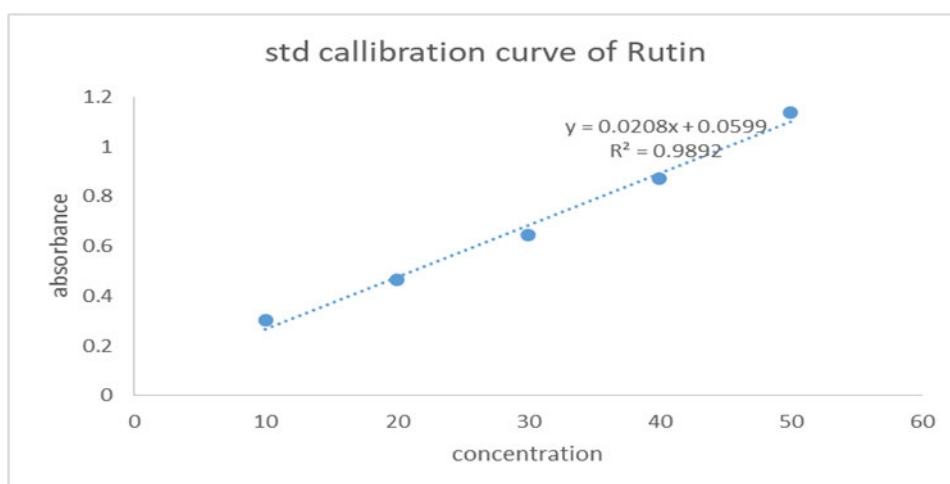
In 100 gm of sample 2%

In above estimation process the total lipid and fat content was found to contain 2 %

3) Estimation of total flavonoids-

Observation and calculation-

Concentration (ppm)	Absorbance
10	0.307
20	0.465
30	0.644
40	0.874
50	1.139



Concentration (ppm)	Absorbance
Aqueous extract 10 ppm	1.306

Alcoholic extract 10 ppm	1.731
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The concentration of unknown sample of water extract (x) = $y-c/m$
 $= 1.306-0.0599/0.0208$
 $= 59.90 \mu\text{g/ml}$

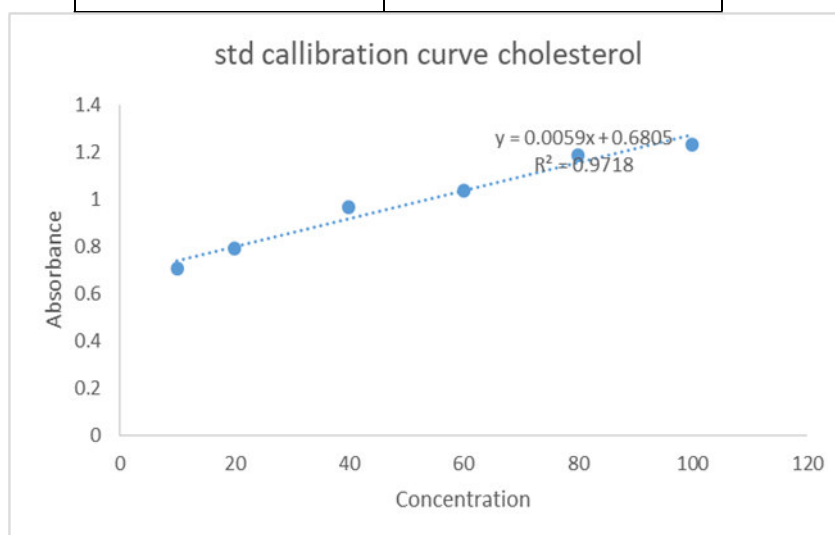
Total flavonoid content for aqueous test sample (T) = $C.V/M$
 $= 0.05990 \times 1 / 0.01 \text{ g}$
 $= 5.9 \%$

Total flavonoid content for alcoholic test sample (T) = $y-c/m$
 $= 1.731- 0.0599/0.0208$
 $= 80.34 \mu\text{g/ml}$

Total flavonoid content for alcoholic test sample (T)= $C.V/M$
 $= 0.08034 \times 1 / 0.01\text{g}$
 $= 8.0 \%$

3) Estimation of total steroids-

Concentration (ppm)	Absorbance
10	0.707
20	0.790
40	0.968
60	1.036
80	1.184
100	1.233



Type of extract	Absorbance
Alcoholic extract	0.949

The concentration of unknown sample of alcoholic extract (x) = $y-c/m$
 $= 0.949-0.6805/0.0059$

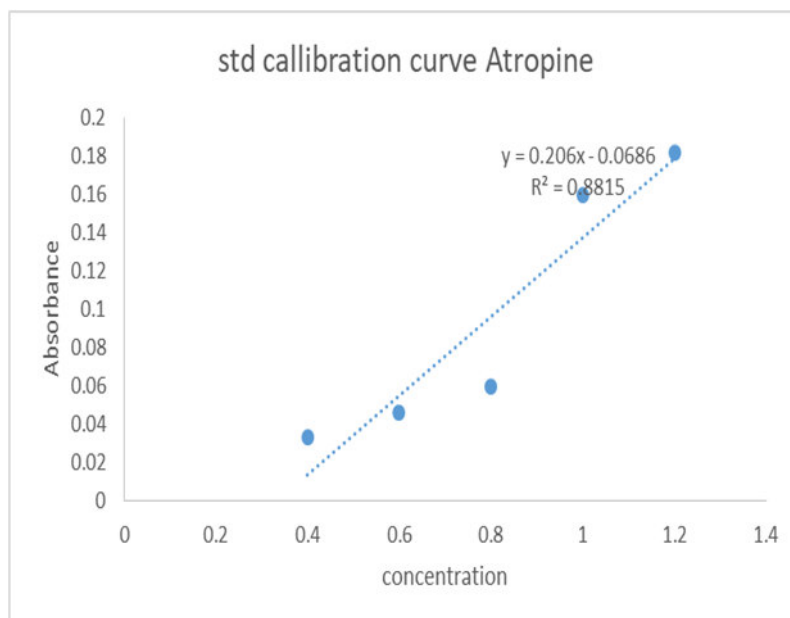
$$= 40.50 \mu\text{g/ml}$$

In 1 mg of alcoholic extract sample = 0.04050 mg of steroid

Thus in 100 mg = 40.50 mg of steroid present i.e 4.0 %

4) Estimation of total Alkaloids-

Concentration (ppm)	Absorbance
10	0.033
20	0.046
40	0.060
60	0.16
80	0.182



Type of extract	Absorbance
Alcoholic extract	0.444

The concentration of unknown sample of ethanolic extract (x) = $y - c/m$

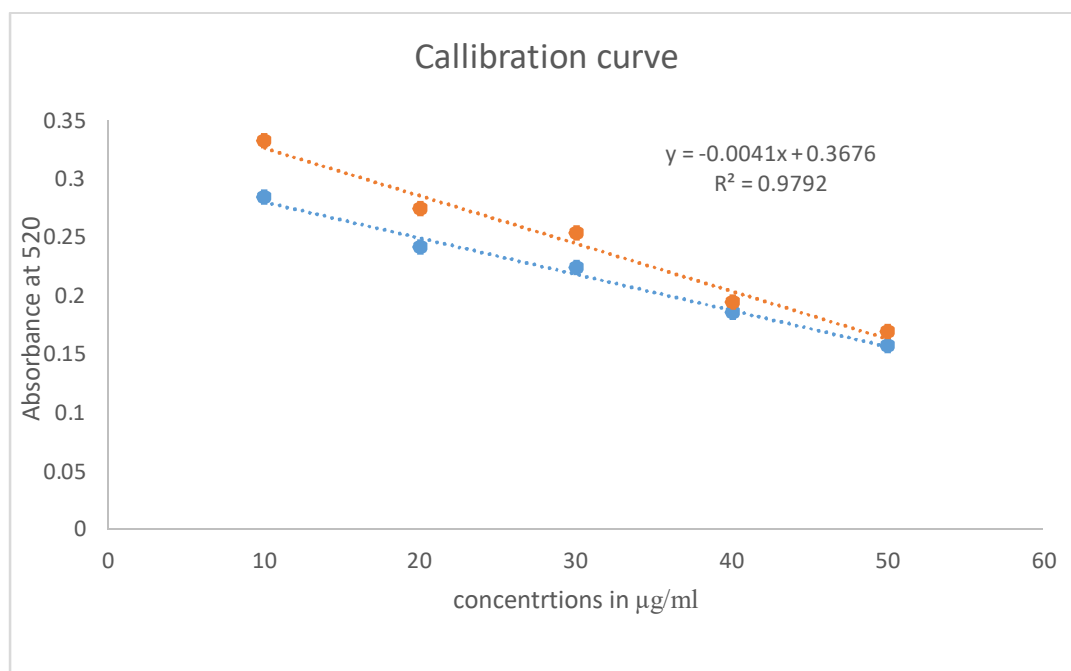
$$= 0.444 - 0.0686/0.206$$

$$= 1.82 \text{ mg/ml}$$

10 mg of alcoholic extract sample contain = 1.82 mg of Alkaloid

Thus in 100 mg contain = 18.2 mg of Alkaloid present i.e 18.2 %

Antioxidant activity by DPPH Method



Sr.no.	Concentration of ascorbic acid µg/ml	Absorbance of blank	Absorbance of ascorbic acid	Percentage of free radical inhibition
1	1	0.440	0.284	35.45%
2	2		0.242	45.00%
3	3		0.224	49.09%
4	4		0.186	57.72%
5	5		0.157	64.31%

Sr.no.	Concentration of sample µg/ml	Absorbance of blank	Absorbance of sample	Percentage of free radical inhibition
1	10	0.408	0.333	18.30%
2	20		0.275	32.59%
3	30		0.254	37.74%
4	40		0.195	52.20%
5	50		0.169	58.57%

Conclusion:

The data in above study indicates WEP *D. alata* has high nutritional content and most phytochemicals show their presence in alcoholic extract. Good radical scavenging activity indicates presence of high antioxidant and May helps to cure various diseases. The current review shows that utilization of wild variety of edible plants could play a crucial role in dietary diversification, improve nutrition and human health.

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