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RESEARCH ARTICLE

STUDY OF NUTRITIVE VALUE AND ANTIOXIDANT ACTIVITY OF LACTUCA RUNCINATA AND PORTULACA QUADRIFIDA

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ABSTRACT

Plants are always used as medicines from long time and there is also a need for studying nutritive value and antioxidant activity of these plants. The present study includes various factors for experimental work and it involves plant extracts preparation from both fresh and dried materials as per methods used and showed significant result for both nutritive values like moisture content, mineral elements, proteins, carbohydrates, reducing and non-reducing sugars. The antioxidant activities of plants like Total Phenols, Vitamin A, Vitamin C and Free radical scavenging activity is considered in this study. There is always a need for an accurate determination of antioxidant capacity as it is gaining importance in most of the areas like food industry, therefore several analytical methods and measuring systems have been developed.

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INTRODUCTION

In the 21st century every human being is very busy in their day to day life that it is impossible for them to concentrate on daily nutrition and it should include Protein, carbohydrate, Vitamin, Total sugar, and Micro and macro- element. Both the plants involved in the study are herbaceous and seasonal since they are wild and not cultivated. So it will be beneficial to use these plants in our diet. *Lactuca runcinata* and *Portulaca quadrifida* both are most commonly used wild vegetables in rural areas of Maharashtra. Since they are wild there are no pesticides in them and no chemicals that may affect human health. Many such plants are commonly used in most of the urban areas too because of their nutritional and antioxidant activity. These are not only easily available but are cheap as compared with other vegetable. In the present study both the plants were analyzed for nutritive and antioxidant analysis, for the experimental work plant material was collected from different localities. Different protocols were used as described by the Indian Pharmacopeia. On an average, vegetables were found to be richer in organic nutrients and minerals. Generally the wild food plant species were found to be richer sources of mineral nutrient than their cultivated relatives.

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MATERIAL AND METHODS

To study nutritive value of the selected wild vegetables, they were collected from different localities of Maharashtra, India. As wild edible vegetables are not found to be grown in one locality and in a particular season of the year, frequent visits were organized to collect the selected plants in various seasons. Efforts were made to collect these plants specifically in flowering and fruiting conditions for the correct botanical identification. The plants were initially identified with the help of The Flora of the presidency of Bombay (Cooke, 1901-1903) and later they were authenticated by "Botanical Survey of India" (Maharashtra) with Authentication letter number (BSI/WC/TECH./2015/137) Dated 19-06-2015.

Plant material collection

The different wild vegetables were collected from the various localities of western Ghats of Maharashtra at the same first-hand information was also collected from tribal communities regarding their utilization, and usually healthy and disease free plant material was collected, cleaned so as to remove soil or any dirt attached to it. After that the material is shade dried and grinded to get a fine powder. This powder is stored in an air tight container for further experiments.

Standardization of plant material: The process of standardization can be achieved by stepwise pharmacognostic

studies. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine which will contribute to its safety and efficacy.

The study includes

Phytochemistry: The chemical investigations of this plant consist of percentage extractives, ash analysis, fluorescence analysis, qualitative tests for the presence of starch, proteins, tannins, saponins, reducing sugars, anthroquinones, flavonoids and glycosides were carried out (Harbone, 1973).

Determination of crude fiber: Extract 2g of ground material with ether or petroleum ether to remove fat (initial boiling temperature 35-38 ^{0}c and final temperature 52^{0}c. If fat content is below 1%, extraction may be omitted. After extraction with ether boil 2 g of dried material with 200 ml of sulphuric acid for 30 min with bumping chips. Filter through muslin and wash with boiling water until washings are no longer acidic. Boil with 200 ml of sodium hydroxide solution for 30 min. Filter through muslin cloth again and wash with 25 ml of boiling 1.25% H_2SO_4, three 50 ml portions of water and 25 ml alcohol. Remove the residue and transfer to ashing dish (preweighed dish W_1). Dry the residue for 2h at 130 ± 2^{0}c. Cool the dish in a desiccator and weigh (W_2). Ignite for 30 min at 600 ± 15^{0}c. Cool in desiccators and reweigh (W_3) (Sadashiv and manikam, 1991)

Determination of Total Carbohydrate by Anthrone method: Weigh 100mg of the sample into a boiling tube. Hydrolyse by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N HCL and cool to room temperature.Neutralise it with solid sodium carbonate until effervescence ceases. Make up the volume to 100ml and centrifuge. Collect the supernant and take 0.5 and 1 ml aliquots for analysis. Prepare the standard by taking 0, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard, "0" serves as blank. Make up the volume to 1ml in all the tubes including the sample tubers by adding distilled water. Then add 4ml of anthrone reagent. Heat for eight minutes in a boiling water bath and Cool rapidly and read the green to dark green colour at 630nm.Draw a standard graph by plotting concentration of the standard on the X – axis versus absorbance on the Y-axis. From the graph calculate the amount of carbohydrate present in the sample tube.

Estimation of reducing sugars by Dinitrosalicylic acid method: Weigh 100mg of the sample and extract the sugars with hot 80% ethanol twice (5 ml each time).Collect the supernatant and evaporate it by keeping on a water bath at 80^{0}C.Add 10ml water and dissolve the sugars. Pipette out 0.5 to 3ml of the extract in the test tube and equalize the volume to 3ml with water in all the tubes. Add 3ml of DNS reagent. Heat the content in a boiling water bath for 5min.When the content of the tubes are still warm, add 1ml of 40% Rochelle salt solution. Cool and read the intensity of dark red colour at 510nm. Run a series of standards using glucose (0 - 500µg) and plot a graph (Sadashiv and manikam, 1991).

Estimation of protein by Bradford method: Prepare a series of protein samples in test tubes in the concentration. This is preferably prepared in PBS. Prepare the experimental samples (a few dilutions) in 100µl of PBS. Add 5ml of diluted dye binding solution to each tube. Mix well and allow the colour to develop for at least 5 min but no longer than 30 min. Then red dye turns blue when it binds protein. Read the absorbance at 595 nm. Plot a standard curve using the standard protein absorbance V concentration. Calculate the protein in the experiment sample using the standard curve (Sadashiv and manikam, 1991).

Estimation of Ascorbic acid: Pipette out 5 ml of the working standard solution into a 100 ml of conical flask. Add 10 ml of 4% oxalic acid and titrate against the dye (V_1ml) end point is the appearance of pink colour which persists for a few minutes. The amount of the dye consumed equivalent to the amount of ascorbic acid. Extract the sample (0.5- 5 g depending on the sample) in 4% oxalic acid and make up to a known volume (100 ml) and centrifuge. Pipette out 5 ml of this supernatant, add 10 ml of 4% oxalic acid and titrate against the dye (V_2 ml)(Sadashiv and manikam, 1991).

Statistical analysis: All data presented are means of six determinations along with standard deviations. Statistical analysis used the MS Excel software (CORREL Statistical function) to calculate ascorbic acid, vitamins, total sugars and proteins content.

RESULT AND DISCUSSION

As we all know green plants prepare their own food in the form of carbohydrates and secondary metabolites or biochemical. These secondary metabolites or biochemicalare nothing but phytochemicals which are used in curing most of diseases. The present study includes physiochemical parameters for the preliminary investigation of biochemical compound present in plants and main concern was to find out nutritive value. As we all know nutrients are very important. Nutrients mainly include carbohydrates, fats, proteins, vitamins and minerals and also crude fiber and moisture content. All are equally important for our well-being.

Below mentioned are the wild vegetables selected for the present investigation

S. No.	Name of the Plant	Family	Location
2	*Lactuca runcinata* DC.	Asteraceae	Pune
3	*Portulaca quadrifida* L.	Portulacaceae	Pune

Table 1. Represents the ash value

S. No.	Plant Sample	Lactuca	Portulaca
1	ASH Value	0.41	O.029
2	ACID Insoluble ASH	0.002	0.013

*Results are the mean of three different readings.

As per our present investigation, *Lactuca runcinata* and *Portulaca quadrifida* were used and if we compare both the wild vegetables for their nutritive values and antioxidant values it is observed that *Lactuca* is more nutritious than *Portulaca* as it contains high value for Carbohydrates i.e.

Table 2. Represents the Percentage extractive value

S. No.	Solvent	L. runcinata	P. quadrifida
I	Distilled Water	32%	64%
II	Petroleum Ether	13%	20%
III	Acetone	16%	25%
IV	Methanol	47%	77%
V	Chloroform	16%	47%

*Results are the mean of three different readings

Table 3. Represents the Total phenol content

Estimation of Total Phenols			
Conc.	Total phenol content (mg/100gm)		
	STD	L. runcinata	P. quadrifida
0.2	0.492	0.46	2.087
0.4	0.568	0.68	3.803
0.6	0.813	0.91	4.993
0.8	0.986	1.14	7.095
1	1.361	1.92	7.868
1.2	1.504	2.98	8.293
1.4	1.801	4.79	10.257
1.6	1.898	6.6	10.921
1.8	2.018	7	12.838
2	2.983	10.11	14.013

*Results are the mean of three different readings

Table 4. Represents the antioxidant value

S. No.	Sample	Extract	0.5	1	1.5	2	2.5
		STD	1.03	1.4	1.93	2.54	2.7
Antioxidant Activity by DPPH Method (mg/100gm)							
			0.5	1	1.5	2	2.5
1	*L. runcinata*	Methanol	4.75	2.95	14.2	23.3	24.7
		Butanol	3.39	4.16	5.01	6.15	14.4
		Ethyl Acetate	12.9	14.9	16.4	17.6	19.9
		Chloroform	4.81	8.12	13.3	16.3	17
6	*P. quadrifida*	Methanol	3.45	8.26	11	18.3	22.2
		Butanol	2.16	4.61	9.74	12	16.1
		Ethyl Acetate	8.37	11.8	15.4	17.2	21.6
		Chloroform	5.24	10.2	13.3	16.1	19.4

*Results are the mean of three different readings

Table 5. Represents the Micro-element

Micro Elements		
Elemement	Concentration in PPM	
	P. quadrifida	L. runcinata
Sulfur	1946	8664
Chlorine	5693	36280
Chromium	4.8	3
Cobalt	0.51	3
Nickel	5.3	2.5
Copper	29.1	16
Zinc	44.6	56.5
Bromine	59.5	424.5
Tin	8.9	12.9

*Results are the mean of three different readings

Table 6. Represents the Macro-element

Macro Elements		
Elemement	Concentration in Precentage (%)	
	L. runcinata	P. quadrifida
Silicon	2.323	3.964
Aluminum	0.332	0.9017
Potassium	0.9712	5.899
Calcium	3.491	1.832
Magnesium	4.072	2.916
Phosphorus	0.4395	0.7398
Iron	0.2508	0.6105

*Results are the mean of three different readings

Table 7. Represents the Nutritive values content in both the plants

S. No.	Plant Sample	Moisture content (%)	Crude Fiber (mg/100gm)	Total protein (mg/100gm)	Total Carbohydrate (mg/100gm)	Total sugars (mg/100g)	Total reducing sugars (mg/100g)	Vit A (mg/100gm)	Vit C (mg/100gm)
1	L. runcinata	90%	13.45	120.89	32.97	19.41	5.89	2.73	45%
2	P. quadrifida	82%	9.89	36.34	16.28	8.58	5.36	2.91	68%

*Results are the mean of three different readings.

Table 8. Represents the Fluroscence analysis

Name of the Plant		L. runcinata			P. quadrifida		
S. No.	Chemical/ Reagent	Short	Day	Long	Short	Day	Long
I	Powder as Such	Green	Green	Black	Brown	Golden yellow	yellow
II	Powder + Nitrocellulose	Dark green	Brown	Purple	Yellow brown	Brown	yellow
III	Powder + Nitrocellulose+ 1n NaOH in Methanol	Black	Brown	Purple	Pale yellow	Brown	Yellow green
IV	Powder + Nitrocellulose + 1n NaOH IN Methanol For 30min.	Dark green	Green	Black	Brown	Brownish green	Brown

14.63mg/100gm in plants. Similarly for protein it shows 110.75mg/100gm, for Reducing Sugars it is 8.56mg/100gm and Non- reducing Sugar is 3.52mg/100gm. Whereas *Portulaca* shows 16.28mg/100gm of protein, 8.58mg/100gm of Total sugar is 5.36mg/100gm of Reducing sugar and 2.22mg/100gm of Non- reducing sugar. For antioxidant analysis fresh plant Material was used and different extract were prepared in Methanol, Ethanol, Butanol and Chloroform according to Protocol. The free radical scavenging activity is observed to be optimum in both the plants in methanol extract and comparatively it is better in *Lactuca*. The main aim of the present investigation was to promote the wild vegetables for the greater production and consumption in day today life. High nutrient and antioxidant value are the common features which would lead to better nutrition and health.

Summary and Conclusion

Plants are the most precious thing in the world as it only donates and never demand anything. They are useful in each and every field as in drugs, in making Buildings, for furniture, for all kind of developmental work, parks etc. so we should respect their qualities as they are useful to us in all senses, and we should go forward for plant based products as they are safe to use without any side effects. This study revealed the importance of wild vegetables in diet. *Lactuca* and *Portulaca* both are herbaceous and seasonal plants and grown in a particular season and are unavailable for the rest of the year. Consumption of these wild vegetables is limited and very important as they show not only nutritional value but antioxidant activity too. Green leafy vegetables are rich in antioxidants. Antioxidants are the free-radical scavenging substances. Whenever there is stress it may be of any kind free- radicals are formed in that cell which damages the cell. To overcome this damage plants prepare antioxidant, which bind to these free-radical and don't allow them to move free which reduces the risk of cell damage. Present work gives an opportunity to say that both the plants are equally important, to include in the diet. But we can see a significant difference in both plants and comparably *Lactuca* is more nutritious and has high antioxidant value. So consumption of both the plants as vegetable is good for health and wealth.

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