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Scientific Presentation of Nutritional Component and Anti-Oxidant Potential of *Draksha* (*Vitis Vinifera* Linn.) and *Kashmari* (*Gmelina Arborea* ROXB.)

[*1]Kumbhar Supriya, [2]Dr. Suma Mallya, [3]Vishwanatha U, [4]Naik TI and [5]Prabhu Suchitra

[*1]PG Scholar, Department of Dravyaguna, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Kuthpady, Udupi, Karnataka, India.

[2]Associate Professor, Department of Dravyaguna, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Kuthpady, Udupi, Karnataka, India.

[4]Assistant Professor, Department of Dravyaguna, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Kuthpady, Udupi, Karnataka, India.

[3, 5]Research Officer, Department of Dravyaguna, Sri Dharmasthala Manjunatheshwara College of Ayurveda, Kuthpady, Udupi, Karnataka, India.

Abstract

Introduction: *Draksha* (*Vitis vinifera* Linn.) and *kashmari* (*Gmelina arborea* Roxb.) are best nutritive fruits mentioned in Ayurveda. Both recommended as balya, pittashamaka, rasayana.

Methods: Fresh fruit samples were collected in the fruiting season collected and used for study. Nutritional value and anti-oxidant activity by in-vitro methods like DPPH assay, reducing power assay, hydroxyl radical scavenging activity and total phenolic content were estimated as per standard protocol.

Discussion and Results: Nutritional assessment of *draksha* showed the presence of 0.26% of fats, 10.2% carbohydrates, 0.33% proteins, fibre content as 1.0% with the total nutritional value of 44.46 Cal/100g. At the same time *kashmari* showed 2.84% fat, 8.0% carbohydrates, 6.0% proteins and 2.30% fibre and nutritional value as 81.56 Cal/100g. On comparing the anti-oxidant activity *Kashmarya* showed better anti-oxidant potential in DPPH assay, Hydroxyl Radical Scavenging Activity and total Phenolic Content compared to *Draksha*. Both fruits did not show any difference in the reducing power activity.

Conclusion: Present study proved both fruits to contain best nutritive component and best antioxidant property.

Keywords: Nutritional value, anti-oxidant activity, *draksha* (*vitis vinifera* linn.), *kashmari* (*gmelina arborea* roxb.)

Introduction

Ayurveda science of life advocates to take care of health through proper diet sleep and disciplined life style [1]. Fruits and vegetables are integrated part of diet, source of vital nutrients. Fruits are indicated as diet as well as therapeutics in traditional texts [2]. *Draksha* (*Vitis vinifera* Linn.) and *Kashmari* (*Gmelina arborea* Roxb.) are among such nutritive fruits which are advised as *Dahaprashamana, Brahmana, Balya, Pittashamaka* [3]. These are also considered as substitute drugs, which are advised in many *Rasayana yogas* [4]. *Fruits of Kashmari (Gmelina arborea* Roxb.) are recommended in many therapeutic conditions like bleeding piles, fever, thirst, gout, atrophy of foetus, internal haemorrhage, anaemia, greying of hairs. Butyric acid, tartaric acid (trace), resinous matter, saccharine matter, cardiac glycosides & Steroids are chief phytochemical constituents of fruit. Fruits are drupe, fleshy, ovoid, turning yellow orange when ripe with 2 seeds available during late summer [5].

Draksha (Vitis vinifera Linn.) cultivated for its fruits ranging from sour to sweet taste, grown in western parts of India, Punjab, Kashmir, Central Europe, Turkey, Morocco, and Portugal. Sugar, gum, tannin, tartaric acid, citric acid, malic acid, potassium chloride, magnesia and alum are chief phytochemical constituents of fruits. These are laxative, cooling, antiallergic, digestant, haemostatic and anti-inflammatory. Both the fruits find mentioned in *madhura skanda*, *phala varga, virechanopaga dashemani* and they also form a part of the trio-'*Madhura Triphala'*, possessing Madhura rasa, Sheeta veerya and rejuvenating [6].

New drug research, adding scientific documentation for the facts mentioned in Ayurveda is a need of the hour. Antioxidants are the molecules that quench free radical damage, there by stabilizing the cells and preventing the

***Corresponding Author:** Dr. Suma Mallya

damage [7]. There are several nutrients in food that contain antioxidants and these found to be essential elements in prevention of hazardous diseases like cancer, cardiovascular diseases etc [8]. Hence with all these backgrounds it has been planned to measure nutritional component and antioxidant potential of these two fruits.

Materials & Methods

Materials

Matured ripened fruits of *Draksha* (*Vitis vinifera* Linn.) and *kashmari* (*Gmelina arborea* Roxb.) were collected from their natural habitat during fruiting season. Authentication was done using floras and the botanist's opinion and the samples were deposited at SDM Centre for Research in Ayurveda and Allied Sciences. Fresh fruit sample used for the study [9].

Methodology

Nutritional Value Assessment

Estimation of fat, fibre, protein and carbohydrate were done as per standard protocol [10, 11, 12].

Total Fat Estimation

In two separate thimbles, 5 g of each fruit sample placed into a Soxhlet apparatus fitted with a condenser. 150 ml Round-bottom flask was filled with 90 ml of petroleum ether (B.P. 40-60°C), which was then heated for six hours. Petroleum ether was evaporated on a water bath while the extracted substance was placed in a pre-weighed conical flask and a vacuum pump was used to eliminate any remaining petroleum ether residues. The weight of fat was taken to a constant weight.

Crude Fibre Estimation

About 5 g of moisture and fat free sample obtained after removal of fat was weighed subjected to standard methods. It was then transferred to a crucible, dried over night at 80-100ºC in hot-air oven and weighed (W_e). The crucible heated in a muffle furnace at 600ºC for 2-3 h, cooled and weighed again (W_a). The difference in the weights (W_e-W_a) represents the weight of crude fibre content in the drug.

$$\text{Crude fibre (g/100g of the sample)} = \frac{[100-(\text{moisture} + \text{fat})] \times (W_e - W_a)}{\text{Wt of the sample taken (moisture and fat free)}}$$

Total Protein Estimation

1 g of each fruit sample mixed with 3ml of water and gently heated with constant stirring. 1 ml of supernatant fluid was taken and was made up to 10 ml with 95% alcohol, mixed well and centrifuged at 3000 rpm for 15 minutes. The precipitate obtained was dissolved in 1 ml of 0.1N NaOH. From that, 10 µl (0.01 ml) was taken for the estimation. Standard method was followed to estimate protein absorbance of samples (Bovine Serum Albumin), and the absorbance of which was read at 650nm against blank. The protein content of the sample was calculated by comparing with the standard and the value of protein was expressed in percentage.

Total Carbohydrate Estimation

100 mg of the sample was weighed and added into a boiling tube. It was hydrolysed by keeping it in a boiling water bath for 3hrs with 5 ml of 2.5 N HCl and cooled to room temperature.

The percentage of carbohydrate was calculated by following formula

Carbohydrate = 100-[percentage of ash + percentage of moisture + percentage of fat + percentage of protein]

Nutritive Value

Nutritive value is calculated by following formula

= 4 x percentage of protein + 9 x percentage of fat + 4 x percentage of carbohydrate

In-Vitro Anti-Oxidant Study[13,14,15]

DPPH Assay

Standard (Vitamin C) and test drug sample were taken at various concentrations and placed in different test tubes. The DPPH assay was done by standard method. Moreover, measurement of absorption at 517 nm was made. Percentage inhibition of the discoloration of DPPH by the extract was expressed as follows:

DPPH scavenging activity (%) = [(OD of Blank ─ OD of Sample)/OD of Blank] × 100

Reducing Power Assay

The reducing power assay of both sample was done using standard method (Oyaizu.1986). [6] The absorbance was measured by spectrophotometer reading at 700 nm after 10 minutes of incubation at room temperature. A higher absorbance of reaction mixture indicates the greater reducing power.

Hydroxyl Radical Scavenging Activity

The capacity of test drug sample to scavenge hydroxyl radicals was assessed using the Halliwell *et al*. technique. Using a spectrophotometer, the solution's absorption at 532 nm was determined. The hydroxyl radical scavenging capacity was evaluated with the inhibition of percentage of 2-deoxy-d-ribose oxidation on hydroxyl radicals. The percentage of hydroxyl radical scavenging activity was calculated according to the following formula:

$$\% \text{ Hydroxyl radical scavenging activity} = [A_0 - (A_1 - A_2)] \times 100/A_0$$

Where A_0 is the absorbance of the control without a sample A_1 is the absorbance after adding the sample and 2-deoxy-D-ribose, A_2 is the absorbance of the sample without 2-deoxy-d-ribose. Then the percentage of inhibition was plotted against concentration, and from the graph IC50 was calculated. The experiment was repeated three times at each concentration.

Total Phenolic Content

Various concentrations of 0.4 ml of test drug samples, a standard sample (gallic acid), and a blank sample were taken separately. The total phenolic content was determined by Folin-Ciocalteu method. After measuring the absorbance at 765 nm, a calibration curve was plotted with gallic acid as the standard. The result was then expressed as Gallic acid equivalent (mg GAE/100 ml).

Result

Nutritional Value Assessment

Table 1: Nutritional value assessment of fruits of *Draksha* (*Vitis vinifera* Linn.) and *Kashmarya*(*Gmelina arborea* Roxb.)

Sl. No.	Parameter	Results n = 3%w/w	
		***Draksha* (*Vitis vinifera* Linn.)**	***Kashmari* (*Gmelina arborea* Roxb.)**
1.	Total fat (%)	0.26	2.84
2.	Total fibre (%)	1.0	2.30
3.	Total carbohydrates (%)	10.2	8.0
4.	Total proteins (%)	0.33	6.0
5.	Nutritive value (Cal/100g)	44.46	81.56

In-Vitro Anti-Oxidant Study

DPPH Assay

Fruit of Kashmarya and *draksha* showed gradual increase in the percentage inhibition of free radicles between 1-1000 µg/mL concentration compared to vitamin C. *kashmarya* showed more percentage inhibition till 100 µg/mL of dilution compared to *draksha. (Table 2 and Figure)*

Table 2: DPPH Assay of *Draksha* (*Vitis vinifera* Linn.) and *Kashmarya*(*Gmelina arborea* Roxb.)

Conc. (µg/ml)		Percentage inhibition			SE		
		Vitamin C	*Kashmari*	*Draksha*	Vitamin C	*Kashmari*	*Draksha*
1 µg/ml	1	44.217	44.996	44.627	0.144	0.287	0.041
2 µg/ml	2	45.035	45.755	44.955	0.103	0.226	0.082
4 µg/ml	4	48.336	48.318	45.406	0.021	0.246	0.369
8 µg/ml	8	52.482	48.339	46.001	0.021	0.226	0.226
10 µg/ml	10	57.732	48.523	46.103	0.021	0.164	0.164
20 µg/ml	20	61.464	51.395	47.518	0.041	0.164	0.062
40 µg/ml	40	73.677	52.051	49.118	0.185	0.164	0.308
80 µg/ml	80	78.560	52.194	49.487	0.062	0.267	0.062
100 µg/ml	100	79.078	54.861	50.103	0.062	0.021	0.349
200 µg/ml	200	82.752	56.214	56.235	0.004	0.226	0.369
400 µg/ml	400	83.696	60.275	61.403	0.062	0.636	0.410
800 µg/ml	800	84.943	67.309	68.970	0.062	0.410	0.349
1000 µg/ml	1000	85.357	73.811	71.308	0.205	0.226	0.554

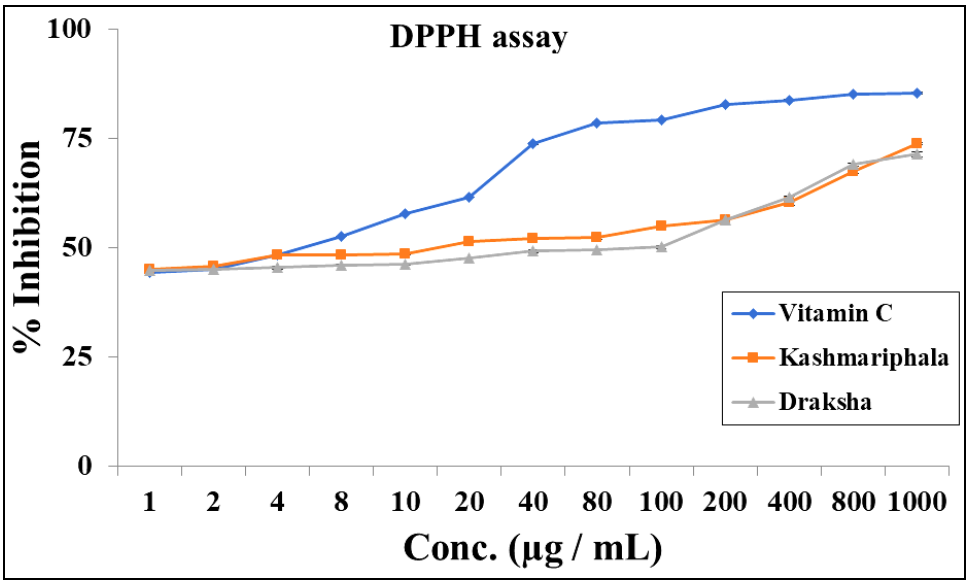

Fig 1: Graphical representation of Anti-oxidant activity by DPPH method

Reducing Power Assay

Both fruit sample did not show any difference in the reducing power activity from 1-1000 µg/mL compared to Vitamin C.(Table and figure)

Table 3: Reducing power Assay of *Draksha* (*Vitis vinifera* Linn.) and *Kashmarya*(*Gmelina arborea* Roxb.)

Conc. (µg/mL)	Percentage inhibition			SE		
	Vitamin C	*Kashmariphala*	*Draksha*	Vitamin C	*Kashmariphala*	*Draksha*
1	0.432	0.001	0.002	0.0006	0.0015	0.0028
2	0.445	0.002	0.004	0.0039	0.0024	0.0008
4	0.464	0.003	0.007	0.0053	0.0014	0.0028
8	0.474	0.004	0.009	0.0094	0.0017	0.0029
10	0.478	0.009	0.011	0.0066	0.0009	0.0022
20	0.486	0.012	0.011	0.0082	0.0001	0.0019
40	0.489	0.012	0.012	0.0061	0.0001	0.0014
80	0.493	0.013	0.013	0.0055	0.0003	0.0017
100	0.495	0.015	0.015	0.0076	0.0024	0.0027
200	0.516	0.016	0.016	0.0193	0.0018	0.0019
400	0.525	0.021	0.019	0.0210	0.0038	0.0052
800	0.529	0.024	0.020	0.0182	0.0006	0.0041
1000	0.534	0.030	0.021	0.0223	0.0048	0.0038

a) Reducing Power Assay

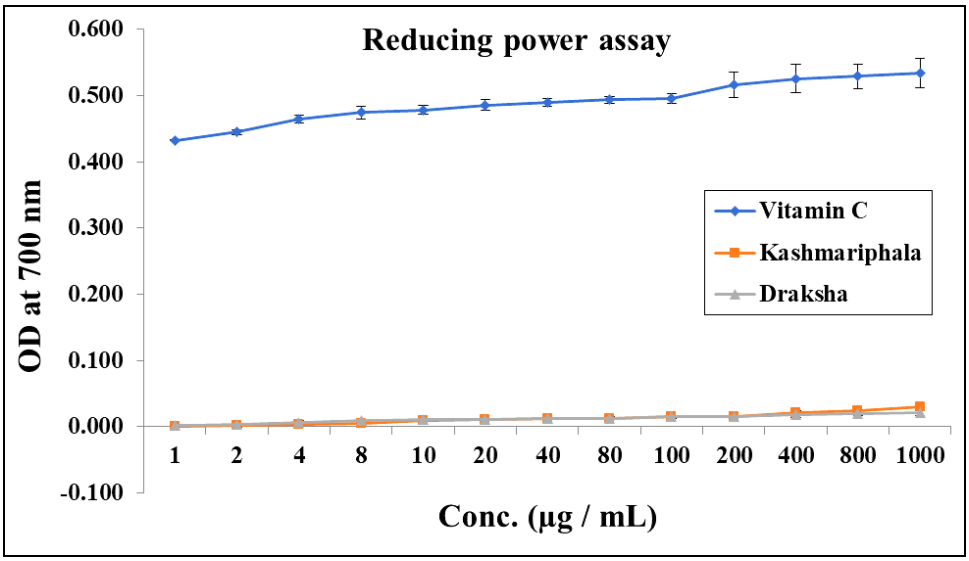

Fig 2: Graphical representation of Anti-oxidant activity by Reducing power assay method

Hydroxyl Radical Scavenging Activity

Kashmarya showed a gradual increase in the percentage inhibition of scavenging activity compared to *draksha* between 1-1000 µg/mL concentrations. Overall, *kashmarya* showed higher hydroxyl radical scavenging activity compared to *draksha*.

Table 4: Hydroxyl Radical Scavenging Activity of *Draksha* (*Vitis vinifera* Linn.) and *Kashmarya*(*Gmelina arborea* Roxb.)

Conc. (µg/mL)	Percentage inhibition		SE	
	Kashmariphala	*Draksha*	*Kashmariphala*	*Draksha*
1	30.095	7.629	0.050	2.408
2	33.794	11.998	0.571	1.018
4	38.287	12.817	0.099	1.787
8	38.461	13.438	0.273	1.763
10	41.017	14.589	0.496	0.730
20	45.585	15.399	0.050	0.199
40	45.982	15.721	0.149	0.372
80	48.639	18.597	0.770	0.525
100	53.231	20.278	0.099	0.184
200	54.298	23.705	0.223	1.355
400	58.742	44.394	0.298	9.384
800	61.622	55.887	0.099	0.472
1000	63.086	59.395	0.074	0.092

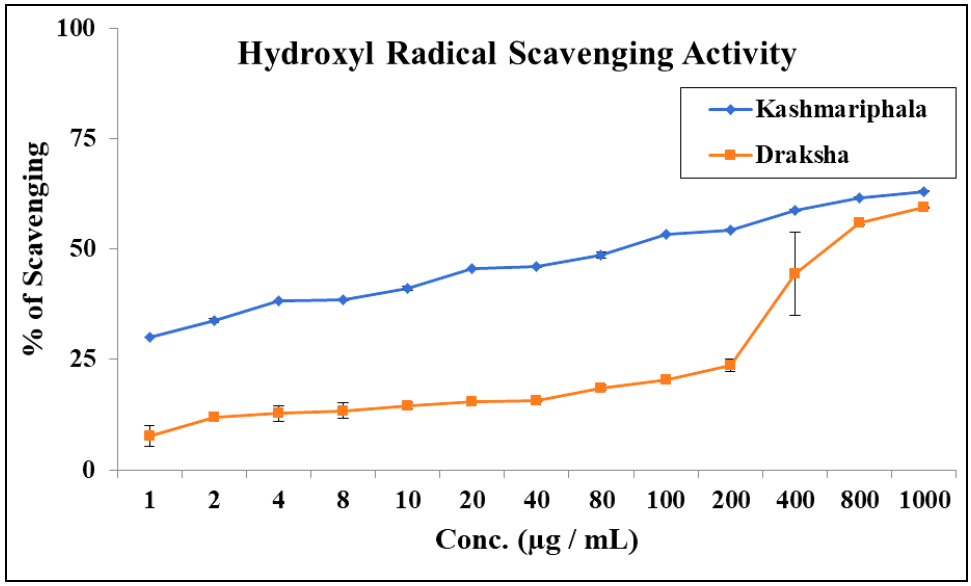

Fig 5: Graphical representation of Anti-oxidant activity by Hydroxyl Radical Scavenging Activity method

Total Phenolic Content

Total phenolic content both sample were compared with reference to gallic acid (µg GAE/100 mL) with a wide concentration of 1-1000 µg/mL. At 1-100 µg/mL concentrations, both fruit showed a gradual increase in total phenolic content where *kashmarya* showed a slight high concentration of total phenolic content compared to *draksha.*

Table 5: Total phenolic content estimation of *Draksha* (*Vitis vinifera* Linn.) and *Kashmarya*(*Gmelina arborea* Roxb.)

Conc. (µg/ml)	Percentage Inhibition		Gallic Acid (mg GAE/100 mL)	
			Kashmari Phala	Draksha
1 µg	1	0.003	2.4	1.35
2 µg	2	0.008	3.65	1.55
4 µg	4	0.009	6.75	2.35
8 µg	8	0.011	7.85	3
10 µg	10	0.016	8.5	4.85
20 µg	16	0.018	9	7.05
40 µg	20	0.024	9.25	8.45
80 µg	40	0.045	9.8	9
100 µg	80	0.092	10.25	10.2
200 µg	100	0.108	10.65	10.5
400 µg	200	0.224	10.7	12.75
800 µg	400	0.407	18	17.4
1000 µg	800	0.763	32.85	21.45

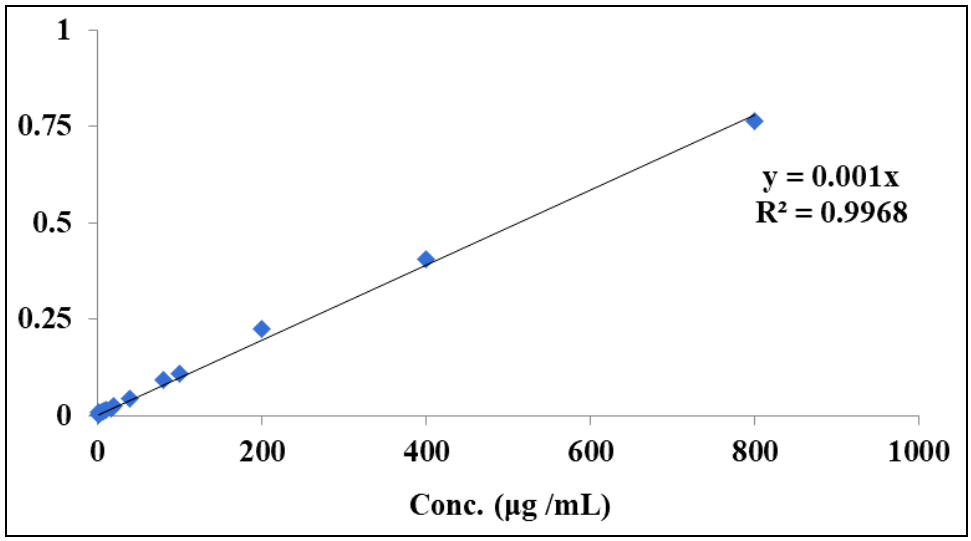

Fig 6: Graph showing protein Absorbance in Vitamin C

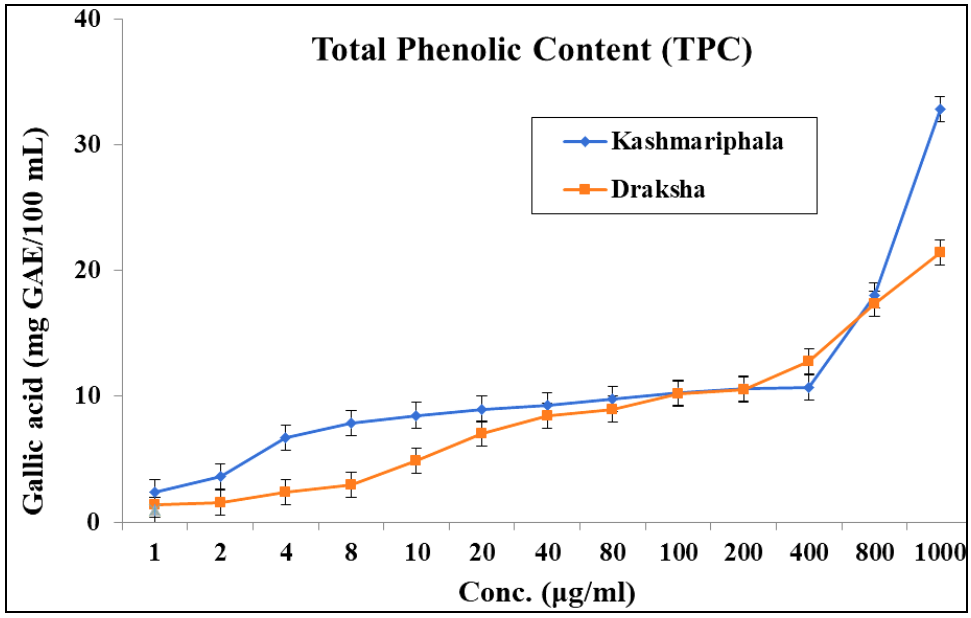

Fig 7: Graphical representation of Anti-oxidant activity by determining Total Phenolic Content method

Discussion

Fruits of Draksha and Gambhari are said to be *pittashamaka, rasayana, brahmaneeya, dahashamana*, mentioned as substitute drugs, indicated in many therapeutic conditions in treatises of Ayurveda. Fruits are edible advised as food condiments as well as medicine in many diseases[16].

Nutritional component assessment indicative of their primary nutrients. Nutritional value estimation depicted that the sample of *Kashmari (Gmelina arborea)* had more total fat, fibre and protein content than that of *Draksha(Vitis vinifera).* But carbohydrate content found more in *Draksha (Vitis vinifera)* (10.2*)* than other.

Natural antioxidants derived from herbs can prevent oxidative stress. Free oxygen radicals play a cardinal role in the aetiology of several diseases like cancer, arthritis, atherosclerosis etc. The oxidative damage to DNA may play a vital role in aging. Natural antioxidants quench these free radicles, reduce oxidative damage, there by cell injury and are termed as rejuvenators[17].

In-vitro Antioxidant activity of ethanolic extract of test drug was performed through DPPH, reducing power activity, hydroxyl radical scavenging activity and estimation of total phenolic content.

DPPH assay of *Kashmari (Gmelina arborea)* showed more percentage inhibition till 100 µg/mL of dilution compared to *Draksha (Vitis vinifera).* But both fruit sample did not show any difference in the reducing power activity from 1-1000 µg/mL compared to Vitamin C.

Kashmari (Gmelina arborea) showed higher hydroxyl radical scavenging activity compared to *Draksha(Vitis vinifera).* Total phenolic content of *Kashmari (Gmelina arborea)* showed a slight high concentration compared to *Draksha(Vitis vinifera).*

Conclusion

Fruits of *Draksha(Vitis vinifera)* and *Kashmari (Gmelina arborea)* are best fruit drugs mentioned as balya, rasayana. Present study proved both fruits to contain best nutritive component and best antioxidant property.

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