

## Diurnal variation in the flavonoids and phenolic content in *Desmostachya bipinnata*: A medicinal plant

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### Abstract

*Desmostachya bipinnata* is a medicinal plant and contain many chemical constituents. Medicinal property of Plant is mostly affected by 'Time (Kala)' factor. As regards to time, it can be seasonal variation, lunar influence and diurnal variation. The concentration of chemical constituents in plants varies at different times of the day. For this work *Desmostachya bipinnata* plant was collected on the same time in morning (7:00 a.m.) as well as in evening (7:00 p.m.) during winter season. Extraction process of collected plant parts were carried out by Soxhlet apparatus. Then extract was analysed for qualitative and quantitative chemical test. This work shows the variation in concentration of flavonoids and phenol constituents. So, this work concluded that plant collection in morning time contain more flavonoids and phenol compound than plant collected in evening time.

**Keywords:** *Desmostachya bipinnata*, diurnal variation, chemical constituents, etc

### Introduction

*Desmostachya bipinnata* is herbaceous, perennial, rhizomatous, C<sub>4</sub> plant and salt tolerant grass. It is found all over in Bharat in hot, dry places and in desert and semi-desert condition. It is a sacred grass because it is used in many Hindu rituals or in ceremonies. It belongs to family Poaceae or Gramineae. *Desmostachya bipinnata* also known as hardiest grass because its roots can go as deep as until they find sub soil water [6]. *Desmostachya bipinnata* commonly known as Darbha, Dabh, Kush etc. [2]. According to AIP (Ayurvedic Pharmacopoeia of Bharat) in different language *Desmostachya bipinnata* known as: Sanskrit - Yagyabhusana, Sucyagra: Hindi - Kush: Gujarati - Dabb: English - Saved gram: Marathi - Darbha.

Traditionally *Desmostachya bipinnata* used as medicinal in plant in urinary tract contamination, syphilis, toothache, dysentery, diarrhoea, etc. [7]. Spiritually *Desmostachya bipinnata* used for protection from all types of negative energies and toxic radiation. It is used for making asana called 'Darbhasana' which is used for prayer and meditation and protect the person from negative spiritual energies. It is also utilized for purification of water during fire ceremony by keeping plant leaf in Kumbha. It is said that, due to the creation of hazardous radiation during solar eclipse as well lunar eclipse, the cooked food become toxic. So, traditionally Darbh is used to protect cooked foods during both eclipse. Darbh leaves containing water sprinkled around the home, workplace, and vehicle to protect from ghosts, demons and black magic [3].

The concentration of plant constituents varies during different times of the day. This variation of the constituents is thought to be due to variation in temperature or light condition. To make good quality medicine, it is necessary to collect the plant or plant parts with concentration of more constituents at a specific time. Therefore, while collecting plant, it is extremely

Significant to show the collection time of plant sample. Thus the collection time of plant sample plays a significant role in the diurnal variation. Hence some plants were accumulated in morning time as well as some in evening time or some at night. So, the constituents with more concentration obtained from plant [4].

### Materials and methods

#### Collection of plant material

The plants of *Desmostachya bipinnata* were collected from Segvi area in Valsad, Gujarat, Bharat in morning at 7:00 a.m. and in evening at 7:00 p.m. The plants were shade dried at room temperature for a duration of one month depending on the water content. The completely air dried materials were powdered by mixture grinder. The powdered material was stored in zip-lock bags or air tight bottle having labels at room temperature. The powdered material was used for the preparation of the extract, estimation and phytochemical test.

#### Preparation of plant extract

The air dried plant was finely grinded and extracted with alcohol (Methanol) using Soxhlet apparatus. About 5 grams of powder was loaded in Soxhlet extraction unit and exhaustively extracted using 150 ml of solvent such as methanol at 60 °c for 6 hours. Thereafter the extract was concentrated by evaporating the methanol using the heating mental and used for testing. The concentrated dried residue was weighed and then diluted with 20 ml methanol and this crude extract kept at 4°C for further analysis.

#### Phytochemical analysis

The extract obtained from plant was subjected to Qualitative and Quantitative tests for the identification of various plant constituents. Phytochemical analysis is essential for the quantitative analysis of chemical compounds [5].

**Qualitative phytochemical analysis****A. Chemical test for Alkaloids****1. Mayer's test****Mayer's reagent**

In a 120 ml. distilled water dissolve 2.72 g  $\text{HgCl}_2$  and in 40 ml distilled water dissolve 10g KI. Make a final volume 200 ml by dissolving both solutions.

**Process**

In 1 ml. plant sample extract added 1 ml Mayer's reagent. Formation of white creamy precipitates represent the presence of alkaloids.

**2. Wagner's test****Wagner's reagent**

2.5 g Iodine dissolves in 12.5 g KI (Potassium iodide). Add 250 ml distilled water to form a Wagner's reagent.

**Process**

Some drops of Wagner's reagent were added to a 1 ml plant extract along the sides of the test tube. Reddish-brown precipitate endorses that this test is positive.

**B. Chemical test for Flavonoids****3. Alkaline reagent test**

1 ml. extract was tested by mixing some drops of NaOH (Sodium Hydroxide) solution. Generation of acute yellow colour, which ultimately becomes colourless on adding dilute acid. This imply the entity of flavonoids.

**4. Lead acetate test**

1 ml extract was examined by mixing some drops of Lead Acetate solution. The creation of yellow colour precipitate represent the entity of flavonoids.

**5. Chemical test for Tannins - Braymer's test**

Add 10% alcoholic ferric chloride solution to 1 ml extract. Production of brownish blue or black color represent the entity of tannins.

**C. Chemical test for Phenol****6. Lead acetate test**

To a 1 ml plant sample extract, 3 ml - 10% lead acetate solution was added. Production of a bulky white precipitate represent the entity of phenolic compounds.

**7. Ferric Chloride test**

To a 1 ml plant sample extract, some drops of neutral 5% ferric chloride solution were added. Formation of a dark green color represent the entity of phenol compounds.

**D. Chemical test for Protein****8. Millon's test****Millon's reagent**

Dilute the solution of 10 g of mercury dissolved in 10 ml of concentrated nitric acid in 20 ml water.

**Process**

To 1 ml of plant sample extract some drops of Millon's reagent were added. Creation of a white precipitate represent the entity of proteins.

**9. Biuret test**

1 ml of plant sample extract was treated with 1 % of NaOH and some drops of 1 % copper sulphate ( $\text{CuSO}_4$ ) solution. Formation of Blue or purple or violet or pinkish colour represent the entity of proteins.

**10. Ninhydrin reagent test**

A couple of drops of ninhydrin solution added into 1 ml of the filtrate will make solutions colour purple which proves the entity of proteins.

**E. Chemical test for Carbohydrates****11. Molish's test**

The mixture of 1 ml sample plant extract and a couple of drops of alcoholic solution of  $\alpha$  - naphthol was shaken well. While shaking, some drops of concentrated sulphuric acid were added slowly from the sides of the test tube. Carbohydrates are occur in this solution as the violet ring is produced inside.

**12. Benedict's test****Benedict's reagent**

To 800 ml distilled water added 173 g  $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$  (Sodium citrate) and 100 g  $\text{Na}_2\text{CO}_3$  (Sodium carbonate) and boiled to make it clear. To 100 ml distilled water added 17.3 g Copper sulphate.

**Process**

To 1 ml plant sample extract, added 0.5 ml Benedict's reagent. For a couple of minutes, this mixture was heated in a boiling water bath. A characteristic coloured precipitate implies the appearance of sugar.

**F. Chemical test for Cardiac glycosides**

To 1 ml plant sample extract added 0.5 ml  $\text{CH}_3\text{COOH}$  (glacial acetic acid) and some drops of 1 % aqueous ferric chloride solution. Creation of brown ring at the interface represent the entity of cardiac glycosides.

**G. Chemical test for Terpenoids**

To 1 ml plant sample extract, 0.5 ml chloroform and some drops of concentrated  $\text{H}_2\text{SO}_4$  (sulphuric acid) were added. Creation of reddish brown precipitates implies the entity of terpenoids in selected plant samples.

**H. Chemical test for Saponins – Foam Test**

To a 2 ml water added 0.5 gm extract was shaken. Formation of foam exists for ten minutes it represent the entity of saponins.

**Quantitative phytochemical analysis****Estimation of total Flavonoids**

Total flavonoid of plant extract was examined by the Aluminium chloride method <sup>[1]</sup>. 100  $\mu\text{l}$  plant sample extract in methanol was taken and added 100 $\mu\text{l}$  20%  $\text{AlCl}_3$  in methanol and some drops of Acetic acid in plant sample extract, then added 5 ml methanol to this mixture. The mixture was kept for 40 minutes, and measured absorbance at 415 nm. Blank sample: Take 100 ml plant extract and added some drops of acetic acid and diluted this mixture with 5 ml methanol. Made standard Quercetin solution in methanol and measured under same conditions.

**Estimation of total Phenol**

The total phenol content of plant sample extract was examined by the method of Folin Ciocalteu's method <sup>[8]</sup>. 1 ml plant extract was taken and added 5 ml of distilled water with 0.5 ml of Folin Ciocalteu's reagent. The mixture was mixed and shaken. After 5 minutes added 1.5 ml of 20% sodium carbonate solution and make the final volume 10 ml with distilled water.

The mixture was kept for 2 hours, and measured absorbance at 750 nm.

The total phenol compound was counted from the calibration curve, and the result were exhibited as mg of Gallic acid equivalent weight per 100 g of dry mass.

## Result and Discussion

### Qualitative phytochemical analysis

In phytochemical study qualitative estimation of alcohol extract of morning and evening

Samples of *Desmostachya bipinnata* (L.) of winter season performed for Alkaloids, Flavonoids, Tannins, Phenol, Protein, Carbohydrates, Cardiac glycosides, Terpenoids and Saponins.

**Table 1:** Phytochemical analysis, Qualitative estimation of alcohol extract of *Desmostachya bipinnata* (L.)

Sr. No.	Plant Constituents	Test / Reagent	Winter	
			Morning	Evening
1	Alkaloids	Mayer's reagent Wagner's reagent	-	-
2	Flavonoids	Alkaline reagent Lead acetate test	+	+
3	Tannins	Braymer's test	-	-
4	Phenols	Lead acetate test Ferric Chloride test	+	+
5	Protein	Millon's test Biuret test Ninhydrin test	+	+
6	Carbohydrates	Molish's test Benedict tsat	+	+
7	Cardiac glycosides		+	+
8	Terpenoids	Salkowski test	+	+
9	Saponins	Foam test	+	+

Legend: + ve: Present, -ve: Absent

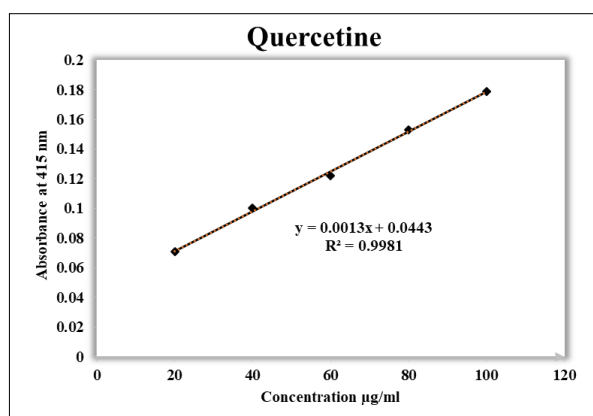
Qualitative phytochemical study of extract of alcohol of *Desmostachya bipinnata* showed (Table: 1) the appearance of phenols, flavonoids, protein, carbohydrates, cardiac glycosides, terpenoids and saponins in both morning and evening samples. Alkaloids and tannins were absent in both morning and evening samples.

### Quantitative phytochemical analysis

#### Total Flavonoids content

**Table 2:** Result of total Flavonoids content in alcohol extract of *Desmostachya bipinnata* (L.) during winter season

Season	Time	Total Flavonoids content in alcohol extract (mg of Quercetine equivalent/ g of dry mass)
Winter	Morning	42.8
	Evening	18.2



**Fig 1:** Standard curve of Quercetine

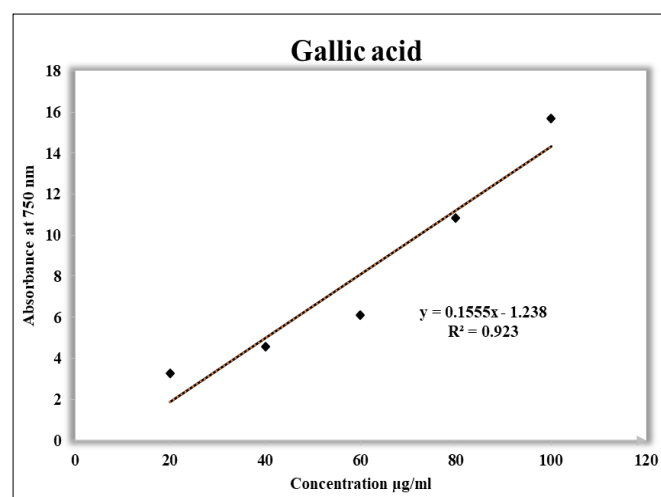
A quantitative phytochemical analysis of alcohol extract (morning and evening sample) of *Desmostachya bipinnata* of winter season was performed. The total flavonoid compound was estimated by Aluminium chloride method (where Quercetine equivalent in mg/ g of dry mass) and calculated with the help of the graph (Figure: 1) and standard curve equation was  $y = 0.0013x + 0.0443$ , where

$R^2 = 0.9981$ . The concentration of total flavonoids of morning sample was 42.8 mg/g and evening sample was 18.2 mg/g. So, the highest flavonoid compound was noted higher in alcohol extract of winter season in morning sample. Flavonoids are a cluster of plant metabolites believed to supply health advantage via cell signalling channels and antioxidant effects. They are important antioxidants and promote several health effects. Flavonoids perform several defensive performance in the human body. Aside from antioxidant activity, these molecules provide the antiviral, anti-cancer, anti-inflammatory and anti-allergic beneficial effects.

#### Total Phenol content

**Table 3:** Result of total Phenol content in alcohol extract of *Desmostachya bipinnata* (L.) during winter season

Season	Time	Total Phenol content in alcohol extract (mg of Gallic acid equivalent/ g of dry mass)
Winter	Morning	16.4
	Evening	11.5



**Fig 2:** Standard curve of Gallic acid

A quantitative phytochemical analysis of alcohol extract (morning and evening sample) of *Desmostachya bipinnata* of winter season was performed. The total phenol content was estimated by Folin Ciocalteu's method (where Gallic acid equivalent in mg/ g of dry mass) and calculated with the help of the graph (Figure: 2) and standard curve equation was  $y = 0.1555x - 1.238$ , where  $R^2 = 0.923$ . The concentration of total phenol of morning sample was 16.4 mg/g and evening sample was 11.5 mg/g. So, the highest phenolic content was noted higher in morning sample. Phenolic compounds were produced by plants and microorganisms. They universally exist in the plant group and are the most broadly assorted secondary metabolites. They are strong antioxidant.

### Conclusions

This study concluded that the morning extract of alcohol of *Desmostachya bipinnata* of winter season shown more concentration of Flavonoids and Phenol contents than the evening extract. So, the most convenient time to collect the medicinal plant for preparing the drug could be given through this study.

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