

# Phytochemical Screening and Evaluation of Antioxidant Activity of Sacred plant *Cynodon dactylon* (Linn.) Pers.: Karuka (God: Lord Surya) in the context of Western Ghats Biodiversity Conservation Kerala

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## Abstract

The Western Ghats region, where in the Kerala state is situated, is one of the 34 biodiversity hotspots in the whole world. A fairly good collection of plants over 200 taxa used in Ayurveda include dashapushpam, ten sacred plants of Kerala tradition and culture. Kerala state is famous for its medicinal plant wealth and the tradition of indigenous system of therapy, specifically the Ayurveda. Among the sacred plants dhasapushpam karuka, the herb is known as Durva, belonging to family Poacea can grow in poor soil. Herbs have been utilized to treat acute and chronic disorders for thousands of years. Natural products, due to their easily availability, low cost and lack of side effects, make themselves attractive for research. The aims of this study were to evaluate antioxidant activities and to screen for phytochemical constituents of one among the plant in Dashapushpam. The phytochemical analysis reveals the presence of Pholobotannin, Alkaloids, Flavonoids, Anthraquinone, Saponins, Steroids, Glycosids, Tannin, Total phenol and Terpenoids and also report the antioxidant activity.

**Keywords:** Phytochemicals, Antioxidants, Dhasapushpam

## Introduction

Western Ghats, also known as the Sahyadri Hills, are well known for their rich and unique assemblage of flora and fauna. Among the 25 hot spots of the world, two are found in India extending into neighboring countries - the Western Ghats or Sri Lanka and the Indo-Burma region (covering the Eastern Himalayas). The Western Ghats are a chain of highlands running along the western edge of the Indian subcontinent, from Bombay south to the southern tip of the peninsula, through the states of Maharashtra, Karnataka, Kerala and Tamil Nadu. Covering an estimated area of 159,000 sq. km, the Western Ghats is an area of exceptional biological diversity and conservation interest, and is "one of the major Tropical Evergreen Forest regions in India". The state contains more than 4,500 species of flowering plants of which above 1,500 taxa are endemic in nature. Kerala state is famous for its medicinal plant wealth and the tradition of indigenous system of therapy, specifically the Ayurveda. During the past 40 to 50 years the plant and animal life has as a whole suffered due to so-called development and urbanization, which has led to the extinction of many spe-

cies and more are in danger of becoming extinct. The medicinal plants having much of phytochemicals and stress releasing antioxidants. Phytochemicals are the dependable sources for the treatment of different health problems. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. So the conservation of Western Ghat biodiversity is one of the major concerns, not expecting any reward to conserve our environment as a mother earth.

*Cynodon dactylon* is an herbaceous plant commonly known as Karuga belonging to family Poaceae can grow in poor soil. It is a creeping grass growing throughout the country. Bermuda grass or Dhub grass was considered as a sacred grass by the Hindus, and it is still used for worships in temples. In ancient days, the Romans squeezed the juice from the stems and used it as a diuretic and also used to stop bleeding. It grows in open areas where there are frequent disturbances such as grazing, flooding, and fire. The various common names are Bermuda grass, Bahama grass, Devil's grass. The parts used medicinally are the stems and the leaves. The leaves are narrowly linear or lanceolate and finely acute with 2-10cm length and 1.25 - 3mm width. The stem is slender, very smooth and yellowish-green in colour. The roots are cylindrical, cream coloured and upto 4 mm thick. The grass is a remedy in epitaxis, haematuria, inflamed tumours, whitlows fleshy excrescences, cuts, cystitis, nephritis and in scabies and other skin diseases. Herb is possessing astringent, antiscabietic, styptic properties. The Ayurvedic Pharmacopoeia of India describes the dried

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fibrous root in menorrhagia, metrorrhagia and burning micturation. It is also reported to be antiseptic, demulcent, diuretic, and emollient. A decoction of the root is used to stop bleeding from piles. Internally it is used in the treatment of chronic diarrhoea and dysentery. The leaf juice has also been used in the treatment of hysteria, epilepsy and insanity. The plant is a folk remedy for headache, haemorrhage, hypertension, measles, snake bite, uro-genital disorders warts and wounds. (Jiny Varghese, *et al.*, 2010).

## Materials and Methods

The collected whole plant materials were washed thoroughly 2-3 times with running tap water, plant material was then air dried under shade to avoid degradation of volatile oil and after shade drying, dried in hot air oven at 55°C for one day. The dried each plant material was grinded in mixer were coarsely powdered, the powder was kept in small polyethylene bags with label for further use. The grinded samples of 5gm weighed using electronic balance and were mixed in 75mL of Distilled water and it was boiled at 50-60°C for 30 minute on waterbath and centrifuged at 15 minute for 2500rpm and the supernatant again centrifuged and it was filtered through cheese cloth. Then the filtrate was stored in air tight sterile bottles at 4°C for further analysis.

Preliminary Phytochemical screening of plant extract was done by the standard procedure (Behera Saiprasanna, *et al.*, and Savithamma, *et al.*, and Sunita Bhatnagar, *et al.*,) with some modification. All the prepared plant extracts were subjected to preliminary phytochemical screening for the presence of Phlobotannin, Alkaloids, Flavonoids, Anthraquinone, Saponins, Steroids, Glycosides, Tannin, Total Phenol, Terpenoids.

### Alkaloids

1mL of aqueous extract was filtered. Then 2mL of 1% aqueous HCl was added to it. Thereafter it was heated for few minutes. 2 drops of Dragendorff reagent was added to the solution. Reddish brown precipitate with turbidity depicts alkaloid presence. This can be measured at 545nm., using a Spectrophotometer. (Sunita Bhatnagar, *et al.*, 2012).

### Anthraquinone

To 1mL of aqueous extract, 2mL of 5% KOH was added. Then the solution was filtered. Change in colour was observed, shows the presence of anthraquinones. Which can be measured at 515 nm. Using as a spectrophotometer. (Sunita Bhatnagar, *et al.*, 2012).

### Flavonoid

To 5mL of aqueous extract, 1mL of 10% NaOH solution was added. From the side of the beaker 2 drops of concentrated HCl was added. Yellow colour turning to colourless is an indication of presence of flavonoids. Which can be measured at 420nm using as a spectrophotometer. (Sunita Bhatnagar, *et al.*, 2012)

### Glycosides

100µL aqueous extract was taken in a test tube and 400µL of acetic anhydride was added to it. Then 1-2 drops of concentrated sulphuric acid was added to it. Blue-Green colour shows the presence of glycosides. Which can be measured at 495nm using as a spectrophotometer. (Sunita Bhatnagar, *et al.*, 2012)

### Phlobotannin

1mL of aqueous 1% HCl was added to 1mL of sample followed by boiling. A red precipitate is indicative of presence of phlobotanins. Which is measured at 505nm using as a spectrophotometer. (Sunita Bhatnagar, *et al.*, 2012)

### Saponin

About 2mL of 1% sodium bicarbonate was added to 1mL of extract and shaken. Lather like formation persistent for some time is indicative of presence of Saponins. Which can be measured at 408 nm using as a spectrophotometer. (Sunita Bhatnagar, *et al.*, 2012)

### Steroid

1mL of the extract was dissolved in 10mL of Chloroform and equal volume of concentrated H<sub>2</sub>SO<sub>4</sub> was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids. This can be measure at 593nm using as a spectrophotometer. (N. Savithamma, *et al.* 2011)

### Tannin

1gm of sample added with 100mL of distilled water, boiled and cooled, and then filtered. 1% ferric chloride was added drop wise to the filtrate. Green black precipitate shows the presence of tannin. Which can be measured 670nm using as a spectrophotometer. (Sunita Bhatnagar, *et al.*, 2012)

### Total Phenol

To 0.5g each of the extract, 2mL of ferric chloride was added. A reddish brown coloration at the interface indicates the presence of phenols. Which can be measured at 765nm using as a spectrophotometer. (Behera Saiprasanna, *et al.*, 1971)

### Terpenoids (Salkowski test)

To 0.5g each of the extract, 2mL of chloroform was added. Concentrated H<sub>2</sub>SO<sub>4</sub> (3mL) was carefully added to form a layer. A reddish brown coloration at the interface indicates the presence of terpenoids. Which can be measured at 235nm using as a spectrophotometer. (Behera Saiprasanna, *et al.*, 1971)

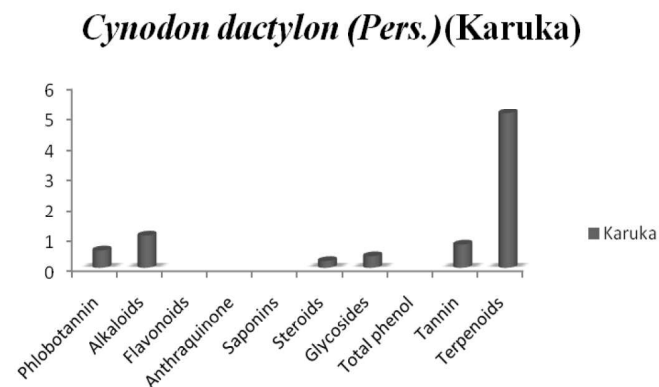
The total antioxidant capacity of different extracts was evaluated according to the method, (K. C. Pushpalatha *et al.*, 2011). The phosphomolybdenum method usually detects antioxidants such as ascorbic acid, some phenolics,

tocopherol, and carotenoids. Samples or standard (1mL) was mixed with 2mL reagent solution (ammonium molybdate (4mM), sodium phosphate (28mM) and sulphuric acid (0.6M). All the reaction mixtures were incubated at 30°C for 60min. Reducing capacity of the extract has been expressed as the ascorbic acid equivalents. The absorbance of the samples were measured at 665nm in UV spectrophotometer. The higher absorbance value indicates higher antioxidant activity.

## Results and Discussion

The Preliminary phytochemical screening of the plant aqueous extracts of showed the presence and absence of Phlobotannin, Alkaloids, Flavonoids, Anthraquinone, Saponins, Steroids, Glycosides, Tannin, Total Phenol, and Terpenoids as chemical constituents. The qualitative result of the Phytochemical Screening are shown in the Table 1. Quantitative Estimation of Phytochemicals are shown in Figure 1. In the qualitative and quantitative analysis, it can be observed from the result, the aqueous extraction contained Phytochemicals are, Tannin (0.749), Steroid (0.218), Glycoside (0.373), Terpenoid (5.070), Alkaloids (1.040), Phenol (1.290) and Terpenoid (2.386). Phytochemicals are biologically active, naturally occurring chemical compounds found in plants, which provide health benefits for humans further than those attributed to macronutrients and micronutrients. They protect plants from disease and damage and contribute to the colour of the plant, aroma and flavour. In general, the plant chemicals that protect

Figure 1. Percentage of Phytochemicals



antioxidants are insufficient to completely remove them and maintain a balance. Still reports on antioxidant properties of Dashapushpam are limited. Since awareness towards natural products in healthcare is rapidly increasing, interest in medicinal plants has been multiply increased. Plants produce many important compounds such as phenolics, Phlobotannin, Alkaloids, Anthraquinone, Saponins, Steroids, Glycosides, Tannin, and Terpenoids and flavonoids which possess antioxidant and antimicrobial properties. Phenolics and flavonoids provide protection against free radicals and regulate various oxidative reactions occurring naturally. Also, they are used to protect food quality mainly by the prevention of oxidative deterioration of constituents of lipids.

Figure 1. Qualitative result of the Phytochemical Screening

Phytochemicals	PT	TN	AL	SN	FN	ST	TS	TP	GS	AQ
Qualitative	-	+	+	-	-	+	+	-	+	-
Quantitative	-	0.749	1.040	-	-	0.218	5.070	-	0.373	-
Antioxidants	NIL									

plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals.

Antioxidants are vital in combating the free radicals which damage human cells under 'oxidative stress' conditions and an imbalance of free radicals may cause grave disturbances in cell metabolism. Oxidative stress conditions can cause DNA and protein damage, lipid peroxidation, cancer, ageing, and inflammatory activities. Free radicals, which are generated in several biochemical reactions in the body, have been implicated as mediators of many diseases, including cancer, atherosclerosis and heart diseases. Although these free radicals can be scavenged by the in vivo produced antioxidant compounds, the endogenous

## Conclusion

Plants are the best source of chemical compounds having different biological properties that could make human life easier by treating various chronic ailments. Unlike the modern drugs that cause many side effects, by using plant derived chemicals many diseases can be cured without any side effects.

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