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Preliminary Phytochemical Screening, *In Vitro and In Vivo* Antioxidant Activities of C*ynodon Dactylon* (L.)Pers.

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Abstract : The present study was carried out to evaluate preliminary phytochemical and antioxidant activity (*in vitro and in vivo)* of the whole plant of C*ynodon dactylon*. Preliminary quality chemical test for different extract revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, sterols, saponins, glycosides, protein, and carbohydrate. The total phenolic, tannin and flavonoid content of aqueous plant extracts were 51.91 $\pm$ 1.20 mg GAE/g, 23.74 $\pm$ 2.99 mg GAE/g and 23.45 $\pm$ 0.07 mg RE/g respectively. The C*ynodon dactylon* exhibited significant *in vitro* antioxidant activity by DPPH method, hydroxy radical scavenging activity, reducing power and lipid peroxide free radical scavenging assay. The *in vivo* antioxidant results of this study showed that phenolic-rich fractions of the plant have strong antioxidant activity by a significant decrease (36.9%) in the liver TBARS levels and increased glutathione peroxidase (GP_X), Superoxide dismutase (SOD) and catalase anti-oxidant enzyme levels.
Keywords : C*ynodon dactylon;* antioxidant activity; *in vitro; in vivo;* aqueous plant extracts.

Introduction

Customary and corresponding prescriptions, especially natural medications are accessible for the treatment of different communicable and non-communicable diseases. Basic focal points of home grown medications are credited to their viability, security, moderateness, and acceptability[1]. Many ethno-natural studies on restorative plants utilized by the neighborhood population have been performed in various parts of the world including Malaysia, India, Morocco, Saudi Arabia, Taiwan and Trinidad and Tobogo.

Utilization of sustenances, containing a huge amount of polyunsaturated fatty acid has expanded the significance and utilization of the antioxidant agents to combat oxidation. The extension of cell reinforcements

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is a strategy for enlarging the time span of applicability, particularly of fat and fatenclose sustenances. Manufactured cell reinforcements have limited use in nutrient as these engineered antioxidant agents are suspected to be cancer-causing. Along these lines, the implication of look for regular antioxidant substance, especially from plant origin, has extraordinarily enlarged in late years[2].

Among various types of medicinal plants developing in the wild in India, Doob Ghas or Durva or Taxonomically the *Cynodon dactylon*(L.) Pers.with the family "Poaceae" possesses its remarkable importunacy in ethnomedicinal rehearses and customary therapeutic (Ayurveda, Unani, Nepalese and Chinese) information frameworks. The naturally grown arrangements of this grass are being founded on legends and customary wisdom[3]. Based on Ayurveda, India's conventional Pharmacopoeia, the medicinal plant is sharp, biting, fragrant, warming, tidbit, vulnerary, anthelmintic, antipyretic and alexiteric. It wrecks revoltingness of breath and valuable in Leucoderma, inflammation of bronchi, Piles, Fistula, Asthma, different types of Tumours and development of Spleen. In homeoopathic frameworks of the solution, it is utilized to treat a wide range of skin inconveniences.

It is a crawling grass, extremely intense, pale green in shading and has a coarse surface, dry spell safe, quickly developing, 3 to 20 mm long, and 2-4 mm in measurement. It is smell less and has a sweet adhesive taste[4].Chemical constituents of *Cynodon dactylon (C. dactylon*) are glycosides, saponins, tannins, flavonoids, and starches. It additionally contains agropyrene, arunodin, and furfural. Furfural liquor, S β ioine, 2-(4' hydroxyl phenyl) propionic corrosive, 2 – (3' – methoxy – 4' – hydroxyl benzoic corrosive, phytol, β – Sitosterol – D – glucoside, stigmasterol acetic acid derivation, phrgostimulantphytone (6,10 – 14 – trimethylpentadecane – 2-one). It additionally contains fundamental oil triticin 12.4%. Cuticular wax in it contains triacontane, docosanol, tetracosanol, hexacosonol, octacosanol, eicosanic corrosive and docosanoic acid[5].

In the perspective of this thought, the present research works we employed *in vitro* and *in vivo* assays to evaluate the antioxidant activities effect of *C.dactylon* in aqueous extract, the *in vivo* antioxidant activity has not been reported yet.

Materials and methods

Drugs and chemicals

Streptozotocin was acquired from Sisco research laboratory Mumbai. Glibenclamide was obtained from Aventis Pharma India Ltd. Various biochemical kits were procured from Agappe diagnostics, Kerala. Every single other substance and reagents utilized as a part of this investigation were of logical review procured from Thermofischer scientific India Ltd, Mumbai.

Collection and authentication of plant materials

*C. dactylon*was gathered from Kanniyakumari area of Tamilnadu, India and confirmed by the Department of Botany, Bharathiar University, Coimbatore, Tamilnadu, India.

Plant extract preparation

The entire plant of *C.dactylon* was washed with tap water, air dried and granulated in a mechanical blender. The dried powder (100 g) of *C.dactylon*whole plant was removed with refined water in a Soxhlet extractor and the resultant concentrate was amassed in a rotational vacuum evaporator. The concentrated extracts were stored in an impermeable holder for later analysis.

Phytochemical qualitative screening

The extracts were screened for the presence of the phytochemical classes which include alkaloids, flavonoids, phenol and tannins, steroids and sterols, saponins, glucosides, protein and amino acid and carbohydrate in accordance with standard methods[1,2].with little modification.

Total antioxidant compounds

The concentration of total phenolic compound was determined in the aqueous extract of *C.dactylon* using Folin Ciocalteu method with slight modifications[3].The flavonoids were estimated by aluminium trichloride method by using rutin as a reference compound[4]. The tannin contents or proanthocyanidin were estimated by modified Broadhurst method using catechin as a reference compound[5].

***In vitro* antioxidant activity**

The DPPH radical-scavenging activity, hydroxyl radical scavenging activities and reducing power assay of whole plant aqueous extract of *C. dactylon* were determined according to the standard methods[3,6,7].The extract providing 50% inhibition (IC_{50}) under the assay condition was calculated through the graph of inhibition value versus sample concentration.

Lipid peroxidation inhibition assay

Goat liver was washed properly in cold phosphate buffer saline (pH 7.4.) and homogenized to give a 10% homogenate. The homogenate was separated and centrifuged at 10000 rpm for 10 min and the supernatant used to carry out for this assay[8].The percentage of lipid peroxidation inhibition was calculated as follows.

$$\% \text{ Lipid peroxidation inhibition} = \frac{\text{Control OD-Sample OD}}{\text{Control OD}} \text{ X } 10$$

In vivo antioxidant activity

a. Experimental animals

Adult male albino Wistar rats (aged 10 weeks, weighing 150-200 g) approximately were acclimatised and housed in the central animal house of SRM medical college hospital and research centre, SRM university campus. Animal experimentation was carried out in accordance with the standards endorsed by the ethical norms, approved by the Institutional animal ethical committee (IAEC) of SRM Medical College, Potheri, Tamilnadu, India. (Ref: 45/IAEC/2011).

b. Experimental design

The rats were arbitrarily isolated into 5 groups of 6 rats in each group. Aqueous extract of *C. dactylon* was given by oral gavage.

Group	Drug treated Animal	Dose/day for 45 days
G1	Control	-
G2	Diabetic control	-
G3	Diabetic rats treated with Glibenclamide	5 mg/kg
G4	Normal rats administered with ACD	500 mg/kg
G5	Diabetic rats treated with ACD	500 mg/kg

***** ACD is Aqueous extract of *C. dactylon*

c. Collection of tissue samples

After 45 days of the experiment, animals were sacrificed, following the guidelines of the animal ethical committee. Parts of the liver tissues were washed in super cold phosphate supported saline at pH 7.4. Tissues were then homogenized with Remi homogenizer. The homogenate centrifuged at 3000 RPM for 10 min, supernatant gathered was utilized for the estimation of *in vivo* antioxidant activity.

Superoxide dismutase (SOD), Lipid peroxidation (TBARS), Glutathione peroxidase (GP_X)andCatalase (CAT) *in vivo* antioxidant activity of aqueous extract of *C. dactylon* accordance with the standard methods[8-11]with little modification.

Statistical analysis

Statistical analysis was done with statistical package for social sciences software (SPSS) version 21. One-way Analysis of Variance (ANOVA) was carried to find the significant difference between the groups which is followed by the Tukey's Honestly Significant Difference (HSD) test for the Post hoc analysis.

Results

Phytochemical qualitative screening

Phytochemical screening tests for different extracts of *C. dactylon* showed the active phytochemical classes as alkaloids, flavonoids, phenols and tannins, phytosterols, saponins, glycosides, proteins, and carbohydrates (Table 1).

Table 1: Qualitative preliminary phytochemical analysis of various extracts of *C.dactylon.*

S. No.	Test	Hexane extract	Petroleum ether extract	Chloroform extract	Acetone extract	Ethanol extract	Aqueous extract
1.	Alkaloids	-	-	+	+	+	-
2.	Flavonoids	-	-	-	-	-	+
3.	Phenols & Tannins	-	-	-	+	+	+
4.	Steroids & Sterols	-	-	-	-	-	+
5.	Saponins	-	-	-	-	-	+
6.	Glycosides	-	-	-	-	+	+
7.	Proteins	+	+	-	-	+	+
8.	Carbohydrate	+	+	+	+	+	+

+ Indicates presence of phytoconstituents, - Indicates absence of phytoconstituents

Total antioxidant compounds

The total phenolic, tannin and flavonoid content of aqueous plant extracts were 51.91 $\pm$ 1.20 mg GAE/g, 23.74 $\pm$ 2.99 mg GAE/g and 23.45 $\pm$ 0.07 mg RE/g respectively, with reference to standard (Table 2).

Table 2:Totalphenolics, tannins and flavonoids content of aqueous extract of *C.dactylon.*

Phytoconstituents	Content
Total phenolic (mg TAE/g extract)	51.91 $\pm$ 1.20
Tannin (mg TAE/g extract)	23.74 $\pm$ 2.99
Total flavonoid (mg RE/g extract)	23.45 $\pm$ 0.07

$\pm$ SD (n=3), TAE – Tannic acid equivalent, RE – Rutin equivalent

In vitro antioxidant activity

In vitro antioxidant studies, the percentage inhibition of aqueous extract of *C. dactylon* plant DPPH radical scavenging activities, reducing power capacity, hydroxy radical scavenging activities, and lipid peroxide assay compares with reference compound. The DPPH radical scavenging activities exhibited in IC_{50} value of > 1000 µg/ml and 3.73µg/ml for *C. dactylon* and ascorbic acid respectively (Fig. 1). Reducing power capacity value of *C. dactylon* increased based on the sample concentration (Fig. 2). The $IC_{50}$of hydroxyl radical assay was found to be 316 µg/ml and 18 µg/ml for*C. dactylon* and gallic acid respectively (Fig. 3). Liquid peroxide inhibiting activity IC_{50} value (Fig. 4) was 39.78 mg/ml, which is almost comparable to that of ascorbic acid (34.55 mg/ml).

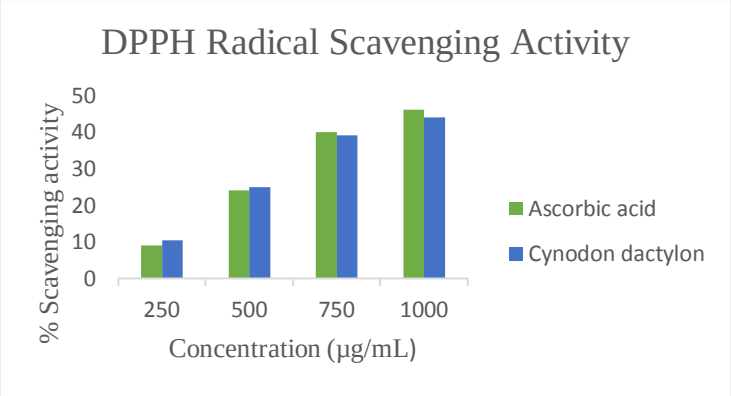

Fig. 1. Effect of ACD on DPPH radical scavenging activity

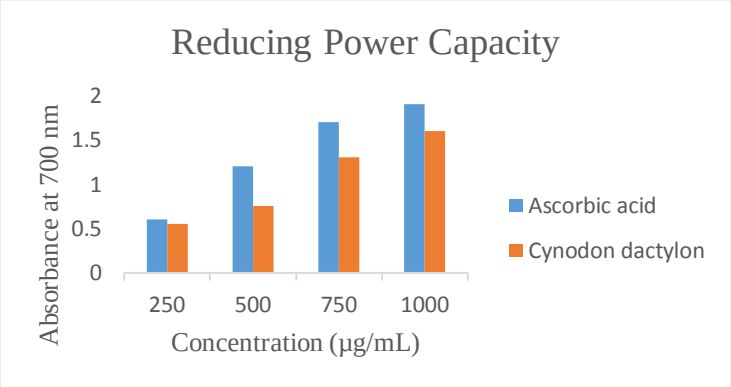

Fig. 2. Effect of acd on reducing power capacity

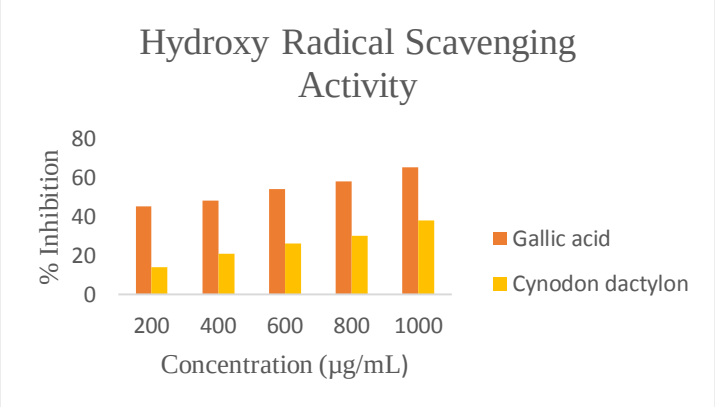

Fig. 3.Effect of ACD on hydroxyl radical scavenging activity

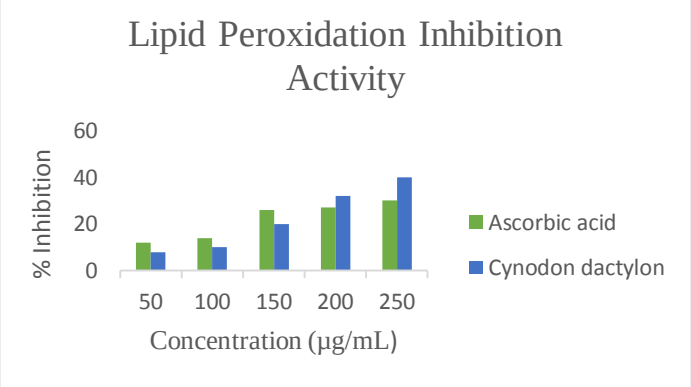

Fig. 4.Effect of ACD on lipid peroxidation inhibiting activity

***In vivo* antioxidant activity**

In vivo antioxidant activity of aqueous *C.dactylon* extract (ACE) on antioxidant enzyme levels shown in Table 3. SOD levels in the diabetic control group (G2) reduced significantly ($p < 0.001$) when compared with the control group (G1). The diabetic and glibenclamide group (G3) and diabetic and ACD group (G5) showed a significant elevation in the SOD levels when compared to diabetic control group (G2). The normal and ACD group (G4) did not demonstrate a significant alteration in the SOD levels when compared with control group (G1). SOD levels of diabetic and ACD group (G5) significantly elevated, when compared to diabetic and glibenclamide group (G3).

GPx levels in the diabetic control group (G2) reduced significantly when compared with the control group (G1). The diabetic and glibenclamide group (G3) and diabetic and ACD group (G5) showed a significant elevation in the GPX levels when compared to diabetic control group (G2). The normal and ACD group (G4) did not demonstrate a significant alteration in the GP_X levels when compared with control group (G1). GP_X levels of diabetic and ACD group (G5) significantly elevated, when compared to diabetic and glibenclamide group (G3).

Catalase levels in the diabetic control group (G2) reduced significantly when compared with the control group (G1). The diabetic and glibenclamide group (G3) and diabetic and ACD group (G5) showed a significant elevation in the catalase levels when compared to diabetic control group (G2). The normal and ACD group (G4) failed to demonstrate a significant alteration in the catalase levels when compared with control group (G1). Catalase levels of diabetic and ACD group (G5) significantly elevated, when compared to diabetic and glibenclamide group (G3).

TBARS levels in the diabetic control group (G2) elevated significantly when compared with the control group (G1). The diabetic and glibenclamide group (G3), diabetic and ACD group (G5) showed a significant reduction in the TBARS levels when compared to diabetic control group (G2). The normal and ACD group (G4) failed to demonstrate a significant alteration in the TBARS levels when compared with control group (G1). TBARS levels of diabetic and ACD group (G5) significantly reduced, when compared to diabetic and glibenclamide group (G3).

Table 3: Effect of ACD on antioxidant enzyme levels of liver homogenate in normal and experimental rats

Groups	SOD (Units/mg protein)	GPx (µM/min/mg protein)	CATALASE (µM/min/mg protein)	TBARS (nM/min/mg protein)
Control (G1)	100.95±1.69	1.7666±0.09	16.79±0.55	1.46±0.01
Diabetic control (G2)	60.318±1.18	0.5833±0.04	5.405±0.24	2.25±0.02
Diabetic +Glibenclamide 5mg/kg (G3)	75.451±1.23	0.8666±0.05	10.726±0.17	1.78±0.01
Normal + ACD 500mg/kg (G4)	100.09±1.30	1.6166±0.06	16.416±0.37	1.41±0.01
Diabetic + ACD 500mg/kg (G5)	96.476±1.36	1.55±0.07	13.88±0.16	1.64±0.01

Values are expressed as Mean ± SEM for each six rats. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (Tukey's HSD). Here all the p values are < 0.001.

Discussion

The phytochemical investigations on aqueous extract of *C. dactylon* revealed the presence of flavonoids, phenols and tannins, phytosterols, saponins, glycosides, proteins and carbohydrates, whereas alkaloids were present in the chloroform, acetone, ethanol extracts. Hexane and petroleum ether extracts demonstrated the presence of proteins and carbohydrates only. The quantitative analysis (total antioxidant compounds) of *C. dactylon* showed that the aqueous extract possessed a significant amount of total phenolics than tannin and total flavonoids. Previous study has already reported the same findings with hydro alcoholic extract of *C. dactylon*[12]. These phytochemical compounds are known to bolster bioactive activities in medicinal plants and thereby responsible for the antioxidant activities of this plant extract.

DPPH free radical scavenging activity of an aqueous extract of *C.dactylon* was concentration dependent. DPPH assay strongly support that the plant aqueous extract contains compounds that are capable of

donating hydrogen to a free radical to remove odd electron which is responsible for radical's reactivity. Hydroxyl radical scavenging activity inhibition was achieved at 1000 µg concentration in the reaction mixture, while in standard gallic acid, 50% inhibition was achieved at 1000 µg concentration. The scavenging activity of this radical by the plant extract compared favorably with the standard reagents such as gallic acid suggesting that the plant is also a potent scavenger of hydroxy radical. Lipid peroxides are responsible for the chemical damage caused by oxygen free radicals to the polyunsaturated fatty acids of cell membranes. The extract showed inhibition of the peroxidation effect in all concentration (50 µg/ml to 250 µg/ml),highest anti-lipid peroxidation activity was found in 200 µg/ml and 250 µg/ml, which were comparable with standard, Vitamin C.

Reducing power assay of aqueous extract of *C.dactylon* was a concentration dependent increase in the absorbance of the reaction mixture and standard ascorbic acid. The Higher absorbance of a reaction mixture indicated greater reducing power. The high reducing power of aqueous extract may be due to its higher phenolic and flavonoid content. Medicinal plants with antioxidant activities have been reported to possess free radical scavenging activity as major contributors to several clinical disorders such as diabetes mellitus, cancer, liver diseases, renal failure and degenerative diseases as a result of deficient natural antioxidant defense mechanism[13].

Antioxidant delays or represses oxidative damage to a target molecule and protects biologically imperative molecules, DNA, proteins and lipids from oxidative damage[14]. Free radicals formation enhances the peroxidation of lipid molecules. The decrease in SOD activity during the progression of diabetes could be due to enzyme glycosylation that occurs in the diabetic state. Decreased SOD activity could likewise be because of aggregation of hydrogen peroxide in the affected tissues[15].

Abnormal large amounts of free radicals cause cell membrane damage, which leads to a decline in antioxidant tissue defence mechanism[16]. The liver is an imperative organ, supports almost every organ in the body and it is key for survival. Due to its multidimensional capacity, the liver is also prone to enormous ailments. However, the liver has incredible ability to regenerate and has a large reserve capacity, in most cases it produced symptoms only after extensive damage[17]. A significant amount of liver damage is prompted by lipid peroxidation and other oxidative damages which are caused by the hepatotoxic chemicals[18,19].In advancing human health, natural antioxidants have gained a great interest, which also reduced the chronic disease state[23]. Oxidants are counteracted by antioxidant enzyme systems like Catalase, superoxide dismutase (SOD) and glutathione (GSH).

Diminished glutathione is a primary factor in detoxification and antioxidant systems, giving a barrier against free radicals and cytotoxins. ROS can be detoxified by an explained battery of enzymatic defence framework, comprising SOD, CAT, and GP_X or non-enzymatic systems by the scavenging actions of GSH, while organic peroxides can be detoxified by the activity of GST[20]. Regulation of these proteins and GSH levels assume a noteworthy part to be determined by redox status through the lessening in ROS and peroxides created in the living being and in addition in the detoxification of xenobiotic[21].

Vitamin E, well-known lipid soluble antioxidant assumes a noteworthy defense part opposing oxidative stress and prohibit the generation of lipid peroxides by scavenging free radicals, especially strong scavengers of hydroxyl radicals in cell membranes[22,23]. Liver damage is related to hepatocyte necrosis, lipid peroxidation and depletion of tissue GSH levels. Raise in malondialdehyde (MDA) levels in the liver recommend increase lipid peroxidation leads to tissue destruction and failure of antioxidants effects to prevent the development of enormous free radicals. Glutathione (GSH), a tripeptide is a critical antioxidant present in all cells[24]. Decreased GSH levels in diabetes have been treated to be an indicator of elevated oxidative stress GSH also acts as a free radical scavenger in the reconstruction of free radical caused biological distruction[25].

Medicinal plants rich in different photochemical derivates such as triterpenes, flavonoids or polyphenols, have been already proven to exhibit antihepatotoxic effects in experimental liver injury models induced by different types of hepatotoxicants, such as carbon tetrachloride, cadmium, acetaminophen etc[26,27].Phenolic compounds in the plants, found to possess strong and significant positive correlation with free radical scavenging potential and inhibition of lipid peroxidation[28]. Flavonoids are a group of naturally occurring compounds distributed widely as the secondary metabolite in the plant kingdom. They have been perceived as having intriguing clinical properties, like anti-inflammatory, antiallergic, antiviral, antibacterial and antitumoral activities[29].

Conclusion

The current research, *C.dactylon* aqueous extract shown to reveal antioxidant capacity in both *invitro* and in*vivo* models, particularly its potential to reduce the oxidant enzyme levels in a diabetic condition. The results of scavenging activity on DPPH radicals, hydroxyl radical scavenging, reducing power and inhibition of lipid peroxidation of all the samples revealed antioxidant activity, Nevertheless, *in vivo* antioxidant enzyme level significantly decreased in the content of a major product of lipid peroxidation (TBARS) in the extract treated group of rats with the diabetic control group. These therapeutic effects may be due to the presence of phenolic compounds such as flavonoids and proanthocyanidins. Further elaborative studies are recommended to prove the efficacy of antioxidant mechanism of this plant extract.

Conflicts of interest

All authors declare no conflicts of interest.

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