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Physico-Chemical Constant, Phytochemical Screening and Antioxidant Activity of Different Extracts of *Commelina clavata*

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ABSTRACT

The present study was designed to phytochemically screen different extracts of the whole plant of *Commelina clavata* (Commelinaceae), a plant generally used in the cure of pain and to establish physicochemical constants and *in vitro* antioxidant activity of these extracts. A physicochemical evaluation was carried out by assessing ash, alcohol and water-soluble extraction values: 5.35, 2.70 and 3.10% (w/w), respectively. Carbohydrates, tannins, alkaloids and flavonoids were present. The ethanolic extract showed potent antioxidant activity, assessed by *in vitro* methods (reducing power and hydrogen peroxide scavenging methods).

Keywords: alkaloid, flavonoid, hydrogen peroxide, reducing power

INTRODUCTION

In a cellular defense system, scavenging of free radicals is an important issue affiliated with the utilization of both exogenous and endogenous antioxidants because the increase in production of free radicals causes damage to cell membranes, enzymes, DNA, lipids and proteins, impairing their function (Gu *et al.* 1998). Although the body possesses defense mechanisms such as enzymes and antioxidant nutrients (Halliwell *et al*. 1995), continuous exposure to chemicals and contaminants may lead to an increase in the amount of free radicals in the body beyond its capacity to control them and cause irreversible oxidative damage (Tseng *et al.* 1997). The use of spices and herbs as antioxidants in processed foods is a promising alternative to the use of synthetic antioxidants (Madsen and Bertelsen 1995) such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), commonly used in processed foods. There may be an inverse relationship between dietary intake of antioxidant-rich foods and the incidence of human disease (Rice-Evans *et al.* 1997). *Commelina clavata* (Commelinaceae) is used traditionally for treating eye pain (Ganesan *et al*. 2006). Since a review of the literature did not reveal any studies on *C. clavata*, basic work on physicochemical constants, phytochemical screening and antioxidant activity of different extracts of the whole plant of *C. clavata* was studied.

MATERIALS AND METHODS

Collection and identification of plant materials

Whole *C. clavata* plants were collected from the Anantapur district during August and identified by Dr. B. Ravi Prasad Rao, Associate Professor, Department of Botany, S. K. University, Anantapur, Andhra Pradesh.

Preparation of extracts

Whole *C. clavata* plants were shade dried, coarsely powdered by using a cutter mill and sieved according to the WHO guidelines of QCMMPM Geneva 1992. Powdered plants were extracted with different solvents (*n*-hexane, chloroform, acetone, methanol, ethanol, water) successively by using a Soxhlet extractor. The whole extraction process was done at once. Extracts were concentrated and dried using a rotavapor (Heidolph) under vacuum.

Preliminary phytochemical screening

Crude extracts from each solvent were subjected to preliminary phytochemical screening (Kokate 1986; Harborne 1998).

Physico-chemical analysis

Physico-chemical values such as ash values, extractive values were performed accordingly to official methods prescribed in IP 1996 and the WHO guidelines on quality control methods for medicinal plant materials (Harborne 1998).

Antioxidant activity

1. Estimation of reducing power

Reducing power (RP) was determined using the ferric reducing - antioxidant power assay and quercetin as a reference standard. Different aliquots of sample maintained to 1 ml, followed by the addition of 2.5 ml phosphate buffer pH (6.6) and 2.5 ml of 1% w/v potassium ferricyanide in each reaction mixture thus obtained were incubated at 50°C for 20 min. After incubation, the reaction was terminated by adding 2.5 ml of 10% (w/v) trichloroacetic acid

Table 1 Percentage yield of the different extracts of whole plant of *Commelina clavata*.

Solvent used	% Yield	Physicochemical constants (% w/w)	
n-Hexane	1.75	Total ash value	5.35
Chloroform	2.53	Acid-insoluble ash	1.30
Acetone	1.05	Water-soluble ash	2.04
Methanol	3.47	Sulphated ash	6.05
Ethanol	3.60	Alcohol-soluble extract	2.70
Water	3.92	Water-soluble extract	3.10

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Research Note

Table 2 Phytochemical screening of the different extracts of whole plant of *Commelina clavata*.

Chemical tests	Hexane	Chloroform	Acetone	Methanol	Ethanol	Water
Test for carbohydrates						
Molisch's test	-	+	+	+	+	+
Fehling's test	-	+	+	+	+	+
Benedict's test	-	+	+	+	+	+
Test for amino acids						
Ninhydrine test	-	+	+	+	+	+
Test for glycosides						
Baljet test	-	-	-	-	+	+
Borntrager's test	-	-	-	-	+	+
Test for fixed oils						
Spot test	+	+	-	-	-	-
Test for saponins						
Froth test	-	-	-	+	+	+
Test for alkaloids						
Hager's test	+	+	+	+	+	+
Wagner's test	+	+	+	+	+	+
Mayer's test	+	+	+	+	+	+
Dragondroff's test	+	+	+	+	+	+
Test for tannins						
Gelatin test	-	-	+	+	+	+
Vanilline HCL test	-	-	+	+	+	-
Test for flavonoids						
Shinoda test	-	-	+	+	+	+
Lead acetate test	-	-	+	+	+	+
Sodium hydroxide test	-	-	-	+	+	+
Test for resins and gums						
Hydrolytic test	-	+	-	-	-	-

+ positive, - negative

solution; 2.5 ml of this solution from each reaction was diluted with an equal amount of distilled water. A 0.5-ml aliquot of FeCl3 (0.1%) was added to each solution and absorbance was recorded after 10 min at 700 nm with an Elico SL210 spectrophotometer. RP was expressed as ascorbic acid equivalents (1 mM = 1 ASE). The ASE/ml value is inversely proportional to its reducing power (Vijaya *et al.* 2002).

2. Scavenging of hydrogen peroxide

A solution of hydrogen peroxide (H_2O_2; 20 mM) was prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of the extract or standard in methanol (1 ml) were added to 2 ml of H_2O_2 solution in PBS. After 10 min the absorbance was measured at 230 nm (Ruch *et al.* 1989).

RESULTS AND DISCUSSION

The preliminary phytochemical screening of *n*-hexane, chloroform, acetone, methanol, ethanol and water extracts of the whole plant of *C. clavata* mainly revealed the presence of carbohydrates, proteins, alkaloids, flavonoids, tannins, and saponins (**Table 2**).

Physico-chemical contents like the ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug. The ash values of the powdered crude drugs showed a higher content of sulphated ash followed by total ash. The extractive values are primarily useful for the detection of exhausted and adulterated drugs phytochemical and pharmacognostical studies on *Stylosanthes fruticosa* Linn. showed amount of ash and extractives obtained (Kumanan *et al.* 2011). The water-soluble extractive was higher than the

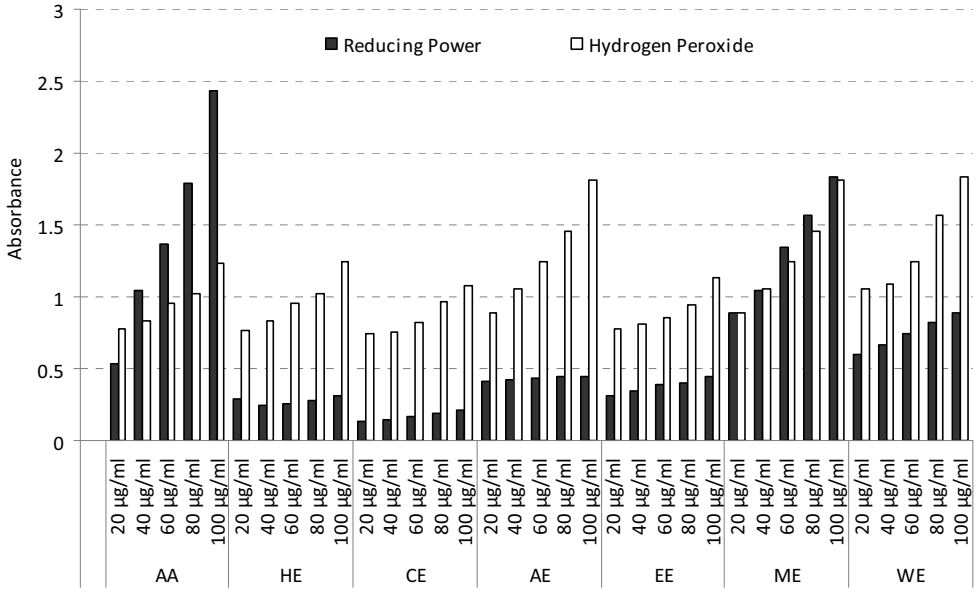

Fig. 1 Reducing power and hydrogen peroxide free radical activity of different extracts of whole plant of *Commelina clavata*. * AA = ascorbic acid, HE = hexane extract, CE = chloroform extract, AE = acetone extract, EE = ethanol extract, ME = methanol extract, WE = water extract.

Table 3 Antioxidant activity, reducing power and free radical scavenging activity (hydrogen peroxide) of different parts of *Commelina clavata*.

Extract	Reducing power (ASE/ml)*	Hydrogen peroxide (AOA; %)*
Ascorbic acid	0.53 ± 0.04	0.78 ± 0.03
	1.05 ± 0.03	0.83 ± 0.05
	1.37 ± 0.06	0.96 ± 0.04
	1.79 ± 0.05	1.02 ± 0.04
	2.43 ± 0.08	1.23 ± 0.03
Hexane extract	0.29 ± 0.08	0.77 ± 0.03
	0.24 ± 0.11	0.83 ± 0.05
	0.26 ± 0.09	0.96 ± 0.04
	0.28 ± 0.05	1.02 ± 0.04
	0.31 ± 0.08	1.24 ± 0.03
Chloroform extract	0.13 ± 0.07	0.74 ± 0.03
	0.15 ± 0.05	0.76 ± 0.05
	0.17 ± 0.04	0.82 ± 0.04
	0.19 ± 0.06	0.97 ± 0.04
	0.21 ± 0.08	1.08 ± 0.03
Acetone extract	0.41 ± 0.06	0.89 ± 0.03
	0.42 ± 0.07	1.06 ± 0.07
	0.43 ± 0.07	1.24 ± 0.05
	0.44 ± 0.05	1.46 ± 0.05
	0.45 ± 0.04	1.81 ± 0.03
Ethanol extract	0.31 ± 0.05	0.78 ± 0.03
	0.34 ± 0.08	0.81 ± 0.05
	0.39 ± 0.07	0.86 ± 0.04
	0.40 ± 0.05	0.94 ± 0.04
	0.44 ± 0.06	1.13 ± 0.03
Methanol extract	0.89 ± 0.05	0.89 ± 0.03
	1.04 ± 0.08	1.06 ± 0.07
	1.34 ± 0.07	1.24 ± 0.05
	1.57 ± 0.05	1.46 ± 0.05
	1.83 ± 0.06	1.81 ± 0.03
Water extract	0.60 ± 0.05	1.06 ± 0.04
	0.67 ± 0.04	1.09 ± 0.08
	0.75 ± 0.07	1.24 ± 0.06
	0.82 ± 0.03	1.57 ± 0.05
	0.89 ± 0.08	1.83 ± 0.04

* Average of 3 values

alcohol-soluble extractives (**Table 1**). *C. clavata* has potent scavenging activity against H_2O_2 and high reducing power (**Table 3; Fig. 1**).

The aqueous leaf extracts of *Commelina nudiflora* were evaluated for nutritional and anti-nutritional composition: it contained saponins, alkaloids and flavonoids (Ujowundu *et al*. 2008). Shibano *et al*. (2008) examined the antioxidant constituents in dayflower (*Commelina communis* L.) and their α-glucosidase-inhibitory activity; the extracts and powder of this herb are important food materials for prophylaxis against type-2 diabetes. Eleven flavonoid glycosides as antioxidants, isoquercitrin, isorhamnetin-3-*O*-rutinoside, isorhamnetin-3-*O*-β-D-glucoside, glucoluteolin, chry-soriol-7-*O*-β-D-glucoside, orientin, vitexin, isoorientin, isovitexin, swertisin, and flavocommelin were identified from the aerial parts of *C. communis*. Further investigations shall be carried out to determine the activities and phytoconstituents of *C. clavata*.

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