International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2015): 6.391

Phytochemical Analysis and Antioxidant Activities of *in vivo* and *in vitro* Shoots of *Cleome gynandra* L

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Abstract: ***Plants can be called as living elixir since they cure almost all types of ailments. Phytochemicals or secondary metabolites are compounds present in plants responsible for the curative properties of them. Free radicals are the causative agents of all forms of metabolic disorders. Nullifying the ill effects of those free radicals can be done through the phytochemicals and secondary metabolites of plant based origin, which will lead to prevent or cure particular disease. The present study deals with the identifying, quantifying of phytochemicals in aqueous, ethanolic extracts of in vivo and in vitro shoots of C. gynandra. This study revealed the presence of metabolically active phytochemicals such alkaloids, flavonoids, phenols, tannins, saponins, terpenoids etc. in the tested extracts. This study also exposed the antioxidant activity of aqueous, ethanolic extracts of in vivo and in vitro shoots of C. gynandra through DPPH scavenging method. The ethanolic extracts of in vitro shoots exhibited higher antioxidant activity than all other extracts tested.***

Keywords: *Cleome gynandra*, *in vivo*, *in vitro*, Phytochemicals, Antioxidant activity.

1. Introduction

Cleome gynandra commonly known as cat's whisker and spider flower in English belongs to the family Cleomaceae (previously capparidaceae). It is one of the staple foods in most of the African countries. It has a wide range of medicinal uses and used in traditional systems of medicine all over the world. The medicinal property of the plant is mainly because of the bioactive substances present in, and the most important of these include flavonoids, phenols, saponins, triterpenes and so on. *C.gynandra* has many scientifically proven medicinal properties such as antidiabetic, anthelmintic and anti microbial, antioxidant, anti-inflammatory, antinociceptive, immunomodulatory, antitumour activity [1]-[8]. All these properties are due to the presence of biologically active phytochemicals present in it. The present study was aimed at identifying and quantifying those phytochemicals and authenticating their medicinal properties through antioxidant study by DPPH scavenging.

2. Materials and Methods

2.1. Plant Material

The shoots of healthy *C. gynandra* plants were selected from Mayanur and Thanthonimalai, villages of Karur District, Tamilnadu and India. From the healthy explants *in vitro* plants were produced using MS medium with optimum concentrations of plant growth regulators [9]. The above said plants samples (*in vivo* and *in vitro*) were used for the entire study.

2.2. Preparation of extracts

The shoots (excluding reproductive parts) of *in vivo* and *in vitro* plants of *C. gynandra* were washed thoroughly with running tap water to remove unwanted particles and placed in bamboo plates at room temperature. The shade dried plant samples were packed in soxhlet apparatus for ethanolic extraction (95% ethanol) and aqueous extraction with distilled water [10]. The yield of aqueous and ethanolic extracts were used for phytochemical and antioxidant studies.

2.3 Preliminary phytochemical screening

The phytochemical screening of secondary metabolites present in the aqueous, ethanolic extracts of *in vivo* and *in vitro* plants of *C. gynandra* was examined by the standard methods [11, 12].

2.4 Quantitative analysis phytochemicals

2.4.1. Estimation of alkaloids

To 1ml of extracts 5 ml phosphate buffer (pH 4.7) and 5 ml BCG (Bacille Calmette-Guerin) solution were added and shaken well with 4 ml of chloroform. The extracts were collected in a 20-ml volumetric flask and then diluted to adjust volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without extract. Atropine is used as a standard material and compared the assay with Atropine equivalents. The values were expressed as mean ± SEM [13].

2.4.2. Estimation of flavonoids

Total flavonoid content was determined by Aluminium chloride method using catechin as a standard. 1ml of test sample and 4 ml of water were taken in a volumetric flask (10 ml volume). After 5 min 0.3 ml of 5 % Sodium nitrite, 0.3 ml of 10% Aluminium chloride was added. After 6 min incubation at room temperature, 2 ml of 1 M Sodium hydroxide was added to the reaction mixture. Immediately the final volume was made up to 10 ml with distilled water. The absorbance of the reaction mixture was measured at 510 nm against a blank spectrophotometrically. Results were expressed as catechin equivalents (mg catechin/g dried extract). The values were expressed as mean ± SEM [14].

2.4.3. Estimation of phenoilc compounds

The total phenolics content in ethanolic and aqueous extracts were determined with Folin- Ciocalteu's reagent (FCR). The extracts were mixed with 0.4 ml FCR (diluted 1:10 v/v);

After 5 min 4 ml of sodium carbonate solution was added. The final volume was made up to 10 ml with distilled water and allowed to stand for 90 min at room temperature. Absorbance of sample was measured against the blank at 750 nm using a spectrophotometer. A calibration curve was constructed using catechol solutions as standard and total phenolic content of the extract was expressed in terms of milligrams of catechol per gram of dry weight. The values were expressed as mean ± SEM [13].

2.4.4. Estimation of saponins

Ethanolic and aqueous extracts were dissolved separately in 80% methanol, 2ml of vanilin in ethanol was added, mixed well and the 2ml of 72% sulphuric acid solution was added, mixed well and heated on a water bath at 60°c for 10min, absorbance was measured at 544nm against reagent blank. Diosgenin is used as a standard material and compared the assay with diosgenin equivalents. The values were expressed as mean ± SEM [11].

2.4.5. Estimation of tannins

1 g of plant sample was dissolved and made up to 50 ml with distilled water in a 50 ml volumetric flask and shaken well. About 5 ml of the above sample was mixed with 2 ml of 0.1 M FeCl3 in 0.1 M HCl and 0.008 M potassium ferrocyanide [K4Fe(CN)6.3H2O]. The absorbance of the sample is measured with a spectrophotometer at 395 nm wavelength within 10 min. Tannic acid was used as standard and compared the assay with tannic acid equivalaents. The values were expressed as mean ± SEM [15].

2.5. *In vitro* antioxidant activity- DPPH scavenging

The antioxidant activity of the aqueous, ethanolic extracts of *in vivo*, *in vitro* plants of *C. gynandra* and the standard (ascorbic acid) was assessed on the basis of the radical scavenging effect against the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH). DPPH free radical scavenging activities were measured using plant extracts in various concentrations (100-500 µg/ml), DPPH solution (methanolic 0.1 mM DPPH) and ascorbic acid (100-500 µg/ml) as standard solution. About 1 ml of DPPH solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Spectrophotometer. Methanolic 0.1mM DPPH solution was used as blank [16]-[18]. The optical density was recorded and % inhibition was calculated using the formula given below.

$$\text{Percent (\%) DPPH scavenging activity} = \frac{A - B}{A} \times 100$$

Where, A = optical density of the blank and B = Optical Density of the sample.

3. Results and Discussion

3.1. Preliminary phytochemical screening

The preliminary phytochemical screening of aqueous extracts of *in vivo* and *in vitro* shoots of *C. gynandra* revealed the presence of alkaloids, flavoinoids, phenols, saponins, tannins, terpenoids, anthroquinones, glycosides, aminoacids and carbohydrates. The screening of ethanolic extracts of *in vivo* and *in vitro* shoots of *C. gynandra* exposed the presence of alkaloids, flavoinoids, phenols, saponins, tannins, steroids, coumarins and glycosides (Table.1). All these chemicals are biologically active and responsible for the therapeutic values [1], [2].

Table 1: Preliminary phytochemical screening of aqueous, ethanolic extracts of in vivo and in vitro plants of C. gynandra L.

S.No	Phytochemicals	*in vivo*		*in vitro*	
		AE	EE	AE	EE
1	Alkaloids	+	+	+	+
2	Flavonoids	+	+	+	+
3	Phenols	+	+	+	+
4	Saponins	+	+	+	+
5	Tannins	+	+	+	+
6	Terpenoids	+	-	+	-
7	Anthroquinones	+	-	+	-
8	Steroids	-	+	-	+
9	Coumarins	-	+	-	+
10	Glycosides	+	+	+	+
11	Aminoacids	+	-	+	-
12	Carbohydrates	+	-	+	-

AE - Aqueous extract, EE – Ethanolic extract, (+) indicates the presence and (-) indicates the absence of phytochemicals**.**

3.2. Quantitative analysis phytochemicals

The alkaloids, flavonoids, phenols, saponins, tannins content of aqueous, ethanolic extracts of *in vivo* and *in vitro* plants of *C. gynandra* were estimated through standard procedures [10]-[15]. Among the tested aqueous extracts phytochemical content of *in vitro* shoots were higher than *in vivo* shoots. In the same way, phytochemical content in ethanolic extracts of *in vitro* were also higher than that of *in vivo*. Amongst the estimated phytochemicals, high amount of phenolics (97.5±0.01 mg/g) were observed in ethanolic extracts of *in vivo* shoots. Other than phenolics all other estimated phytochemicals (alkaloids-19.5± 0.10 mg/g, flavonoids-45.3±0.15 mg/g, saponins-27.7±0.20 mg/g, tannins-18.4± 0.12 mg/g) were more in ethanolic extracts of *in vitro* shoots. Aqueous extracts of both tested plant samples yielded comparatively lesser quantity of phytochemicals when compared to their ethanolic extracts (Table.2, Fig.1). These compounds were indentified and quantified by many scientists in different extracts of various plants [14],[17].

Table 2: Quantitative analysis of phytochemicals in aqueous, ethanolic extracts of in vivo and in vitro C. gynandra L.

Phytochemical	Phytochemical content (mg/g)			
	in vivo		*in vitro*	
	AE	EE	AE	EE
Alkaloids	10.2± 0.80	18.7± 0.40	11.4± 0.12	19.5± 0.10
Flavonoids	19.6± 0.40	41.3± 0.21	21.3± 0.13	45.3± 0.15
Phenols	55.1± 0.11	97.5± 0.01	59.4± 0.14	90.1± 0.16
Saponins	14.3± 0.13	25.2± 0.14	14.9± 0.14	27.7± 0.20
Tannins	10.1± 0.10	17.6± 0.12	10.7± 0.11	18.4± 0.12

AE – Aqueous extract, EE – Ethanolic extract. All the values in the table were mean of five replicates and were expressed as mean ± SEM.

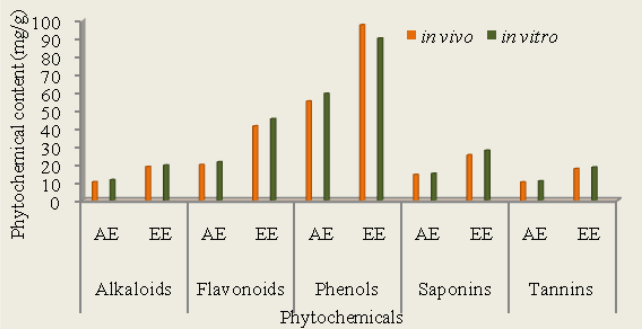

Figure 1: Quantitative analysis of phytochemicals in aqueous, ethanolic extracts of *in vivo* and *in vitro C. gynandra* L. AE – Aqueous extract, EE – Ethanolic extract.

3.3. *In vitro* antioxidant activity

The antioxidant activity of aqueous, ethanolic extracts of *in vivo* and *in vitro* plants of *C. gynandra* was studied by scavenging of DPPH. All the tested extracts showed radical scavenging activity in a dose dependant manner. Among the tested extracts, ethanolic extract at a concentration 500 µg/ml of *in vitro* shoots produced maximum DPPH scavenging activity of 37.67±0.91%. When compared to the standard ascorbic acid (18.96±0.69%) all other tested extracts produced maximum radical scavenging activity (Table.3, Fig.2). Similar DPPH scavenging activities of different plant extracts had been observed by different researchers [16]-[18].

Table 3: Antioxidant activity of aqueous, ethanolic extracts of in vivo and in vitro plants of C. gynandra L.

S. No	Concentration of extract (µg/ml)	DPPH scavenging activity (%)				
		Aqueous		Ethanol		Ascorbic acid
		in vivo	*in vitro*	*in vivo*	*in vitro*	
1	100	16.11± 0.34	18.15± 0.67	17.21± 0.23	27.58± 0.84	15.00±0.38
2	200	18.25± 0.65	20.92± 0.59	20.42± 0.41	29.97± 0.57	17.23±0.86
3	300	19.86± 0.28	22.12± 0.91	21.36± 0.46	32.16± 0.69	17.86±0.81
4	400	21.34± 0.42	25.11± 0.78	22.90± 0.61	34.63± 0.87	18.32±0.57
5	500	23.48± 0.71	26.97± 0.65	27.33± 0.43	37.67± 0.91	18.96±0.69

All the values in the table were mean of five replicates and were expressed as mean ± SEM.

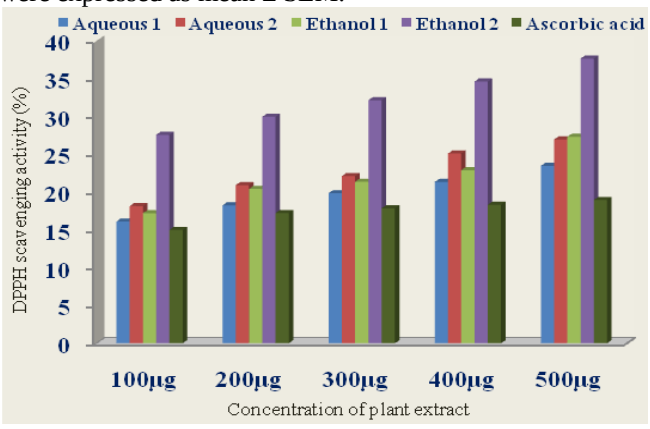

Figure 2: Antioxidant activity of aqueous, ethanolic extracts of *in vivo* and *in vitro* plants of *C. gynandra* L**.**
1 – *In vivo*, 2 – *In vitro*

4. Acknowledgement

The Corresponding author (Dr. A.V.P. Karthikeyan) is thankful to *UGC, New Delhi* for its financial assistance in the form of major research project.

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