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Nephroprotective and antioxidant activities of *Caralluma umbellata* Roxb.

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Abstract

The present study was carried out to investigate the preliminary phytochemical screening, *in vitro* antioxidant activity and nephroprotective activity of methanolic (70%v/v) extract of *Caralluma umbellata* Roxb. stem extract. The phytochemical screening was carried out using standard phytochemical tests. The *in vitro* antioxidant activity was tested on superoxide, hydroxyl and DPPH free radicals. The nephroprotective activity of *C. umbellata* stem extract was carried out using cisplatin and gentamicin induced renal injury. The plant extract showed the positive results for the presence of different phytochemicals like steroids, triterpenes, alkaloids, glycosides and flavonoids. The extracts gave negative results for the amino acids, oils, quinines, tannins and saponins. The stem extract of *C. umbellata* reduced the amounts of free radicals at different concentrations on tested free radicals, *i.e*., superoxide, hydroxyl and DPPH by *in vitro* free radical antioxidant activity. The mean IC_{50} values for ascorbic acid to superoxide anion, hydroxyl radical and DPPH radical were found to be 54.4 µg, 68 µg and 22 µg. The serum parameter levels such as blood urea, serum creatinine, serum total protein and serum albumin were estimated by standard biochemical procedures for evaluation of the nephroprotective activity of *C. umbellata*. The % protection of hydroalcoholic, *C. umbellata* stem extract expressed as Mean ± SEM, n = 6 and significant values were expressed as $p<0.01$ and $p<0.05$ levels of probability.

Key words: *Caralluma umbellata* Roxb., stem, *in vitro* antioxidant activity and nephroprotective activity

1. Introduction

Plants have been providing shelter, clothing, food, flavors and fragrances and last not the least medicine. Plants have been using in the treatment of diseases since thousands of years and are basis for traditional medicine. Some of the oldest known medicinal systems of the world such as Indus civilization medication "Ayurveda", Mesopotamia's Arabian medicine, Japanese's Kempo medicine and mainly Yellow river civilization's Chinese and Tibetan's medicine are all based mostly on plants (Vedavathy *et al.*, 1997; Rachel Wynberg, 2009).

The medicinals are basis for the modern medicine in the isolation of new chemical compounds, having different biological benefits with less side effects (Izzo and Ernst, 2009). It is estimated that at least 25% recent pharmaceuticals (antibitics, antitumoral, steroids and their devrivatives) either directly or indirectly derived from the medicinal plants. Recent studies around the world applying the modern technology in the isolation of bioactive molecules based traditional medicinal plants knowledge (Cotton, 1997; Gurib-Fakim, 2006; Shabana Praveen *et al.*, 2015). The search for new molecules from the medicinal plants is popularized now-a-days and many researchers validating traditional medicinal plants biological activities (Kidd and Kidd, 2006; Kennedy and Wightman, 2011; Rao, 2013; Mallikarjuna Rao *et al.*, 2012, 2013), and reporting many new compounds in them. In this point of view, the authors selected a thick, erect, branching succulent thorny perennial herb, belonging to the family, Asclepiadeceae. It is usually found in hilly regions of Andhra Pradesh, Kerala and Karnataka. In Telugu, it is called as kundete kommulu. The present study was carried out to identify the phytochemicals present in it and evaluation of its *in vitro* antioxidant and nephroprotective activity, because stem of *C. umbellata* have been using in the treatment of stomach disorders, abdominal pains and ulcers in by different tribal people (Vedavathy *et al.*, 1997).

2. Materials and Methods

2.1 Collection of *C. umbellata* Roxb. and extraction preparation

The plant *C. umbellata* was collected from Araku valley, Visakhapatnam, Andhra Pradesh, India. The plant material was taxonomically identified by Dr. Prayaga Murty Pragada, Botanist, Andhra University, Andhra Pradesh, India. Voucher specimen (BGR/ KLB/ 11/2010) have been kept in our laboratory for future reference. Freshly collected stem from plant was dried under shade for 14 days and the dried material was milled to obtain a coarse powder. The coarse powder was used for the extraction, using maceration at room temperature with methanol (70% v/v).

2.2 Preliminary phytochemical studies

The extract of the *C.umbellata* stem was subjected to different phytochemical tests for the identification of its phytochemical

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constituents, using standard procedures (Kokate, 2002; Wallis, 1995; Wagner *et al.*, 1984; Shah and Quadry, 1980; Rosenthaler and Ghosh, 1930) .

2.3 Quantification of total phenolic content (Rao, 2013; Singleton and Rossi, 1965)

Total phenolic content was determined using the Folin-Ciocalteau reagent. The method is based on blue light absorption measurement due to the chemical reduction of tungsten and molybdenum oxides of Folin-Coicalteau reagent, when combined with the compounds present in the extracts using colorimetry at 765 nm. The phenolic content in the extract was meassured in the gallic acid equivalents as mg/gm (GAE), using gallic acid calibration curve. The results showed in mean values.

2.4 Quantification of total alkaloid content (Rao, 2013; Fazel Shamsa *et al.*, 2008)

The plant extract (1mg/ml) was dissolved in 2 N HCl and the solution was filtered. The phosphate buffer's pH was neutralized 0.1N NaOH. 1 ml of extract solution, 5 ml of phosphate buffer and 5 ml of bromocresol green (BCG) solution placed in separation and then mix the solution well. The complex formed in the solution was extract with chloroform. The absorbance of complex color in chloroform was measure at 470 nm. Overall experiment was performed thrice and results were reported in atropine equivalents. The results showed in mean value.

2.5 *In vitro* antioxidant activity

For the assessment of antioxidant activity, the extract of *C. umbellata* was dissolved in dimethyl sulphoxide (DMSO). The results showed in Mean ± SE.

2.5.1 *Superoxide radical scavenging activity*

Superoxide scavenging activity of the selected plant extract was evaluated as per method (McCord and Fridovich, 1969). It is by absorption of light at 560 nm induction of superoxide free radical generation by riboflavin and corresponding reduction by nitroblue tetrazolium.

2.5.2 *Hydroxyl radical scavenging activity*

The hydroxyl radical scavenging activity is measured as per method established by Kunchandy and Rao (1990). It was studied by the competition between deoxyribose and the extract's antioxidant molecules for hydroxyl radicals generated from the Fe+2/EDTA/ H_2O_2 system.

2.5.3 *2, 2-Diphenyl-1-picrylhydrazyl* (DPPH) *radical scavenging activity*

The DPPH radical scavenging activity was measured as per methods of Braca *et al.* (2003) and Anita *et al.* (2011). These methods are based on measure of color absorbance of alcoholic DPPH solution (Blue color) after addition of antioxidant solution (extract/ compound). If antioxidants present in the test compound, blue color/yellow color appears due to diphenyl-picrylhydrazine.

2.6 Acute toxic studies

The acute toxicity study was conducted for methanol (70%v/v) *C. umbellata* stem extract as per OECD guidelines, 420 (OECD. 2001) and regulations of the institutional Animal Ethics Committee (Regd. No. 516/01/A/CPCSEA). The albino mice of single sex, were selected in to two groups of consisting of 6 animals. They were maintained for one week before the experiment, under room temperature and allowed free access to water and diet. The animals were subjected for acute toxicity study, using each extract at a dose of 2000 mg/kg orally in 2 groups at regular intervals of time, *i.e.*, 1, 2, 4, 8, 12 and 24 h. During this time, the animals were under observation to note different conditions like skin changes, morbidity, aggressiveness, oral secretions, sensitivity to sound and pain, respiratory movements and finally their mortality.

2.7 Nephroprotective activity

2.7.1 *Selection of animals*

Healthy albino rats of either sex weighing between 130-250 g aged 60-90 days were used for the study. The rats were taken care of at standard light and humidity by supplying proper food and water.

2.7.2 *Cisplatin induced renal injury in rats* (Sara *et al.*, 2009)

12 rats weighing between 130-250 g were divided into four groups of three rats each, were used in the study. Group I (Control) rats were treated with equivalent volumes of 1% sodium carboxy methylcellulose suspension orally for 10 days. Blood samples were withdrawn on 16th day to assess renal function. Group II (Negative control) rats were treated with equivalent volumes of 1% sodium carboxymethylcellulose suspension orally up to 10 days and on the 11th day, cisplatin (5 mg/kg b.w) (Lusania *et al.*, 2000) one dose given intraperitonially. Blood samples were withdrawn on 16th day to assess renal function. Group III (Extract treatment) rats were treated with suspension of stem extract of *C. umbellata* (250 mg/kg body weight) orally for 10 days and on the 11th day, cisplatin (5 mg/kg body weight) single dose was given intraperitonially. Blood samples were withdrawn on 16th day to assess renal function. Group IV (Extract treatment) rats were treated with suspension of stem extract of *C. umbellata* (500 mg/kg body weight) orally for 10 days and on the 11th day, cisplatin (5 mg/kg body weight) single dose was administered intraperitonially. Blood samples were withdrawn by retro orbital plexus on 16th day to assess renal function.

2.7.3 *Gentamicin induced renal damage* (Menaka *et al.*, 2010)

12 rats weighing between 130-250 g were divided into four groups of three rats each, were used in the study. Group I (Control) rats were treated with equivalent volumes of 1% sodium carboxy methylcellulose suspension orally for 8 days. Blood samples were withdrawn on 9th day to assess renal function. Group II (Negative control) rats were first treated with equivalent volumes of 1% sodium carboxymethylcellulose suspension orally and after 2 h., gentamicin (80 mg/kg body weight) was administered intraperitonially for 8 days. Blood samples were withdrawn on 9th day to assess renal function. Group III (Extract treatment) rats

were first treated with suspension of stem extract of *C. umbellata* (250 mg/kg body weight) orally and after 2 h., gentamicin (80 mg/kg body weight) was administered intraperitonially for 8 days. Blood samples were withdrawn on 9th day to assess renal function. Group IV (Extract treatment) rats were first treated with suspension of stem extract of *C. umbellata* (500 mg/kg body weight) orally and after 2 h., gentamicin (80 mg/kg body weight) was administered intraperitonially for 8 days. Blood samples were withdrawn on 9th day to assess renal function.

2.7.4 *Parameters assessed for renal function*

Blood samples were withdrawn by retro orbital plexus from rats and serum parameter levels were estimated by standard biochemical procedure by using an Auto analyzer (BS Biosciences, Italy).The serum parameters estimated for the study are:

Blood urea: Urea concentration in blood was estimated by NED-DYE method (Levinson, 1978), using urea estimation kit by Span Diagnostics Ltd.

Serum creatinine: Creatinine level in serum was estimated by Modified Jaffe's reaction method (Folin and Wu, 1919), using creatinine estimation kit by Span Diagnostics Ltd.

Serum total protein: Protein level was estimated by Biuret method using protein estimation kit by Span Diagnostics Ltd.

Serum albumin: Albumin level was estimated by bromocresol green (BCG) method using albumin estimation (Parikh *et al*., 2003), kit by Span Diagnostics Ltd.

3. Results and Discussion

3.1 Phytochemical studies

Qualitative phytochemical screening of *C. umbellata* stem extract revealed the presence of steroids, triterpenes, alkaloids, glycosides, carbohydrates and flavonoids. The extract gave negative results for the amino acids, oils, quinines, tannins and saponins.

The quantified phenolic content and alkaloid content of *C. umbellata* methanol extract was found to contain 3.93 mg/gm and 26.99 mg/gm. The results of qualitative phytochemical screening and quantified total phenolic and alkaloid were shown in Tables 1 and Table 2.

Table 1: Qualitative phytochemical analysis of *C. umbellata* Roxb. stem extract

Phytochemical constituents	Methanolic (70%v/v) stem extract
C. umbellata Roxb.	
Carbohydrates	+
Glycosides	+
Saponins	-
Tannins	-
Phytosterols	+
Terpenoids	+
Flavonoids	+
Alkaloids	+
Quinones	-
Amino acids	-

+ = Present: - = Absent

Table 2: Total phenolic and alkaloid content (mg/gm) of *C. umbellata* Roxb. stem extract

Name of the extract	Total phenolic content(mg/gm)	Total alkaloid content(mg/gm)
C. umbellata stem	3.93	26.99

3.2 *In vitro* antioxidant activity

The stem extract of *C. umbellata* produced a dose dependent inhibition on free radicals (superoxide, hydroxyl and DPPH) were ranging from 12.4 ± 0.5 to 72.5 ± 1.4, 10.3 ± 0.3 to 67.8 ± 1.6 and 15.3 ± 0.5 to 80.23 ± 2.1. The mean IC_{50} values for superoxide anion, hydroxyl radical and DPPH radical of *in vitro* antioxidant activity were found to be 190.50 µg, 266.30 µg and 175 µg. The mean IC_{50} values for ascorbic acid to superoxide anion, hydroxyl radical and DPPH radical were found to be 54.4 µg, 68 µg and 22 µg. The results clearly indicate the free radical scavenging activity of methanolic *of C. umbellata* stem extract and this activity comparable with that of the standard drug ascorbic acid. The results were given in Table 3.

Table 3: 50% inhibition concentration (IC_{50}) of *C. umbellata* Roxb. stem extract on superoxide, hydroxyl and DPPH free radicals

Extract/Ascorbic acid	IC_{50} value (µg)		
	Superoxide radical	Hydroxyl radical	DPPH radical
Methanol extract of *C.umbellata*	190.50±1.20	266.30±1.30	175.00±0.50
ascorbic acid	54.4±1.1	68.00±1.3	22.0±0.5

3.3 Acute toxicity studies

The selected plant extract showed neither visible sign of toxicity nor mortality. The results undoubtedly be a sign of non-toxic at the amount, they tested, *i.e*., 2000 mg/kg B.W. Hence, there is no LD_{50} and all the extracts tested and are considered safe and non-toxic.

3.4 Nephroprotective activity

3.4.1 *Effect of C. umbellata stem extract in cisplatin induced nephrotoxicity in rats*

3.4.1.1 *Effect on serum creatinine and Blood urea nitrogen* (BUN)

Rats treated with cisplatin (Group II) developed a significant renal damage, observed as elevated serum levels of creatinine and blood urea nitrogen when compared to normal control (Group I). Pretreatment of stem extract of *C. umbellata* (Methanol 70%v/v) to group III with 250 mg/kg .W. and 500 mg/kg B.W. along with cisplatin showed dose dependent significant ($p<0.01$) reduction in serum levels of creatinine and blood urea nitrogen as compared to cisplatin treated group. The % protection against raised serum creatinine and blood urea levels by stem extract of *C. umbellata* was 66.42% and 54.43 % at doses of and 73.88 % and 68.54 % at 500mg/kg was found. The results were provided in Table 4.

3.4.1.2 *Effect on serum albumin and total protein*

Rats treated with cisplatin (Group II) developed a significant renal damage observed as decreased serum levels of albumin and total protein when compared to normal control (Group I). Pretreatment with stem extract of *C. umbellata* (Methanol 70%v/v) along with cisplatin at doses of 250 and 500 mg/kg (Group III and Group IV) significantly increased the levels of albumin and total protein in

serum compared to cisplatin treated group. The % protection against decreased albumin and total protein levels were 34.32%, 37.8% and 70.88% and 62.99% at doses of 250 and 500 mg/kg B.W. The results were provided in Table 4.

Table 4: % Protection of *C. umbellata* Roxb. stem extract on cisplatin induced nephrotoxicity

Groups	Creatinine (mg/dl)	BUN (mg/dl)	Albumin (g/dl)	Total protien (g/dl)
Group I Vehicle Control	0.85 ± 0.01	21.17 ± 0.32	4.57 ± 0.11	6.13 ± 0.11
Group II Cisplatin 5mg/kg,i.p	1.52 ± 0.04	32.15 ± 0.61	3.45± 0.10	4.86 ± 0.12
Group III CUHAE 250mg/kg	1.07 ± 0.02** (66.42)	26.17 ± 0.32** (54.43)	3.83± 0.06** (34.32)	5.34 ± 0.03** (37.80)
Group IV CUHAE 500mg/kg	1.02 ± 0.04* (73.88)	24.62 ± 0.40** (68.54)	4.24± 0.03** (70.88)	5.66 ± 0.10** (62.99)

Values are Mean ± SEM, n=6, % protection= Negative control – Treatment/Negative control-Vehicle control´ 100

* p<0.05 compared to group II (negative control)

**p<0.01 compared to group II (negative control)

3.4.2 *Effect of C. umbellata* Roxb. *stem extract in gentamicin induced nephrotoxicity in rats effect*

3.4.2.1 *Effect on serum creatinine and blood urea nitrogen* (BUN)

Rats treated with gentamicin (Group II) developed a significant renal damage, observed as elevated serum levels of creatinine and blood urea nitrogen when compared to normal control (Group I). Pretreatment with stem extract of *C. umbellata* (Methanol 70%v/v) along with gentamicin at doses of 250 and 500 mg/kg (Group III and Group IV) produced dose dependent significant ($p<0.05$) reduction in serum levels of creatinine and blood urea nitrogen as compared to gentamicin treated group. The % protection against rise in serum creatinine levels and blood urea levels by stem extract of *C. umbellata* at doses of 250 and 500 mg/kg was found to be 49.14%, 77.71% and 43.51%, 73.74%, respectively. The results were shown in Table 5.

Table 5 : % Protection of *C. umbellata* Roxb. stem extract on gentamicin induced nephrotoxicity

Groups	Creatinine (mg/dl)	BUN (mg/dl)	Albumin (g/dl)	Total protien (g/dl)
Group I Vehicle Control	0.85 ± 0.01	21.17 ± 0.32	4.57 ± 0.11	6.13 ± 0.11
Group II Gentamicin 80mg/kg, i.p	1.72 ± 0.03	36.22 ± 0.49	3.10± 0.03	4.20 ± 0.04
Group III CUHAE 250mg/kg	1.29 ± 0.02** (49.14)	29.67± 0.37** (43.51)	4.08± 0.04** (66.82)	5.41 ± 0.07** (62.69)
Group IV CUHAE 500mg/kg	1.04 ± 0.02** (77.71)	25.12 ± 0.29** (73.74)	4.27± 0.02** (79.77)	5.71 ± 0.07** (78.24)

Values are Mean ± SEM, n=6, % protection= Negative control – Treatment/Negative control-Vehicle control ´ 100

* p<0.05 compared to group II (negative control)

**p<0.01 compared to group II (negative control)

3.4.2.2 *Effect on serum albumin and total protein*

Rats treated with gentamicin (Group II) developed a significant renal damage, observed as decreased serum levels of albumin and total protein when compared to normal control (Group I). Pretreatment with stem extract of *C. umbellata* (Methanol 70%v/v) along with gentamicin at doses of 250 and 500 mg/kg (Group III and Group IV) showed dose dependent significant ($p<0.01$) increase in serum levels of albumin and total protein as compared to gentamicin treated group. The % protection against decrease in serum albumin levels and total protein levels by stem extract of *C. umbellata* at doses of 250 and 500 mg/kg was found to be 66.82%, 79.77% and 62.69%, 78.24%, respectively. The results were showed in Table 5.

Phytochemical screening of *C. umbellate* stem extract indicates that plant contain different biologically phytochemicals like sterols, terpenoids, alkaloids, glycosides and phenols. There were reports about the phytochemicals responsible for different activities from *Caralluma* species and other plants (Habibuddin, 2008; Latha *et al.,* 2004; Shanmugam *et al.,* 2013; Yokozawa *et al*., 1992; Fadila Maiza-Benabdesselam, 2007; Yokozawa *et al.,* 1999; Meesala Sreenivasarao *et al.,* 2015). The *C. umbellate* stem extract showed antioxidant activity as standard drug ascorbic acid. Free radicals present in the body majorly contribute in damage of body organs and causes different disorders like diabetes, cancer, liver and kidney failures. All these are because of short natural antioxidants in the body (Ali, 2003; Shirwaikar *et al*., 2004; Kiyohara *et al*., 1995). The phytochemicals like phenols and alkaloids present in the stem extract of *C. umbellate* may increase stabilization of the free radicals (Rajeswari *et al.,* 2014). The stabilization of free radicals reduces the free radicals reaction with the stabilized organic molecules present in the cells and it did not damage the permeability of cell membrane systems.

C. umbellate stem extract showed the significant nephroprotective activity on cisplatin and gentamicin induced nephrotoxicity. Acute renal failure refers to the sudden and usually reversible loss of renal function due to several compounds like radiocontrast agents, cyclosporine, antibiotics, chemotherapeutic agents, laboratory chemicals and pollutants, *etc*. These may directly affect membrane permeability by increasing the activity of lipase enzymes activity, these damage the cell structure and its capacity in the maintenance of solvents, ions and organic materials pathways in cells (Davison *et al.,* 1988; Chaturvedi *et al.,* 2003). Cisplatin and gentamicin cause the renal failure in the rats by producing the different free radicals, *i.e*., by reduction in sulphydryl groups in the rat renal cortex, increase in calcium in renal cortex and mitochondria. The produced free radicals causes the oxidative stress to the lipid molecules in the membranes and finally damages the structure and function of the renal cells. The compounds responsible for antioxidant activity present in the stem extract of the *C. umbellate* may be responsible in the protection of cell membrane (nephrons) of the kidney from free radicals formation due to the toxicity of cisplatin and gentamicin (Barry, 2000; Sara *et al.,* 2009). The isolation pure compounds from the *C. umbellate* may reveals the complete mechanism of antioxidant and nephroprotective activity.

4. Conclusion

The formation of free radicals in the human body causes the severe damage in the cell of every organ in the body. The free radical reacts with the other molecules to stabilize themselves in this process, the damage the cell membrane structure of cells. In the current investigation, the stem methanol extract of *C. umbellata* was found to possess significant antioxidant and nephroprotective activities and, further, studies needful to identify and isolate the active molecules responsible for its activities.

Conflict of interest

We declare that we have no conflict of interest.

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