

# Evaluation of Phenolic Content and Antioxidant Property of *Crossandra infundibuliformis* Leaves Extracts

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Received 5 January 2014; revised 5 March 2014; accepted 15 March 2014

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## Abstract

The present study was aimed to investigate the phenolic content and antioxidant property of *Crossandra infundibuliformis* leaves. Shade dried leaves were successively extracted and determined phenolic and flavonol content of the extracts. Six different *in vitro* antioxidant assays such as 1, 1'-diphenyl-2-picrylhydrazyl (DPPH), lipid peroxidation (LPO), nitric oxide, hydrogen peroxide radical scavenging, reducing power of the extracts and total antioxidant capacity (values were expressed as mg ascorbic acid (vitamin C) equivalent antioxidant capacity per gram of extracts) assays were followed to determine the antioxidant capacity of the extracts. The results of this study indicated that methanol extract possess higher level of phenolic (98.52 mg of gallic acid/g of extract) and flavonol (84.59 mg of rutin/g of extract) constituents. The antioxidant property of ethyl acetate extract was significantly higher than other extracts in DPPH method. Moreover, methanol extract has significantly higher antioxidant activity in LPO inhibition (IC<sub>50</sub> value of 70.66 µg/ml), hydrogen peroxide (IC<sub>50</sub> value 130.33 µg/ml) and reducing capacity of the extract (1.19) than other chloroform and ethyl acetate extracts. The strong correlation was observed for phenolic content and antioxidant activities of the extracts ensuring the involvement of phenols in antioxidant activity. However, results suggests that *Crossandra infundibuliformis* leaves possess antioxidant activity.

## Keywords

*Crossandra infundibuliformis*; DPPH; Hydrogen Peroxide; Nitric Oxide; Ascorbic Acid; Gallic Acid

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## 1. Introduction

Free radicals generated by increasing the cell oxidation contribute to many diseases like cardiovascular disease, tumor growth, wrinkled skin, cancer, Alzheimer's disease and even a decline in energy and endurance [1]-[4]. Several free radicals such as superoxide anions, hydroxyl radicals and hydrogen peroxide are toxic to cell membrane present in the body organs [5]. Natural antioxidants provide a protection to living organisms from damage caused by reactive oxygen species and concomitant lipid peroxidation, protein damage and DNA strand breaking [6]. At the present time, the most commonly used antioxidants butylated hydroxyanisole (BHA) and butylated hydroxy toluene (BHT) which are suspected of being carcinogenic and causing liver damage. Therefore, there is a growing interest on natural and safer antioxidants [7]. Naturally occurring antioxidants such as ascorbic acid, vitamin E, phenols and flavonols possess the ability to inhibit the free radical generation associated with many diseases, including cardiovascular disease, cancer, diabetes, arthritis, immune deficiency diseases and aging [8].

*Crossandra infundibuliformis* L. Leaf belongs to the family *Acanthaceae*, traditionally used in the treatment of migraine in Belgaum district, Karnataka, India. It is distributed in India, Bengal, Malaya, Sri Lanka, and Nepal. The plant has aphrodisiacal properties [9] and crushed plant material applied to a cattle is bitten by a dog [10]. The leaves of *C. infundibuliformis* reported for several activities including hepatoprotective [11] and antimicrobial properties were studied [12]. So far, no *in vitro* antioxidant study has been reported on *C. infundibuliformis* leave extracts. Hence, the present study is designed to give information and application for traditional use of *C. infundibuliformis* by conducting *in vitro* antioxidant assays of leave extracts.

## 2. Experimental

### 2.1. Plant Material

The leaves of *C. infundibuliformis* were collected from Raibag Taluk, Belgaum District, Karnataka, India. This plant was authenticated by Dr. Shiddamallayya N, Regional Research Institute Bangalore, where a voucher specimen has been preserved for future identification. Collected leaves were then washed with running water to remove the dirt, shade dried and ground to obtain course powder.

### 2.2. Chemicals

Folin-Ciocalteu reagent, ascorbic acid, gallic acid and DPPH were purchased from Sigma Chemicals, Germany. Potassium dihydrogen phosphate, potassium hydroxide, ferric chloride, ferrous sulphate, potassium ferricyanide, butylated hydroxy anisole (BHA) and thiobarbituric acid (TBA), trichloro acetic acid (TCA) were purchased from Merck (Mumbai, India). All the other solvents used in this study were of analytical grade and purchased from Sigma Aldrich, Bangalore.

### 2.3. Extraction

The powdered plant material (250 g) was extracted successively with 1500 ml each of petroleum ether (60°C - 80°C), chloroform, ethyl acetate and methanol in Soxhlet extractor for 18 - 20 h. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40°C - 50°C) in rotary evaporator. The percentage yield of all the extracts was given in **Table 1**. The extracts were then stored at 4°C in refrigerator for further analysis.

### 2.4. Determination of Total Phenol Content

The total phenol content of the extracts was measured by using Folin-Ciocalteu method with minor modifications [13]. Aliquots of the extracts 0.1 ml was mixed with 2.5 ml of Folin-Ciocalteu reagent (previously diluted with distilled water 1:10 v/v) and 2 ml (7.5%) of sodium carbonate. After incubation at 40°C for 30 min, the absorbance of the reaction mixture was measured at 760 nm by using a spectrophotometer (Shimadzu UV-160 spectrophotometer). Gallic acid was used as a standard and total phenol content of the extracts was expressed in milligram gallic acid equivalents (mg GAE) per gram of extract. All determinations were carried out in triplicates.

**Table 1.** Percentage yield, total phenolic, flavonol and total antioxidant capacity of successive extracts of *Crossandra infundibuliformis* leaves.

Extracts/Standard	% yield of extract	Total phenols mg/g <sup>a,b</sup>	Total flavonol mg/g <sup>a,c</sup>	Total antioxidant capacity <sup>d</sup>
Petroleum ether	2.48%	9.71 ± 0.09 <sup>f</sup>	47.12 ± 1.015 <sup>f</sup>	1.05 ± 0.04 <sup>g</sup>
Chloroform	2.56%	68.02 ± 1.06 <sup>e</sup>	66.93 ± 1.00 <sup>g</sup>	5.03 ± 0.01 <sup>i</sup>
Ethyl acetate	2.80%	47.66 ± 0.40 <sup>g</sup>	56.39 ± 0.57 <sup>g</sup>	0.52 ± 0.00 <sup>f</sup>
Methanol	2.88%	98.52 ± 0.29 <sup>e</sup>	84.59 ± 0.61 <sup>i</sup>	2.12 ± 0.01 <sup>g</sup>

<sup>a</sup>Mean of three replicate determination ± SEM, standard error mean, <sup>b</sup>gallic acid, <sup>c</sup>rutin, equivalent in mg/g of extract. <sup>d</sup>The total antioxidant capacity expressed as millimolar equivalent to ascorbic acid. Values with different symbols in the same column were significantly different ( $p < 0.05$ ) from each other.

## 2.5. Determination of Total Flavonol Content

Total flavonol content of extracts was determined by using the aluminium chloride method with some modifications [14]. Aliquots of the extracts 0.5 ml was mixed with 1.5 ml aluminium chloride (10%) and 0.1 ml potassium acetate (1 M). After incubation at room temperature for 30 min, 2.8 ml of distilled water was added. The absorbance of the reaction mixture was measured at 415 nm. Total flavonol content was expressed as milligram rutin equivalents per gram of extract. All determinations were carried out in triplicates.

## 2.6. In Vitro Antioxidant Activity

### 2.6.1. Total Antioxidant Capacity Assay

The total antioxidant activity of successive extracts was determined by phosphomolybdenum method [15]. An aliquot of 0.1 ml of extract or standard in DMSO was combined with 1 ml of reagent solution (sulphuric acid 0.6 M, sodium phosphate 28 mM and ammonium molybdate 4 mM) in an Eppendroff tube. The tubes were capped and incubated in water bath at 95°C for 90 min. The blank solution contained 1 ml of reagent solution and 0.1 ml of methanol. After cooling to room temperature, absorbance was measured at 695 nm against a blank. The results were expressed as millimolar equivalents of ascorbic acid.

### 2.6.2. DPPH Radical Scavenging Assay

DPPH radical scavenging activity of the extracts was determined by minor modifications [16]. Different concentrations of extracts 1 ml was added to 1 ml of DPPH (0.2 mM in methanol). The reaction mixture incubated at 30°C for 30 min. The control was prepared as above without any extract and methanol was used for the baseline correction. The changes in absorbance were measured at 517 nm using a spectrophotometer. All the measurements were done in triplicate.

### 2.6.3. Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging assay was determined by the method prescribed Jaishree *et al.*, (2008) [17]. A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS, pH 7.4). Various concentration of the extract or standard in methanol (1 ml) was added to 2 ml of hydrogen peroxide solution in PBS. After 10 min, the absorbance was measured at 230 nm.

### 2.6.4. Lipid Peroxidation Inhibitory Assay

Lipid peroxidation inhibitory activity was measured according to the modified method described by Jaishree *et al.*, (2008) [17]. Lipid peroxidation was induced by adding ferric chloride 10 µl (400 mM) and L- ascorbic acid 10 µl (400 mM) to a mixture containing egg lecithin (3 mg/ml) in phosphate buffer solution and different concentration of extracts (100 µl). After incubation for 1 h at 37°C, the reaction was stopped by adding 2 ml of 0.25 N hydrochloric acid containing 15% w/v trichloroacetic acid and 0.375% w/v thiobarbituric acid, boiled for 15 min, cooled, centrifuged and absorbance of supernatant was measured at 532 nm.

### 2.6.5. Nitric Oxide Scavenging Assay

Nitric oxide scavenging assay was determined with minor modifications [17]. The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), PBS (1 ml) was incubated at 25°C for 150 min. After incubation,

0.5 ml of the reaction mixture was removed and 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was mixed and allowed to stand for 5 min for completion of diazotization reaction. Naphthyl ethylene diamine dihydrochloride (1 ml) was added to the mixture and allowed to stand for 30 min in diffused light. The absorbance was measured at 540 nm.

#### 2.6.6. Reducing Power Assay

Reducing capacity of extract was measured with minor modifications [18]. 1.0 ml of extract was mixed with 2.5 ml of phosphate buffer (200 mM, pH 6.6) and 2.5 ml of potassium ferricyanide (30 mM). The mixture was incubated at 50°C for 20 min. Thereafter, 2.5 ml of trichloroacetic acid (600 mM) was added to the reaction mixture and centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (6 mM) and absorbance was measured at 700 nm.

### 3. Statistical Analyses

All assays were carried out in triplicates and results are presented as Mean  $\pm$  SD (n = 3). One way analysis of variance was performed (one way ANOVA) and the significant values were determined by Tukey's test level of significance of  $p < 0.05$ . The statistical analyses were carried out by using PRISM 5.

### 4. Results and Discussion

The total phenol and flavonol content of successive extracts of *C. infundibuliformis* were shown in **Table 1**. The methanol extract was found to be the highest amount of total phenols ( $98.52 \pm 0.61$  mg GAE/g of extract) and flavonols ( $84.59 \pm 0.29$  mg rutin equivalent to/g of extract) contents than other extracts.

DPPH radical is stable organic free radical with absorption band at 517 nm. The antioxidants donate the electron to DPPH radical on accepting electron, the absorption range will change in a visible to nonvisible coloration from purple to yellow [19]. Methanol extract has shown highest percentage of inhibition. Successive ethyl acetate extract of *C. infundibuliformis* was found to be highest IC<sub>50</sub> value ( $9.79 \pm 0.55$   $\mu$ g/ml) with excellent scavenging activity. Whereas, other extracts possess lower antioxidant activity. There was significant difference in the DPPH radical scavenging activity observed among the extracts. Reactive oxygen species such as hydroxyl radical were found by different mechanism. This is considered causative factor for many diseases [20]. Hydroxyl radicals easily penetrate in cell membranes and readily react with biomolecules including proteins, carbohydrates and lipids. These cause cell death or tissue injury. Natural antioxidants plays important role in neutralizing such type of free radicals. Among all the extracts, methanol and petroleum ether extracts of *C. infundibuliformis* have shown the highest scavenging ability. The IC<sub>50</sub> values of all the extracts confirming antioxidant activity, when compared with the standard ascorbic acid (**Table 2**).

Free radicals induce lipid peroxidation in polyunsaturated lipid rich areas like brain and liver [21]. The assay used to evaluate lipid peroxidation inhibitory activity of *C. infundibuliformis* leave extracts. In this study, our results demonstrate excellent antioxidant activity. The IC<sub>50</sub> value of methanol extract was found to be  $70.66 \pm 0.76$   $\mu$ g/ml and standard BHA  $75.83 \pm 0.58$   $\mu$ g/ml. All other extracts exhibited moderate antioxidant activity. Nitric oxide radicals are well known inducer of tissue pathogenesis leading to several diseases such as cancer, diabetes mellitus and age related disorders [22]. Many plant species with antioxidants acts as protective agents with these diseases. Among the four extracts, chloroform extract was found higher percentage inhibition and all other extracts possess moderate percentage inhibition. The IC<sub>50</sub> value of chloroform extract is  $240.50 \pm 0.50$   $\mu$ g/ml and other extracts have shown decreasing order of antioxidant activity; petroleum ether > methanol > ethyl acetate extracts with IC<sub>50</sub> values  $370.66 \pm 0.58$ ,  $814.66 \pm 0.29$  and  $970.50 \pm 0.87$   $\mu$ g/ml, respectively. The reducing power assay was usually used to determine the reduction capacity of antioxidants from ferric (Fe<sup>3+</sup>) to ferrous (Fe<sup>2+</sup>) [23]. Natural antioxidants play a vital role in breaking the chain reaction of free radicals by donating electron. In this study, the yellow color of test solution changes to various shades from green to blue depending on the reducing power of extracts. These results may serve significant antioxidant activity. The reducing capacity of all the extracts was shown in the **Table 2**. The methanol extract showed excellent reducing capacity with the absorbance  $2.03 \pm 0.005$ . However, petroleum ether, chloroform and ethyl acetate extracts exhibited moderate reduction capacity. In phosphomolybdenum method, molybdenum reduces to form green phosphate molybdenum complex by antioxidants, which have an absorption peak at 695 nm. Total antioxidant capacity of all the successive extracts of *C. infundibuliformis* was measured by monitoring the absorbance of the reaction mixture

**Table 2.** *In vitro* antioxidant activity of successive extracts of *Crossandra infundibuliformis* leaves in different methods.

Extracts/Standards	IC <sub>50</sub> values ± SEM (µg/ml) by assays				Reducing capacity at 500 µg/ml
	DPPH	Lipid peroxidation	Nitric oxide	H <sub>2</sub> O <sub>2</sub>	
Petroleum ether	63.38 ± 0.85	90.33 ± 0.29	814.66 ± 0.58	230.50 ± 0.50	0.07 ± 0.00
Chloroform	62.86 ± 0.17	110.83 ± 1.04	240.50 ± 0.50 <sup>a</sup>	154.66 ± 0.76	0.10 ± 0.00
Ethyl acetate	9.79 ± 0.55 <sup>a</sup>	74.33 ± 0.763	970.5 ± 0.87	201.16 ± 0.58	0.05 ± 0.00
Methanol	33.35 ± 0.59 <sup>a</sup>	70.66 ± 0.763 <sup>a</sup>	370.66 ± 0.29 <sup>a</sup>	130.33 ± 0.29 <sup>a</sup>	1.19 ± 0.00 <sup>a</sup>
Ascorbic acid	4.80 ± 0.22	NA	57.66 ± 0.76	90.5 ± 0.5	0.16 ± 0.00
BHA	NA	75.83 ± 0.58	NA	NA	NA

Values are means of three replicate determination ± SEM, standard error mean; NA- not analysed. <sup>a</sup>Values are significant higher reducing capacity than other groups and <sup>b</sup>values are significantly higher antioxidant activity when compared to other groups ( $P < 0.05$ ).

at 695 nm. A high absorbance value of the reaction mixture produces higher millimolar concentration, which proves a stronger antioxidant capacity as summarised in **Table 1**. Chloroform extract of *C. infundibuliformis* has shown higher antioxidant capacity ( $5.03 \pm 0.02$  mM), followed by methanol, petroleum ether and ethyl acetate extracts of *C. infundibuliformis*  $2.12 \pm 0.01$ ,  $1.05 \pm 0.04$  and  $0.52 \pm 0.00$  mM, respectively.

## 5. Conclusion

This study was designed to evaluate the antioxidant and free radical scavenging properties of successive extracts of *C. infundibuliformis* by using six different *in vitro* antioxidant methods. The methanol extract demonstrated the highest total phenol and flavonol contents. Among all the tested methods, methanol and chloroform extracts of *C. infundibuliformis* have shown strong antioxidant activity in the most of the tested methods. Considering both total phenol and flavonol contents, the antioxidant activity of methanol and chloroform extracts of *C. infundibuliformis* was optimal. It is indicated by the results of work that, methanol and chloroform extracts of *C. infundibuliformis* could serve as source of natural antioxidants.

## References

- [1] Finkel, T. and Holbrook, N.J. (2000) Oxidants, Oxidative Stress and the Biology of Ageing. *Nature*, **408**, 239-247. <http://dx.doi.org/10.1038/35041687>
- [2] Radisky, D.C., Levy, D.D., Littlepage, L.E., Liu, H., Nelson, C.M. and Fata, J.E., et al. (2005) Rac1b and Reactive Oxygen Species Mediate MMP-3-Induced EMT and Genomic Instability. *Nature*, **436**, 123-127. <http://dx.doi.org/10.1038/nature03688>
- [3] Houstis, N., Rosen, E.D. and Lander, E.S (2006) Reactive Oxygen Species Have a Causal Role in Multiple Forms of Insulin Resistance. *Nature*, **440**, 944-948. <http://dx.doi.org/10.1038/nature04634>
- [4] Fresquet, F., Pourageaud, F., Leblais, V., Brandes, R.P., Savineau, J.P. and Marthan, R., et al. (2006) Role of Reactive Oxygen Species and gp91phox in Endothelial Dysfunction of Pulmonary Arteries Induced by Chronic Hypoxia. *British Journal of Pharmacology*, **148**, 714-723. <http://dx.doi.org/10.1038/sj.bjp.0706779>
- [5] Jainu, M. and Shyamaladevi, C.S. (2005) *In Vitro* and *In Vivo* Evaluation of Free Radical Scavenging Potential of *Cissus quadrangularis*. *African Journal Biomedical Reseach*, **8**, 95-99.
- [6] Srinivasan, R., Chandrasekar, M.J.N. and Nanjan, M.J. and Suresh, B. (2007) Antioxidant Activity of *Caesalpinia digyna* Root. *Journal of Ethnopharmacology*, **113**, 284-291. <http://dx.doi.org/10.1016/j.jep.2007.06.006>
- [7] Jayaprakasha, G.K., Selvi, T. and Sakariah, K.K. (2003) Antibacterial and Antioxidant Activities of Grape (*Vitis vinifera*) Seed Extract. *Food Research International*, **36**, 117-122. [http://dx.doi.org/10.1016/S0963-9969\(02\)00116-3](http://dx.doi.org/10.1016/S0963-9969(02)00116-3)
- [8] Pietta, P., Simonetti, P. and Mauri, P. (1998) Antioxidant Activity of Selected Medicinal Plants. *Journal of Agriculture and Food Chemistry*, **46**, 4487-4490. <http://dx.doi.org/10.1021/jf980310p>
- [9] Kirtikar, K.R. and Basu, B.D. (2001) Indian Medicinal Plants. 2nd Edition, Oriental Enterprises, Uttaranchal, Volume 8, 2604.
- [10] Abhijit, D. and De, J.N. (2010) Ethnoveterinary Uses of Medicinal Plants by the Aborigines of Purulia District, West Bengal. *India Science Alert*, **95**, 551-559.
- [11] Madhumitha, G., Saral, A.M., Senthilkumar, B. and Sivaraj, A. (2010) Hepatoprotective Potential of Petroleum Ether Leaf Extract of *Crossandra infundibuliformis* on CCl<sub>4</sub> Induced Liver Toxicity in Albino Mice. *Asian Pacific Journal of*

*Tropical Medicine*, **3**, 788-790.

- [12] Madhumitha, G. and A. M. Saral (2011) Preliminary Phytochemical Analysis, Antibacterial, Antifungal and Anticandidal Activities of Successive Extracts of *Crossandra infundibuliformis*. *Asian Pacific Journal of Tropical Medicine*, **4**, 192-195.
- [13] Meda, A., Lamien, C.E., Romito, M., Millogo, J. and Nacoulma, O.G (2005) Determination of the Total Phenolic, Flavonoid and Praline Contents in Burkina Fasan Honey, As Well As Their Radical Scavenging Activity. *Food Chemistry*, **91**, 571-577. <http://dx.doi.org/10.1016/j.foodchem.2004.10.006>
- [14] Willet, W.C. (2002) Balancing Life-Style and Genomics Research for Disease Prevention. *Science*, **296**, 695-698. <http://dx.doi.org/10.1126/science.1071055>
- [15] Huong, N.T.T., Malsumato, K., Kasai, R., Yamasaki, K. and Watana, B. (1998) *In Vitro* Antioxidant Activity of Vietnamese ginseng saponin and Its Components. *Biological and Pharmaceutical Bulletin*, **21**, 978-981. <http://dx.doi.org/10.1248/bpb.21.978>
- [16] Blois, M.S. (1958) Antioxidant Determinations by the Use of a Stable Free Radical. *Nature*, **181**, 1199-1200. <http://dx.doi.org/10.1038/1811199a0>
- [17] Jaishree, V., Badami, S. and Suresh, B. (2008) *In Vitro* Antioxidant Activity of *Enicostemma axillare*. *Journal of Health Science*, **54**, 524-528. <http://dx.doi.org/10.1248/jhs.54.524>
- [18] Athukorala, Y., Kim, K.N. and Jeon, Y.J. (2006) Antiproliferative and Antioxidant Properties of an Enzymatic Hydrolysate from Brown Alga *Ecklonia cava*. *Food and Chemical Toxicology*, **44**, 1065-1074. <http://dx.doi.org/10.1016/j.fct.2006.01.011>
- [19] Hseu, Y.C., Chang, W.H., Chen, C.S., Liao, J.W., Huang, C.J., Lu, F.J., *et al.* (2008) Antioxidant Activities of *Toona sinensis* Leaves Extracts Using Different Antioxidant models. *Food and Chemical Toxicology*, **46**, 105-114. <http://dx.doi.org/10.1016/j.fct.2007.07.003>
- [20] Aoshima, H., Tsunoue, H., Koda H. and Kiso, Y. (2004) Aging of Whiskey Increases 1, 1-Diphenyl-2-Picrylhydrazyl Radical Scavenging Activity. *Journal of Agriculture and Food Chemistry*, **52**, 5240-5244.
- [21] Coyle, J.T. and Puttfarcken, P. (1993) Oxidative Stress, Glutamate and Neurodegenerative Diseases. *Science*, **219**, 1184. <http://dx.doi.org/10.1126/science.6338589>
- [22] Ialenti, A., Moncada, S. and Dirosa, M. (1993) Modulation of Adjuvant Arthritis by Endogenous Nitric Oxide. *British Journal of Pharmacology*, **110**, 701. <http://dx.doi.org/10.1111/j.1476-5381.1993.tb13868.x>
- [23] Oyaizu, M. (1986) Studies on Product of Browning Reaction Prepared from Glucose Amine. *Japanese Journal of Nutrition*, **44**, 307-315. <http://dx.doi.org/10.5264/eiyogakuzashi.44.307>

## Abbreviations

- 1, 1'-Diphenyl-2-picrylhydrazyl (DPPH)
- Lipid peroxidation (LPO)
- 50 Percent inhibition concentration (IC<sub>50</sub> value)
- Butylated hydroxyanisole (BHA)
- Butylated hydroxy toluene (BHT)
- Dimethyl sulfoxide (DMSO)