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PHYTOCHEMICAL PROPERTIES AND ANTIOXIDANT ACTIVITY OF *CALOTROPIS PROCERA* (Ait.) R. Br.

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ABSTRACT

The communication deals with the antioxidant activity of aqueous leaf extract of *Calotropis procera* (Ait.) R. Br., along with the phytochemical, pharmacological and nutritive properties. The total antioxidant activity was not very pronounced, however, the reducing potential of the extract was prominent, and showed concentration dependence. The phytochemical analysis showed high amount of total ash content (18.3 ± 0.8 mg/g), moderate amount of flavonoids (25.08 ± 0.6) and tannin (9.1 ± 0.3) contents and low amount of phenol (15.2 ± 0.3 mg/g). The protein content (181.0 ± 6.0 mg/g) was found high, while the fat (41.4 ± 3.0 mg/g) and carbohydrate content (79.1 ± 3.5 mg/g) as well as the nutritive potential (1.41 ± 0.3 Cal/g) were in the moderate range. *C. procera* leaves showed high swelling and foaming indices (195 ± 2.0 and 125 ± 2.5 % respectively), which is an indicative of good drug release characteristics. Present findings suggest that *C. procera* is a potential source of phytochemicals like phenols, tannins and flavonoids, nutrients and natural antioxidants.

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INTRODUCTION

As a result of normal cellular events like aerobic respiration, phagocytosis of infected cells, degradation of fatty acids and natural toxins, as well as some exogenous interference, like exposure to low wavelength electromagnetic radiations, free radicals are generated within the body (Krishnaiah *et al.,* 2007). In form of reactive oxygen species (ROS), and reactive nitrogen species (RNS) when present in low moderate concentrations, these free radicals are known to play beneficial roles in cellular response to noxia, in defence against infective agents and in various cell signalling pathways (Valko *et al.,* 2007). However, when present in high concentrations, these free radicals react with membrane lipids, proteins, nucleic acids, enzymes and other biomolecules resulting into cellular damage (Shivaprasad *et al.,* 2005). Body employs antioxidants to scavenge these free radicals and hence to keep in check the free radical-induced cellular damage (Wayner *et al.,* 1987). At times, the rate of generation of free radicals exceeds their rate of neutralization by the endogenous antioxidants, the resulting condition is known as oxidative stress (Niki, 2010; Sen *et al.,* 2010). Recent investigations attest the involvement of oxidative stress in various ailments including cardiovascular disorders and cancer (Niki, 2010; Kumar *et al.,* 2013a). This has attracted the much demanded attention of the workers as well as the general public towards antioxidants contained in natural sources like fruits, herbs and spices, which could be employed for maintenance of health by prevention and treatment of diseases (Niki, 2010; Kumar *et al.* 2013b).

One important plant of the rich medicinal flora of India is *Calotropis procera* (Asclepiadaceae). It is a perennial shrub found in the tropical parts of the world, including Asia, Africa, and Arabian Peninsula. It has also been reported in Australia, Mexico, South-Central America and in few Caribbean and Pacific islands (Rahman and Wilcock, 1991). In traditional and folk medicine systems, *C. procera* has been used to treat a variety of ailments like leprosy, fever, menorrhagia, malaria, headache, and rheumatism (Singh *et al.,* 2010; Tomar *et al.,* 2012) in the Sudani, Unani, Arabic and Indian traditional medicine systems (Sheth, 2011). There is however, an insufficiency of information regarding the antioxidant efficacy of the aqueous leaf extract of the plant. For acceptance of the herbal health claims, the assessment of phytochemical, pharmacological and nutritional parameters is also essential. With this background, the antioxidant properties of aqueous leaf extract of *Calotropis procera* along with its phytochemical, pharmacological and nutritive properties have been studied.

MATERIALS AND METHODS

Collection of plant material: The fresh mature parts of stem were collected, chopped, dried in a shade under room temperature for six to seven days and then crushed into coarse powder using electric grinder. The powder was sieved to get fine powder using fine plastic sieve which was stored in air tight bottle in the laboratory until required.

Extract preparation: 50g of the powder was subjected to extraction by soxhlet using distilled water. The extracts obtained were filtered, concentrated after dryness in rotary flash evaporator maintained at 45ºC; percentage yield of each extract was calculated and the dried extract was stored in air tight containers at room temperature for further studies.

Phytochemical analysis: Following WHO (1998), 2g of powder was incinerated at 500-600ºC to free the sample from carbon. The percentage of ash was calculated with reference to air dried powder. The total ash obtained was boiled in 25mL of distilled water for 5 min and the insoluble matter was collected in an ash-free filter paper and incinerated at temperature not exceeding 450ºC. Subtracting the weight of the insoluble matter from the weight of the ash gives the percentage of water soluble ash. For the acid-insoluble ash, the total ash was boiled with 25 mL of 2N HCl for 5 min, the insoluble matter was collected, washed, dried and weighed.

The moisture content was determined in terms of the loss in weight of the plant material on overnight heating at 150ºC (Sadasivam and Manickam, 1996).

Total phenol was determined by Folin Ciocalteau reagent, following Ramamoorthy and Bono (2007). Tannins were quantified as stated in the Quality control methods for medicinal plant materials (1998). Aluminium chloride colorimetric method was used to determine flavonoids content (Lin and Tang, 2007).

Pharmacological properties: The swelling index and foaming index were calculated using 1g of dry power of the sample (WHO, 1998).

Nutritive value: Micro Kjeldahl method was used for the determination of protein. Crude fat, carbohydrate and nutritive value were calculated, following Nile and Khobragade (2009).

Antioxidant activity: The reducing power of the extract was evaluated spectrophotometrically as it reduced potassium ferricyanide to potassium ferrocyanide (Jayanthi and Lalitha, 2011). The total antioxidant capacity was determined by the spectrophotometric quantification of phosphomolybedate complex (Prieto *et al.,* 1999).

RESULTS AND DISCUSSION

Physicochemical analysis: The results are depicted in Tables 1-3 and Fig. 1 and 2.

The total ash content of *C. procera* (18.3±0.8mg/g) stands higher than that of some other medicinal plants like *Butea monosperma* (6.5±0.21%), *Cassia fistula* (5.3±0.22%), *Q. infectoria* (2.0±0.12%), *T. cordifolia* (7.2±0.21%) and

Table 1: Quantitative analysis of phytochemical properties of *C. procera* leaves (M±SD; n = 3)

S. No.	Attributes	Values in mg/g
1.	Total ash	18.3 ± 0.8
2.	Water soluble ash	19.0 ± 1.1
3.	Acid insoluble ash	16.0 ± 0.5
4.	Phenols	15.2 ± 0.3
5.	Flavonoids	25.08 ± 0.6
6.	Tannins	9.1 ± 0.3
7.	Moisture content	112.6 ± 2.1

Table 2: Pharmacological properties of *C. procera* leaves (M±SD; n = 3)

S. No.	Attributes	Values in %
1.	Swelling index	195 ± 2.0
2.	Foaming index	125 ± 2.5

Table 3: Nutritive potential of *C. procera* leaves (M±SD; n = 3)

S. No.	Attributes	Values in mg/g
1.	Fat	41.4 ± 3.0
2.	Protein	181.0 ± 5.2
3.	Carbohydrate	79.1 ± 3.5
4.	Nutritive value	1.41 ± 0.3

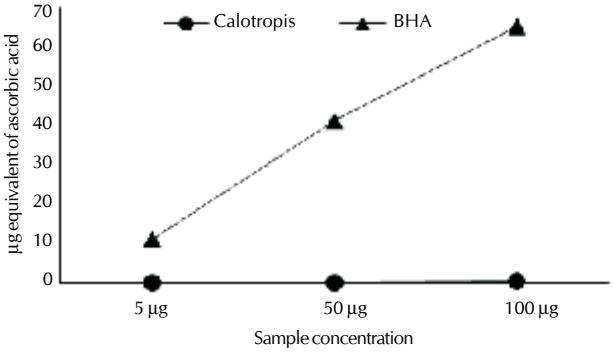

Figure 1: Total antioxidant capacity of aqueous leaf extract of *C. procera*

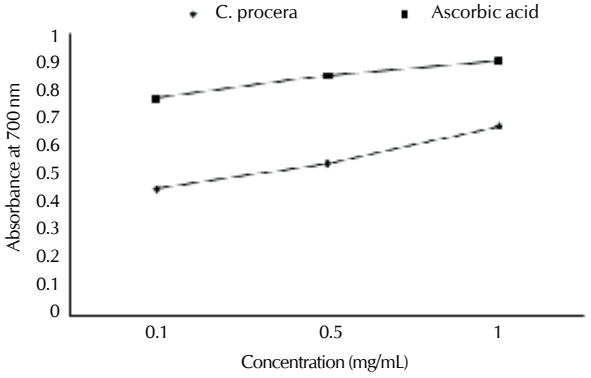

Figure 2: Reducing power of aqueous leaf extract of *C. procera*

Cedrela toona (7.4±0.19%), as reported by Vermani *et al.* (2010). Similarly, the values for water soluble ash and acid insoluble ash (table 1) were also proportionately high. The ash content analysis measures the amount of non-ignitable (silicious) materials present in the sample (WHO, 1998). The amount and composition of ash remaining after combustion of plant material varies considerably with the part of plant, age, treatment etc. The constituents of ash also vary with time and from organ to organ since it mainly represents the inorganic part of the plant (Vermani *et al.,* 2010). The higher ash content is an indicative of greater amount of inorganic material in the sample.

Phenol and flavonoids found in the edible and inedible parts

of plants portray antioxidant activity, and hence are of immense importance (Djeridane *et al.*, 2007). The antioxidant capacity of phenol and flavonoids is mainly due to their redox properties, which allows them to cut as reducing agents, hydrogen donors' singlet oxygen quenchers or metal chelators (Kanimozhi *et al.*, 2011). The total phenolic content of *C. procera* was found to be 15.2 ± 0.3mg/g (Table 1), which is of lower range as compared to that in the leaves of some other medicinal plants like *B. pinnatum* (18.4mg/g), *Ipomea aquatic* (18.8mg/g), *Terminalia bellerica* (29.6mg/g), *T. cordifolia* (17.03 ± 0.4mg/g), and *Xanthium strumarium* (71.6mg/g), while the flavonoid content in *C. procera* (25.08 ± 0.6 mg/g) was found higher than that in *B. pinnatum* (8.4mg/g) and *T. cordifolia* (6.5 ± 0.2 mg/g). The flavonoid content of *C. procera* however was found lower than that of *Xanthium strumarium*, *Ipomea aquatic* and *Terminalia bellerica i.e.* 28.8mg/g, 37.5mg/g and 42.8mg/g respectively (Yadav and Agarwala, 2011; Kumar *et al.* 2013b).

Tannins are major secondary metabolite of higher-order plants, and these phytophenol-related chemicals are thought to be principal molecular defence mechanism against herbivores and viruses alongside their antioxidant property, which is of immense importance and for that green tea is taken all over the world (Beart *et al.*, 1985). The tannin content in a number of foods items consumed in different parts of India ranged between 1.5 – 2.0g% (Bagepalli and Rao, 1982) Comparing these to our result, tannin content in *C. procera* leaves (9.1 ± 0.3 mg/g) stands near to these values.

Pharmacological analysis: A direct relationship exists between swelling index and polymer concentration (Nayak *et al.,* 2011). The swelling index of *C. procera* (195 ± 2.0%) was found very high as compared to the standard polymers like pectin (55%) and Xanthan (44%). Medicinal plants are known to contain saponins that forms persistent foam when as aqueous decoction is shaken, which is indicated by foaming index. Not much work has been done on foaming index, and of those available, none has reported any significant value against the index. Thus, the foaming index of *C. procera* (125 ± 2.5%) can be considered high, which again indicates a higher drug release rate. The Swelling and foaming indices are indicatives of the drug release characters (Jain *et al.,* 2008). The release of drug from hydrophilic matrices occurs as a result of complex interaction between diffusion, dissolution and erosion mechanisms. On coming in contact with water, hydrophilic matrices undergo gel formation, and progressive phase transition from glassy to rubbery state takes place. This results in solvation of individual polymer chains (Nayak *et al.*, 2011).

Nutritional properties: Under nutritional properties, amongst the investigated attributes (Table 3), species was found rich in protein content (181.0 ± 6.0mg/g), while the fat and protein contents were moderate (41.4 ± 3.0 and 79.1 ± 3.5mg/g respectively). The nutritive value of *C. procera* was found 1.41 ± 0.3 Cal/g. The fat, protein and carbohydrate content of some other medicinal plants have been reported as 19.83 ± 0.7%, 12.5 ± 0.5 %, 0.78 ± 0.4% respectively for *Hydrocotyl rotendifolia*; 13.2 ± 0.7%, 12.5 ± 0.5%, 1.36 ± 0.3% respectively for *Oxalis corniculata*; 9.9 ± 1.0%, 1.8 ± 0.3%, 0.19 ± 0.1% respectively for *Potentilla mooniana*; 7 ± 0.6%, 2.1 ± 0.3%, 0.75 ± 0.2% respectively for *Murraya koengigi*; and 6.175 ± 0.2%, 4.59 ± 0.1%, 0.27 ± 0.1% respectively for *Pogostemon benghalensis*. The nutritive potential of various tropical and subtropical fruits and vegetables have been reported as 348.45 Cal/100g for *Nelumbo nucifera* seeds, 370.33 Cal/100g for *Embelia ribes* seeds, 267.22 Cal/100g for *Eugenia jambola* seeds and 124.10 Cal/100g for *Artocarpus heterophyllus* leaves (Hoe and Siong, 1999; Indrayan *et al.,* 2005). Comparing these values to the present results, we may infer that *C. procera*, with high protein content, along with moderate fat and carbohydrate content, showing moderate nutritive value, supports its use as, fodder and a handy supplement for various important phytochemicals (Abbas *et al.,* 1992; Nehra *et al.,* 1987).

Antioxidant potential: Total antioxidant capacity (TAC) means the capacity of free radical scavenging by the bioactive constituents contained in the test sample (Niki, 2010). Comparing the TAC of aqueous leaf extract of *C. procera* against the BHA standard (Fig. 1), we may infer that the TAC of *C. procera* leaf is not very pronounced.

Reducing power is associated with the antioxidant activity and may serve as a significant reflection of the antioxidant activity. Compounds with reducing power indicate that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation processes, so that they can act as primary and secondary antioxidants (Jayanthi and Lalitha, 2011). The reducing power of *C. procera* (Fig. 2) shows concentration-dependence. Comparing the present result with ascorbic acid standard, we may conclude that aqueous leaf extract of *C. procera* exhibits good reducing potential.

On the basis of above results, it can be concluded that *C. procera* possess considerable nutritive potential and can be considered a good source of phytochemicals like phenols, flavonoids and tannins, and reducing agents.

REFERENCES

Abbas, B., El Tayeb, A. E. and Sulleiman, Y. R. 1992. *Calotropis procera*: feed potential for arid zones. *Veterinary Record.* **131(6):** 132.

Bagepalli, S. and Rao, N. 1982. Tannin content of foods commonly consumed in India and its influence on ionisable iron. *J. Sci. Food and Agriculture.* **33(1):** 89-96.

Beart, J. E., Lilley, T. H. and Haslam, E. 1985. Plant polyphenols, secondary metabolites and chemical defense: some observations. *Phytochemistry.* **24:** 33-38.

Djeridane, B., Yousfi, M., Vidal, N., Nadjemi, N., Lesgards, J. F. and Stocker, P. 2007. Screening of some Algerian medicinal plants for the phenolic compounds and their antioxidant activity. *European Food Research and Technology.* **224(6):** 801-809.

Hoe, V. B. and Siong, K. H. 1999. The nutritional value of indigenous fruits and vegetables in Sarawak. *Asia Pacific J. Clinical Nutrition.* **8(1):** 24-31.

Indrayan, A. K., Sharma, S., Durgapal, D., Kumar, N. and Kumar, M. 2005. Determination of nutritivev value and analysis of mineral elements of some medicinally valued plants from Uttaranchal. *Current Science.* **89(7):** 1252-1255.

Jain, S., Yadav, S. K. and Patil, U. K. 2008. Preparation and evaluation of sustained release matrix table of furosemide using natural polymers. *Res. J. Pharma. and Tech.* **1(4):** 374-376.

Jayanthi, P. and Lalitha, P. 2011. Reducing power of the solvent

extracts of *Eichhornia crassipes* (Mart.) Solms. *Int. J. Pharmacy and Pharmaceutical Sci.* **3(3):** 126-128.

Jayanthi, P. and Lalitha, P. 2011. Reducing power of the solvent extracts of *Eichhornia crassipes* (Mart.) Solms. *Int. J. Pharmacy and Pharmaceutical Sci.* **3(3):** 126-128.

Kanimozhi, D., Kandhymathi, K., Bharathidasan, R., Mahalingam, R., Deepa, S. and Panneerselvam, A. 2011. Antioxidant activity, estimation of total phenolic content and tannin of *Lecuas aspera* and *Sassia ariculata*. *World Journal of Science and Technology*. **1(9):** 11-17.

Krishnaiah, D., Sarbatly, R. and Bono, A. 2007. Phytochemical antioxidants for health and medicine- A move towards nature. *Biotechnology and Molecular Biology Review*. **1(4):** 97-104.

Kumar, A., Kumar, M., Dandapat, S. and Sinha, M.P. 2013. Antioxidant activity and pharmocological screening of Tinospora cordifolia. The Bioscan. 8(2): Supplement on Medicinal Plants. pp. 689-693.

Kumar, M., Kumar, A., Dandapat, s. and Sinha, M. P. 2013. Phytochemical screening and antioxidant potency of Adhatoda vasica and Vitex negundo. The Bioscan. 8(2): Supplement on Medicinal Plants. pp. 723-730.

Lin, J. Y. and Tang, C. Y., 2007. Determination of total phenolic and flavonoids contents in selected fruits and vegetables, as well as their stimulatory effects on mouse splenocyte proliferation. *Food Chem.* **101(1):** 140-147.

Nayak, R.K., Narayana Swamy, V.B., Senthil, A. and Mahalaxmi, R. 2011. An *in vitro* evaluation of *Mangifera indica* gum as a potential excipient for oral controlled-release matrix tablet. *Pharmacology online.* **2:** 360-391.

Nehra, O. P.; Oswal, M. C. and Faroda, A. S. 1987. Management of fodder trees in Haryana. *Indian Farming.* **37(3):** 31-33.

Niki, E. 2010. Assessment of antioxidant capacity *in vitro* and *in vivo*. *Free Radical Biol. and Med.* **49:** 503-515.

Nile, S.H. and Khobragade, C.N.N. 2009. Determination of nutritive value and mineral elements of some important medicinal plants from western part of India. *J. Medicinal Plants*. **8(5):** 79-88.

Prieto, P., Pineda, M. and Aguilar, M. 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybedate complex: specific application to the determination of vitamin E. *Analytical Biochem*. **269:** 337-341.

Quality control methods for medicinal plant materials. 1998. WHO Library Cataloguing in Publication data. pp**.** 28, 44-46.

Rahman, M.A. and Wilcock, C.C. 1991. A taxonomic revision of Calotropis (Asclepiadaceae). *Nordiac Journal of Botany*. **11(3):** 301-308.

Ramamoorthy, P. K., and Bono, A. 2007. Antioxidant activity, total phenolic and flavonoids content of *Morinda citrifolia* fruit extacts from various extraction processes. *J Engg Sci. and Tech.* **2(1):** 70-80.

Sadasivam, S. and Manickam, A. 1996. Biochemical Methods. *New age International, Delhi*. **2:** 159-160.

Sen, S., Chakraborty, R., Sridhar, C., Reddy, Y. S. R. and De, B. 2010. Free radicals, antioxidants, diseases and phytomedicines: current status and future prospect. *Int. J Pharmaceutical Sci.* **3(1):** 91-100.

Sheth, F. 2011. Range of seasonal phytochemical variations in Calotropis procera (Ait.) R. Br. *Int. J. Medicinal and Aromatic Plants*. **1(2):** 180-183.

Shivaprasad, H. N., Mohan, S., Kharya, M. D., Shiradkar, R. M., Lakshman, K. 2005. *In-vitro* models for antioxidant activity evaluation: a review. *Latest Reviews*. **3(4).**

Singh, H., Krishna, G., and Baske, P.K. 2010. Plants used in the treatment of joint diseases (rheumatism, arthritis, gout and lumbago) in Mayurbhanj district of Odisha, India. *Report and Opinion*. **2(9):** 22-26.

Tomar, J.B., Bishnoi, S.K. and Saini, K.K. 2012. Healing the tribal way: Ethanomedicinal formulations used by the tribes of Jharkhand. *International Journal of Medicinal and Aromatic Plants*. **2(1):** 97-105.

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M. and Telser, J. 2007. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. and Cell Bio.*. **39:** 44-84.

Vermani, A., Navneet, Prabhat and Chauhan, A., 2010. Physicochemical analysis of ash of some medicinal plants growing in Uttarakhand, India. *Nature and Science*. **8(6):** 88-91.

Wayner, D. D., Burton, G. W., Ingold, K.U., Barclay, L. R., Locke, S. J. 1987. The relative contributions of vitamin E, urate, ascorbate and proteins to the total peroxyl radical-trapping antioxidant activity of human blood plasma. *Biochem. Biophys. Acta.* **924:** 408-419.

Yadav, R.N.S. and Agarwala, M. 2011. Phytochemical analysis of some medicinal plants. *Journal of Phytology*. **3(12):** 10-14.