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Research Article

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *BOUCEROSIA TRUNCATO-CORONATA* (SEDGW.) GRAVELY MAYUR

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ABSTRACT
As a measure of testing the medicinal properties of *B. truncato-coronata* ethanolic extract obtained from both *in vivo* and *in vitro* plants were studied for phytochemical test and antioxidant activity by DPPH and FRAP. Quantitative phytochemical analysis confirms the presence of various secondary metabolites. The *in vivo* and *in vitro* extracts exhibited appreciable level of scavenging activity 94.22 and 91.20 at 600 µg/ml respectively. Better reducing activity in both *in vivo* and *in vitro* extracts is 17.44 and 16.47 respectively was observed in 600 µg/ml concentrations.
Keywords: Antioxidant, Phytochemical, *B. truncato-coronata* and Secondary metabolites

INTRODUCTION

Traditional healing systems play an important role in maintaining the physical and psychological wellbeing of the vast majority of tribal people in India. For centuries, People have been using plants for their therapeutic values. More than Eighty-five thousand plants have been documented for therapeutic use globally[1]. Efforts are currently being made to look for the products of natural origin. India is endowed with rich wealth of medicinal plants which are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines[2]. India is well known for significant geographical diversity which has favored the formation of different habitats and vegetation types. The reason for using them as medicine lies in the fact that they contain chemical components of therapeutic value[3]. The medicinal values of plants lie in some secondary metabolites present in it that produce a definite physiological action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavanoids, tannins and phenolics[4]. The genus *Caralluma* (Asclepiadaceae) includes 13 species distributed in all the tropical and subtropical regions of the India. The members of this genus is a morphological diverse. These plants are succulent, erect, perennial herb and fleshy, grow up to the height of 1 to 10 feet. The stem is quadrangular in nature with very small leaves and small dark colour flowers. The medicinal and pharmacological actions of medicinal herbs are often depended on the presence of bioactive compounds, the secondary metabolites[5]. The phytochemistry of genus *Caralluma* is characterized by many pregnane glycosides while recently megastimane glycosides also have been isolated[6]. *Caralluma* extracts have also been found to be appetite suppressant a property which is well known to Indian tribal and Hunters. Indian folk lore records its use as a potent appetite suppressant and weight loss promoter[7]. The main constituents of medicinal plants, such as saponins, flavanoids and polyphenols are known to be major bioactive compounds in Ayurvedic medicine[8]. Most antioxidants isolated from medicinal plants are polypherols, which show biological activities include anti bacterial, antiviral, anti carcinogenic and immune stimulating effect. The use of herbal remedies for arthritis treatment has been gaining momentum in recent years[9]. An antioxidant is "any substance that delays, prevents or removes oxidative damage to a target molecule"[10]. Antioxidants can act by diverse mechanisms in the oxidative sequence. The human body complex antioxidant defense system consists of the dietary intake of antioxidants, as well as the endogenous production of antioxidative compounds, like glutathione, etc[11]. Antioxidants may work either alone, or in association with each other against different types of free radicals[12]. To our knowledge there is no report on antioxidant activity. So the present study deals with the activity of *in vivo* and *in vitro* plant extracts of *B. truncato-coronata* were tested for their antioxidant activity by DPPH and FRAP along with phytochemical studies.

MATERIALS AND METHODS

Source of plant materials

In vivo plant

Plants of *Boucerosia truncato-coronata* (synonym: *Caralluma truncato-coronata*) were collected from Madukkarai hills, located at 10.9^0N and 76.97^0E along the hill sides of the southern Western Ghats of Coimbatore, Tamil Nadu, India and also a part of Nilgiri Biosphere Reserve and maintained in earthen pots in shade house at Government Arts College (Autonomous), Coimbatore, Tamil Nadu, India. The proper identification of the plant is the most important aspect of any research programme. The plant material collected was identified and authenticated by Botanical Survey of India, Coimbatore (BSI/ SRC/ 5/23/2012-13/ tech 1375).

In vitro plant

Multiple shoots formed from nodal segments cultured on Murashige and Skoog's medium supplemented with 2.0 mg/L BAP and 0.2 mg/L of NAA. (Kalimuthu *et al*., 2014) was used with this study.

Analysis of Bioactive Compounds

Preliminary phytochemical studies

The extract was subjected to preliminary phytochemical tests to determine the group of secondary metabolites present in the plant material as follows.

Preparation of plant extracts

The powdered *in vivo* and *in vitro* plants were successively extracted using 50 ml of ethanol using the Soxhelt extractor for 8-10 h[13]. The extract was filtered through Whatmann No.1 filter paper to remove all undissolved matter including cellular materials and other constitutions that are insoluble in the extraction solvent.

Phytochemical screening

The condensed ethanolic extracts were used for preliminary screening of phytochemicals such as alkaloids, tannins, steroids[14], lavonoids[15], glycosides[16], saponins[17], terpenoids[18], Phenols[19].

Antioxidant activity

The *in vivo* and *in vitro* ethanolic plant extract was subjected to antioxidant activity by DPPH and FRAP activity.

Preparation of Extract

The *in vivo* and *in vitro* fresh plant was carefully washed with tap water, rinsed with distilled water, and air dried for one hour. Then it was cut into small pieces, dried in room temperature for two weeks and powdered in a local mill. Two grams of each pulverized plant material was initially extracted by maceration with ethanol for one week. The extracts were concentrated in vacuum (Rota vapor) and the residues from the ethanol extracts were weighed and stored in sealed vials in a freezer until tested.

DPPH radical scavenging activity

Scavenging activity on DPPH free radicals by the test compound was assessed according to[20]. Different concentrations (100, 200, 300, 400, 500 and 600 µg/ml) of the test sample was dissolved in DMSO was mixed individually with 1 mL of 0.1 mM DPPH in ethanol solution and 450 µL of 50 mM Tris-HCl buffer (pH 7.4) was added. The solution was incubated at 37ºC for 30 minutes and reduction of DPPH free radicals was measured by reading the absorbance at 517 nm (Shimadzu, 1601). This activity is given as % DPPH scavenging and calculated according to the following equation:

$$\% \text{ Inhibition} = [(A_B - A_A)/A_B] \times 100,$$

Where A_B, absorption of blank sample, A_A, absorption of test sample

Reducing power activity (FRAP)

The reducing power of the test compound was determined by the method of [21]. Different concentration (100, 200, 300, 400, 500 and 600 µg/ml) of the test compound was mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of 1 % potassium ferricyanide. The reaction mixture was incubated at 50ºC for 30 minutes 2.5 mL of 10 % trichloroacetic acid (TCA) was added to the above mixture and centrifuged for 10 minutes at 3000 rpm. 2.5 mL of supernatant solution was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1 % ferric chloride. Absorbance was measured at 700 nm.

RESULT AND DISCUSSION

Preliminary phytochemical screening of *in vivo* and *in vitro* plant extracts of *B. truncato-coronata* was carried out. Qualitative phytochemical analysis of this plant confirm the presence of various secondary metabolites like alkaloids, glycosides, tannins, saponins, flavonoids, steroid, triterpenes and phenol (Table 1). Both the extracts of *B. truncato-coronata* had significant scavenging effect which increased with increasing concentration of extracts in the 100 µg/ml to 600 µg/ml concentration. The extracts exhibited superior ferric reducing antioxidant power. Scavenging of free radicles found to use with increasing concentration of the extract tested and was presented in Table 2. DPPH free radicle have the ability to take electron from the antioxidants that is why it is used for the *in vitro* antioxidants scavenging assays of the medicinal plants for its estimation. Table 2 shows the percentage scavenging activity of *in vivo* and *in vitro* plant extracts. Positive scavenging activity of both *in vivo* and *in vitro* explants was observed with the increasing concentrations; percentage of scavenging activity by 88.4, 79.65, at 100 mg/l and 94.22, 91.20 at 600 mg/l respectively (Table 2) in both *in vivo* and *in vitro* explants. The scavenging percentage of *in vivo* extract is more or less equal when compare to *in vitro* extracts. The similar observation was also observed in *Ceropegia pusilla* tuber extract[22]. Table 2 shows the result of reducing power of solvent extracts (FRAP). Better ferric reducing power with increasing concentration of both *in vivo* and *in vitro* ethanol extracts of *B. truncato-coronata* was observed. The absorbance is 10.67, 08.21 at the concentration 100 µg/ml and in the absorbance 17.44 and 16.47 in 600 µg/ml in both the extracts respectively (Table 2). In conclusion there is no much difference in the activity of *in vivo* and *in vitro* extracts reducing power. Phytochemical constituents are responsible for medicinal activity of plant species. In the present study, preliminary phytochemical screening of *B.truncato-coronata* for *in vivo* and *in vitro* plant was carried out. Qualitative phytochemical analysis of this plant confirm the presence of various secondary metabolites like alkaloids, glycosides, tannins, saponins, flavonoids, steroids, triterpenes and phenols. The results suggest that the phytochemical properties for curing various ailments and possess potential anti inflammatory, antimicrobial and antioxidant and leads to the isolation of new and novel compounds. The use and search for, drugs and dietary supplements derived from plants have accelerated in recent years. Pharmacologists, microbiologists, biochemists, botanists and natural-products chemists all over the world are currently investigating medicinal herbs for phytochemicals and lead compounds that could be developed for treatment of various diseases[23]. Alkaloid compounds are found in ethanolic extracts alkaloids have a bitter taste while many to toxic to other organisms[24]. Glycosides are herbal secondary metabolites made up of two components, a carbohydrate component known as the glycone and a non carbohydrate component known as the aglycone. Glycosides are important in medicine because of their action on heart and are used in cardiac insufficiency[25]. Tannins are complex moieties produced by majority of plants as protective substances, they have wide pharmacological activities. They have been used since past as tanning agents and they possess astringent, antioxidant activities[26]. Flavonoids are free radical scavengers, super antioxidants and potent water soluble which prevent oxidative cell damage and have strong anti-cancer activity[27]. Flavonoids have several proven medicinal properties, such as anti inflammatory, antioxidant, anticancer, antibacterial and antiviral properties[28,29].

Table 1: Preliminary phytochemical analysis of *Boucerosia truncato-coronata*

S. No.	Secondary metabolites	*In vivo* plant		*In vitro* plant	
		Methanolic Extract	Ethanolic Extract	Methanolic Extract	Ethanolic Extract
1	Alkaloids	+	-	+	-
2	Glycosides	+	+	+	+
3	Tannins	+	-	+	-
4	Saponins	-	+	-	+
5	Flavonoids	+	-	+	-
6	Steroid	+	-	+	-
7	Triterpenes	-	+	-	+
8	Phenol	+	-	+	-

Table 2: DPPH and FRAP activity of different concentration of *in vivo* and *in vitro* ethanolic extracts of *Boucerosia truncato-coronata*

S. No.	Concentration (µg/ml)	DPPH Assay % of inhibition *in vivo*	DPPH Assay % of inhibition *in vitro*	Ferrous iron chelating assay % of inhibition *in vivo*	Ferrous iron chelating assay % of inhibition *in vitro*
1	100	88.94	79.65	10.67	08.21
2	200	91.73	82.32	11.40	10.41
3	400	93.10	89.11	12.75	11.29
4	500	93.91	90.13	13.42	12.03
5	600	94.22	91.20	17.44	16.47

Phenolic compounds have been extensively used in disinfections and remain the standards with which other bactericides are compared[30]. Phenolic herb secondary metabolites are widely distributed in herbs and are responsible for colour development, pollination and protection against UV radiation and pathogens[31]. *In vivo* and *in vitro* ethanolic extracts of *B. truncato-coronata* significantly scavenged the DPPH and FRAP. The 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) assay was widely used as the model system to investigate the scavenging activity of several natural compounds such as extract of plants in a relatively short time[32]. DPPH radical is scavenged by antioxidants through the donation of proton, forming the reduced DPPH, illustrated a significant decrease in the concentration of DPPH radical due to the scavenging ability of the extracts. With the increasing in the concentration of extracts the scavenging activity of the ethanolic extracts of both *in vivo* and *in vitro* was increased. The scavenging percentage is higher (94.22) in *in vivo* and 91.20 in *in vitro* extracts at 600 µg/l respectively. The result obtained in the present study may be attributed to several reasons scavenging of H_2O_2 or by changing / converting Fe^{3+} /Fe^{2+}, [33]. The reducing capacity of compound may serve as significant indicator of its potential antioxidant activity[34]. An increase in the absorbance revealed that the reducing power of *in vivo* and *in vitro* plant extracts was increased in dose, dependent manner. The antioxidant activity has been reported to be the concomitant development of reducing power[35,36]. Flavonoid and tannins are the phenolic compounds responsible for the antioxidant potentials of medicinal plants[37]. Superoxide anion is oxygen centered radical with selective activity. It can reduce certain iron complexes like cytochrome. The availability of steroids, terpenoids, flavonoids, phenolic compounds tannins are reported by many scientists. From this study it is confirmed that both the *in vivo* and *in vitro* plant extracts of *B. truncato-coronata* showed the high level of antioxidant activity in *in vitro* system.

CONCLUSION

In vivo and *in vitro* plant extracts had significant scavenging activity which increased with the increase in concentration of extracts from 100 µg/ml to 600 µg/ml. The *in vivo* and *in vitro* extracts exhibited appreciable level of scavenging activity 94.22 and 91.20 at 600 mg/l respectively. The percentage of scavenging activity of *in vivo* extract is almost equal when compare to *in vitro* extracts. *In vivo* and *in vitro* ethanolic extracts of *B. truncato-coronata* yields better ferric reducing power with increasing concentration. At the concentration 600 µg/ml the absorbance is 17.44and 16.47 in *in vivo* and *in vitro* extract respectively. The % of scavenging activity of *in vivo* extract is almost equal when compare to *in vitro* extracts. In both the extracts no major difference has been observed between *in vivo* and *in vitro* plant extracts reducing power.

REFERENCES

1. Liu Y, Wang MW. Botanical drugs: Challenges and opportunities: Contribution to Linnaeus Memorial Symposium. Life Science 2008; 82: 445-449. http://dx.doi.org/10.1016/j.lfs.2007.11.007
2. Alagesboopathi C. Ethnomedicinal Plants used as Medicine by the Kurumba Tribals in Pennagaram Region, Dharmapuri District of Tamil Nadu, India. Asian Journal of Experimental Biological Science 2011; 2(1): 140 -142.
3. Nostro A, Germano MP, Angelo V, Cannatelli MA. Extraction methods and bio autography for evaluation of medicinal plant antimicrobial activity. Letters in Applied Microbiology 2000; 30: 379-384. http://dx.doi.org/10.1046/j.1472-765x.2000.00731.x
4. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology 2005; 4: 685-688. http://dx.doi.org/10.5897/AJB2005.000-3127
5. Heinrich M, Barnes J, Gibbons S, Williamson EM. Fundamentals of Pharmacognosy and Phytotherapy. Churchill Livingstone, Elsevier Science Ltd., UK; 2004.
6. Jayakar B, Rajkapoor B. Efforts of *Caralluma attenuata* in normal and alloxen induced diabetic rate. Journal of Herbal Pharmacotherapy 2004; 4: 35-40. http://dx.doi.org/10.1080/J157v04n01_04
7. Raja Sreelatha V, Pullaiah T. Induction of somatic embryogenesis and plant regeneration from internodal explants of *Caralluma stalagnifera*. Botany Research Internatinal 2010; 3(1): 17-20.
8. Andrzej L, Dawidowicz Dorota Wianowska, Barbara. The antioxidant properties of alcoholic extracts from *Sambucus nigra* L. (antioxidant properties of extracts). In: LWT-food science and technology. Volume 39; 2006.
9. Matsui T, Tanaka T, Amura S, Toshima A, Miyata Y, Tanaka K. Alpha glycosidase inhibitory profile of catechius and theaflavins. Journal of Agricultural and food chemistry 2007; 55: 99-105. http://dx.doi.org/.1021/jf0627672
10. Halliwell B. Biochemistry of Oxidative Stress, Biochemical Society Transactions 2007; 35(5): 1147-1150. http://dx.doi.org/10.1042 BST0351147
11. Clarkson TW, Magos L. The toxicology of mercury and its chemical compounds. Critical reviews in toxicology 2006; 36: 609-62. http://dx.doi.org/10.1080/10408440600845619
12. Feher J, Csomos G, Vereckei A. Free radical reactions in medicine. In: Springer Verlag, Berlin Heidelberg, USA; 1987. p. 40-43. http://dx.doi.org/10.1007/978-3-642-83104-1
13. Gafner S, Woffender JL, Nianga M, Hostettmann K. In: Phytochemistry; 1998. p. 215.
14. Ciulci I. Methodology for the analysis of vegetable drugs. Chemical industries branch, Division of industrial operations. UNIDO, Romania; 1994. p. 24-67.

15. Sofowora A. Medicinal plants and Traditional Medicines in Africa. In: Chichester John Wiley and Sons New York; 1993. p. 34-36.
16. Gokhale SB, Kokate CK, Purohit AP. A text book of pharmacognosy, Naiali Prakashan, Maharashtra, India; 2008. p. 889.
17. Brain KR, Turner TD. The practical evaluation of phytochemicals. Wright. Science technical Bristol; 1975. p. 57-58.
18. Salkowski FB, Ross C. Plant physiology. Wadsworth Publishing Company, Inc., California; 1991. p. 747.
19. Krishnamurthy KV. Methods in Plant Histochemistry. Chennai: S. Vishwanathan Printers and Publishers Pvt. Ltd; 1988.
20. Blois MS. Antioxidant determination by the use of a stable free radical. Nature 1958; 181: 1199-1200. http://dx.doi.org/10.1038/1811199a0
21. Yildrim A, Oktay M, Bilaoglu V. The antioxidant activity of the leaves of *Cydonia vulgaris*. Turkish Journal of Medical Sciences 2001; 31: 23-27.
22. Prabakaran R, Kalimuthu K, Vani C, Brindha C. Angiogenesis and antioxidant activity of *in vitro* and *in vivo* tuber of *Ceropegia pusilla* Wight and Arn. British Journal of Pharmaceutical Research 2014; 4(5): 608-616. http://dx.doi.org/10.9734/BJPR/2014/7481
23. Acharya D, Shrivastava K. Indigenous Herbal Medicines. In: Tribal Formulations and Traditional Herbal Practices, Aavishkar Publishers Distributor, Jaipur- India; 2008. p. 440.
24. Gupta VK, Singh GD, Singh S, Kaul A. Medicinal Plants: Phytochemistry, Pharmacolgoy and Therapeutics. Daya Publishing House, Delhi; 2010.
25. Balch JF, Balch PA. Prescription for Nutritional Healing. New York. A very, Penguin Putnam Inc; 2000. p. 267-270.
26. Killedar SG, More HN. Estimation of tannins in different parts of *Memecylon umbellatum.* Burm. Journal of Pharmacy Research 2010; 3(3): 554-556.
27. Salah W, Miller N, Pagauga G, Tybury G, Bolwell E, Rice E, Evans C. Polyphenolic flavonoids as scavenger of aqueous phase radicals and chain breaking antioxidants. Archives of Biochemistry 1995; 2: 239-346.
28. Cheynier V. Polyphenols in foods are more complex than often thought. American Journal of Clinical Nutrition 2005; 81: 223-229.
29. Manach C, Scalbert A, Morand C, Rémésy C, Jimenez L. Polyphenols food sources and bioavailability. American Journal of Clinical Nutrition 2004; 79: 727-747.
30. Okwu DE. Evaluation of Chemical composition of indigenous species and flavouring agents. Global Journal of Pure and Applied Sciences 2001; 458-459.
31. Bruneton J. Pharmacognosy, Phytochemistry and Medicinal Plants. Intercept. Ltd. England, U.K; 1999.
32. Kadam Balaji, Manasa G, Amarender Reddy A, Nagaraju M, Srikanth T, Saarangi Ramesh. Evaluation of *in vitro* antioxidant and cytotoxicity activity of aqueous extract of *Pergularia daemia.* Scholars Academic Journal of Pharmacy 2013; 2(2): 125-129.
33. Rajan S, Shalini R, Bharathi C, Aruna V, Thirunalasundari T, Brindha P. *In vitro* antioxidant screening of *Hemidesmus indicus* root from South India. Asian Journal of Pharmaceutical and Biological Research 2011; 1(2): 222-231.
34. Meir S, Kanner J, Akiri B, Hadas SP. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. Journal of Agriculture and Food Chemistry 1995; 43: 1813-1819. http://dx.doi.org/10.1021/jf00055a012
35. Yang JH, Lin HC, Mau JL. Antioxidant properties of several commercial mushrooms. Food Chemistry 2002; 77: 229-235. http://dx .doi.org/10.1016/S0308-8146(01)00342-9
36. Ashwini SK, Kiran R, Soumya KV, Sudharshan SJ, Prashith Kekuda TR, Vinayaka KS, Raghavendra HL. Insecticidal and *in vitro* antioxidant potency of extracts of *Cryptolepis buchanani* Roem. and Schult. International Journal of Pharmaceutical Sciences 2010; 2(1): 418-425.
37. Sadhu SK, Okuyama E, Fujumoto H, Ishibashi M. Separation of *Leucas aspara* medicinal plant of Bangladesh, guided by prostaglandin inhibitory and antioxidant activities. Chemical and Pharmaceutical Bulletin 2003; 51(5): 595-598. http://dx.doi.org/10.1248/cpb.51.595

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