Investigation of antioxidant activity of an ayurvedic formulation *Chandraprabha vati*

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Abstract

Numerous epidemiological studies have indicated that herbal medicines rich in antioxidant properties provide protection against oxidative stress induced diseases and disorders. *Chandrapraprabha vati:* an ayurvedic herbo-mineral formula is widely used for many kinds of diseases in the Ayurvedic medicinal field. Antioxidant activity of *Chandraprabha vati* has not been reported and the present study was aimed to investigate the antioxidant activity of *Chandrapraprabha vati.* Water extract of *Chandraprabha vati* prepared using 10.0 g of *Chandraprabha vati* and 100.0 mL cold distilled water. The diluted supernatant was used for the antioxidant assays. Antioxidant activity was investigated in terms of 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) free radical scavenging activity, ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) and 6-hydroxy-2-5-7-8-tetramethylchroman-2-carboxylic acid (Trolox) was used as a standard antioxidant. Cold water extract of Chandraprabha vati contained 39.0 ± 0.9 mg/mL dry matter. The extract showed $ABTS^{+}$ radical scavenging activity in dose dependent manner (1.6 ± 0.8, 7.6 ± 4.5, 15.1 ± 4.0, 29.0 ± 1.7 and 57.1 ± 2.1% inhibition for 1.5, 3.0, 6.0, 12 and 25 µg/mL concentrations respectively). The results showed comparable radical scavenging activity 50% inhibitory concentration (IC_{50}) of the extract *vs* standard was 20.9 *vs* 6.8 µg/mL. Further, the results showed ferric reducing (119.4 ± 8.1 µmole trolox equivalents (TE)/g sample) and oxygen radical absorption capacities (139.7 ± 6.9 µmole TE/g sample) of *Chandraprabha vati.* In conclusion, *Chandraprabha vati* possess potent antioxidant activity in terms of free radical scavenging, reducing power and oxygen radical absorption.

Key words: *Chandraprabha vati,* antioxidant activity, free radical scavenging, ferric reducing antioxidant.

Introduction

Oxygen is one of the most essential components for living being and it is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called "free radicals". These free radicals are capable of attacking the healthy cells of the body. This may lead to damage, disease and severe disorders. Cell damage caused by free radicals appears to be a major contributor to aging and many diseases. Antioxidants are substances that protect the cells against the effects of free radicals. *Chandraprabha vati* is an effective and very popular ayurvedic formula prescribed for many diseases. It is extremely useful in all types of *Prameha* a syndrome described in the ancient texts that includes clinical conditions involved in obesity, pre-diabetes, diabetes mellitus, and metabolic syndrome and also prescribed for diseases occurs in urinary tract, skin and gastro intestinal tract. Apart from these conditions *Chandraprabha vati* is recommended to improve strength, for anticipate aphrodisiac and as an anti-aging remedy [1]. *Chandraprabha vati* plays a key role in helping to cure above mentioned diseases. Therefore the present study was aims to investigate the *in vitro* antioxidant activity of *Chandraprabha vati* using ABTS [2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) free radical scavenging activity] [2], FRAP (ferric reducing antioxidant power) [3] and ORAC (oxygen radical absorbance capacity) [4] assays.

Material and methods

Preparation of *Chandraprabha vati*

Rhizomes of *Acorus calamus, Zingiber officinale* and *Curcuma longa,* tubers of *Cyperus rotandus,* whole plant of *Berberis aristatam, Andrographis paniculata* and *Tinospora cordifolia,* heartwood of *Cedrus deodara,* roots of *Ipomea turpethum, Aconite heterophyllium, Plumbago zeylanica, Baliosperum montanum* fruits and dried spikes of *Piper lomgum,* fruits of *Coriandrum sativum, Terminalia belarica, Terminalia chebula, Emblica officinale, Emblica ribes, Scindasus officinalis, Piper nigrum, Piper cheba, Elettaria cardomomum,* bark of *Cinnamomum zeylanicum* and sugar were purchased from registered ayurvedic drug sales outlets in Colombo, Sri Lanka, and shade dried for 3 days. Leaves of *Cinnamomum tamala,* 3 salts (Rock salt, Black salt, Ammonium chloride), 2 alkali (Sodium chloride and Potassium carbonate) and metal ashes (Ferrum and Copper), Aspelt

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mineral pitch (Shilajatu), The manne of bamboo (Vanshalochana) and purified resinous gum of *Balsamodendron mukul* (Gugul) and Camphor were purchased from the Indian Medical Practitioners Co-operative Pharmacy and Stores, Ltd. Chennai, India and all ingredients were authenticated by the Department of Dravya Guna Vinjnana, Institute of Indigenous Medicine,University of Colombo, Sri Lanka. Using appropriate each of these ingredients of *Chandraprabha vati* was made according to details description given in the Ayurvedic text [5].

Sample preparation

Chandraprabha vati (10.0 g) was extracted into 100.0 mL distilled water for 5 hours. The extract was filtered and then centrifuged using refrigerated centrifuged (HERMAl Z - Germany) at 10,000 rpm for 10 minutes at 10°C temperature. Resulted supernatant was used as a cold water extract of *Chandraprabha vati* in all antioxidant assays. Total soluble solid (dry matter content) of the cold water extract was determined using oven method (5.0 mL of the extract in triplicates was poured into a weighted oven-disk and evaporated to dryness at 105°C). This process is followed to calculate the concentrations in respective dilutions used to determined antioxidant activity. The other way of doing this is concentration of the cold water extracts to dryness and then dissolves a weighted amount in respective buffer or water. However, it was observed that most of herbal medicinal extracts do not dissolve completely in water or buffer once they are lyophilized, possibly due to structural alteration resulted with dehydration. Therefore, use of first water extract without concentrating gives better results in these types of bioassays. Therefore, in order to calculate activity in dry mater basis, soluble solid content of the extract which was determined by standard oven method and dilution factor were used.

Determination of 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^+$) free radical scavenging activity of *Chandraprabha vati* water extract

Water extract of *Chandraprabha vati* was serially diluted with 50 mM phosphate buffered saline (PBS) (pH 7.4) to serve 5 different concentrations (1.5, 3.0, 6.0, 12 and 24 µg/mL) in $ABTS^+$ radical scavenging assay (reaction volume of 200 µL). In the assay, 50 µL of sample was mixed with 110 µL of 0.2 M PBS in 96 well micro plate and pre plate reading was recorded at 734 nm. Then 40 µL of diluted $ABTS^+$ solution was added to each well and incubated at 25±2°C for 10 minutes before obtaining the reading at 734 nm using the micro-plate plate reader (Spectra MAXPLUSE^{348}, Molecular Devices, USA). Phosphate buffer served as a blank and five concentrations (2.5, 5.0, 10.0, 20.0 and 40.0 µM) of Trolox was used as a standard antioxidant. Antioxidant potential of the *Chandraprabha vati* was expressed as an equivalent of standard antioxidant (Trolox) per g of *Chandraprabha vati* (wet basis).

$ABTS^+$ radical scavenging activity of *Chandraprabha vati* extract was calculated using following formulas

Radical scavenging % = 100* (A-B)/A

Where;

A – absorbance of control at 734 nm (Only $ABTS^+$ radicals and buffer)

B – absorbance of sample at 734 nm ($ABTS^+$ radicals, sample and buffer)

Determination of ferric reducing antioxidant power (FRAP) of *Chandraprabha vati* water extract

In the assay, 25 µL of diluted *Chandraprabha vati* water extract (0.78 mg/mL concentration) in phosphate buffer was mixed with 250 µL of 0.2 M phosphate buffer (pH 6.6) and 250 µL of 1% potassium ferricyanide in 2.0 mL micro centrifuged tube and incubated at 50°C in a water bath for 20 minnutes. Then 250 µL of 10 % tricholoro acetic acid was added and centrifuged at 10,000 rpm for 10 minutes. From the supernatant 125 µL was mixed with 55 µL of deionized water and 20 µL from 0.1% ferric chloride solution in 96 well micro plate and the plate was read at 700 nm. Same concentrations of Trolox as described in above ABTS assay was used as a standard antioxidant. FRAP value of *Chandraprabha vati* was expressed as an equivalent of Trolox per g of *Chandraprabha vati* (in wet base). FRAP value of *Chandraprabha vati* was calculated using following formula

FRAP value = [(A -c)/m]/S

Where;

A – Absorbance of sample at 700 nm

c – Intercept of the standard curve (Trolox)

m – Slope of the standard curve (Trolox)

S – Concentration of the sample

Determination of oxygen radical absorption capacity (ORAC) of *Chandraprabha vati* water extract

Water extract of *Chandraprabha vati* was diluted in 75 mM phosphate buffer (pH 7.4) to serve 14 µg/mL concentrations in the ORAC assay (final volume 200 µL). In the assay, 10 µL from diluted water extract (0.28 mg/mL concentration), 40 µL from 75 mM phosphate buffer (pH7.4) and 100 µL from fluoresein were mixed in microplate and pre incubated at 37°C for 5 minutes and 50 µL from AAPH solution were added to start the reaction. Then the plate was read using Spectra MAX GEMINI EM flurosence microplate reader (Molecular Devices Inc, USA). Kinetic parameters set were as follows, excitation; 494 nm, emission; 521 nm, kinetic chase duration 35 min, reading interval 1 minute and incubation temperature 37°C. Trolox (0.8 µg/mL) was used as a standard antioxidant in the ORAC assay. ORAC value of *Chandraprabha vati* was expressed as an equivalent of Trolox per g of *Chandraprabha vati* (in wet base). ORAC value of *Chandraprabha vati* was calculated using following formula.

ORAC value of sample = (Net AUC sample/Net AUC Trolox) x (A/B)

Where;

Net AUC sample = Area Under Curve of blank – Area Under Curve of sample

Net AUC Trolox = Area Under Curve of blank – Area Under Curve of Trolox

A = Concentration of Trolox (µg/mL)

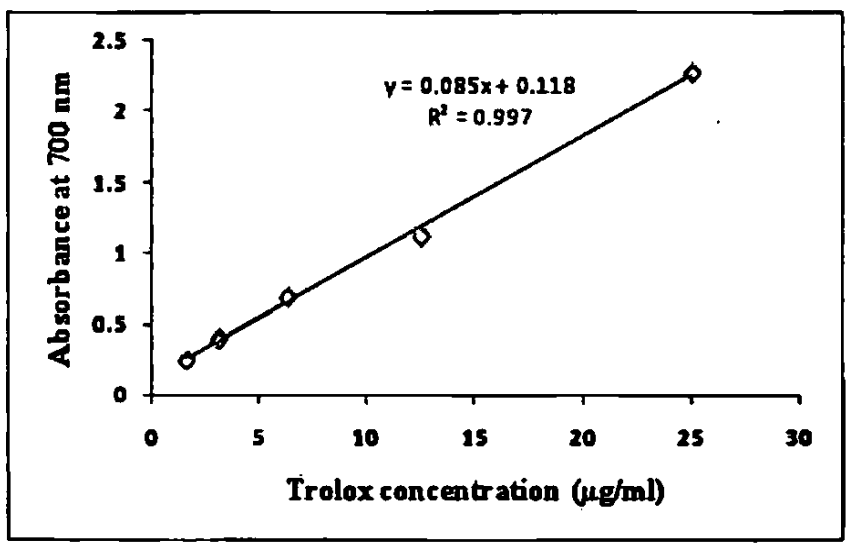
B = Concentration of Sample (µg/mL)

Results

ABTS⁺ radical scavenging activity of *Chandraprabha vati*

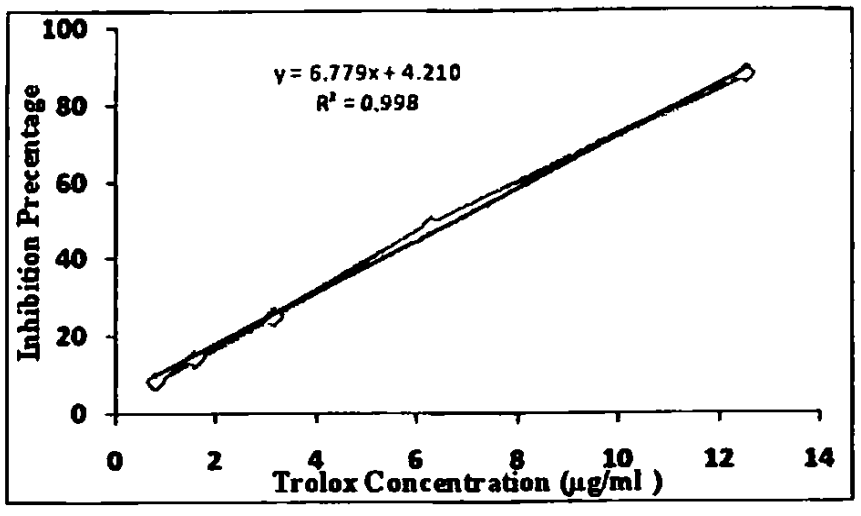
Chandraprabha vati water extracts showed ABTS+ radical scavenging activity at all tested concentrations in dose dependent manner with R2=0.99 (Figure 1).

Figure 1: ABTS⁺ radical scavenging percentages of *Chandraprabha vati* water extract.



Cold water extract of *Chandraprabha vati* showed highest inhibition (-57.1 ± 2.1%) at 24 µg/mL concentrations. According to the results inhibitory concentration 50 (IC50) of the extract was 20.9 µg/mL whereas standard (Trolox) showed 6.8 µg/ml (Figure 2). Further, ABTS⁺ radical scavenging activity of *Chandraprabha vati* was equivalent to 412 0.113 µmole Trolox (103 mg) per g of sample.

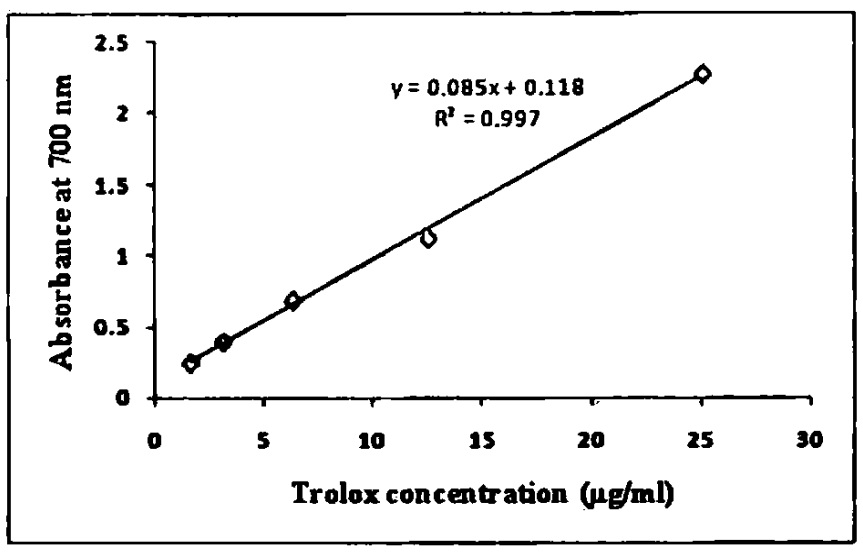
Figure 2: ABTS+ radical scavenging percentages of *Chandraprabha vati* water extract.



Ferric reducing antioxidant power of *Chandraprabha vati*

Standard curve of FARP assay is given in Figure 3. According to results *Chandraprabha vati* showed FRAP activity equivalent to 119.4 8.1 µmole Trolox (29.8 mg) per g of sample.

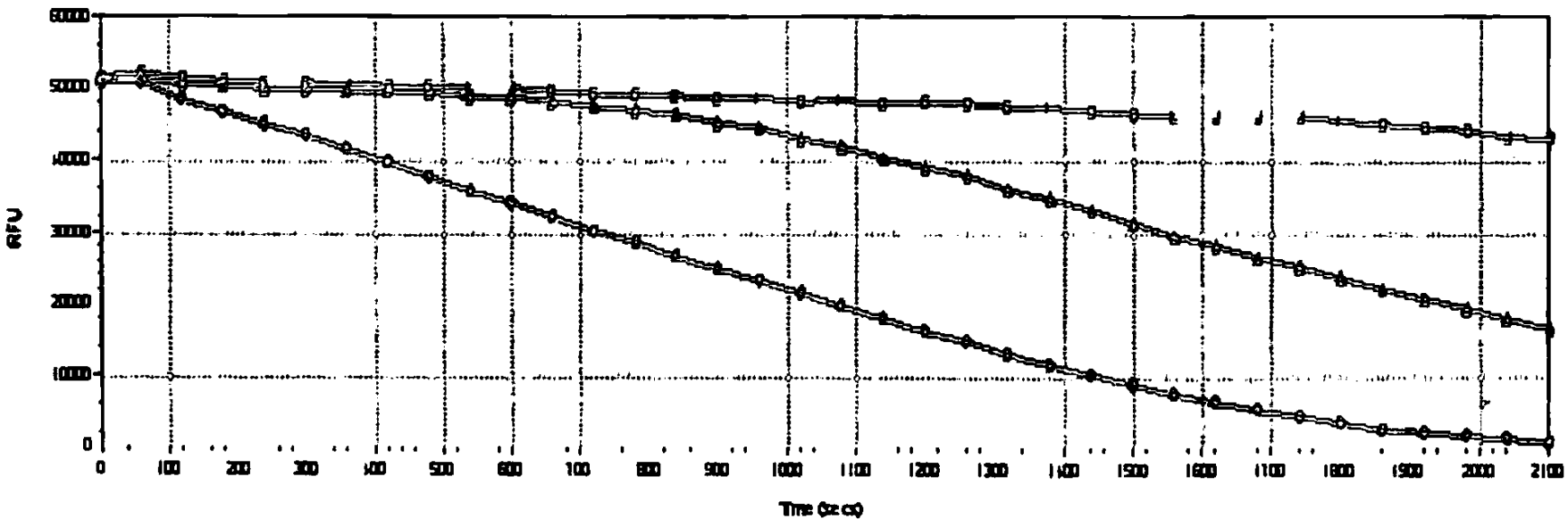
Figure 3: Standard (Trolox) curve of FRAP assay.



Oxygen radical absorption capacity of *Chandraprabha vati*

Kinetic graph for Trolox (standard antioxidant) and sample is given in Figure 4. *Chandraprabha vati* showed ORAC activity equivalent to 139.7 6.9 µmole Trolox (34.9 mg) per g of sample.

Figure 4: Kinetic graphs of fluoresce decay in ORAC assay for Trolox and sample.



Discussion

Natural compounds in herbal medicines posses variety of mode of actions to neutralize the free radicals generate inside the body before they launch oxidative damages to cellular macro-molecules [6]. Free radical scavenging, oxygen radical absorption, total reducing power, superoxide scavenging are some of important mechanisms of actions of antioxidants [7]. Therefore, in this study, three different assays representing free radical scavenging (ABTS), total reducing power (FRAP) and oxygen radical absorption capacity (ORAC) were used to determine antioxidant activity of *Chandraprabha vati.* The results clearly exhibited that *Chandraprabha vati* posses free radical scavenging, reducing power and oxygen radical absorption capacity in varying strengths. *Chandraprabha vati* showed comparatively higher radical scavenging activity (412 µmole Trolox per g of sample) over reducing capacity (119.4 µmole Trolox per g of sample) and oxygen radical absorption capacity (139.7 µmole Trolox per g of sample). However, these assays represent different mechanism of actions where some different phytochemicals may involve in reactions. Therefore, *Chandraprabha vati* may have many different types of photochemical with radical scavenging, reducing capacity and oxygen radical absorption capacity in varying concentration thus it expressed significantly different values for different antioxidant assays.

Biologically generated free radicals and oxidative stress are associated with numerous physiological disorders and diseases such as diabetics, cardiovascular diseases, malignancy and inflammation [8]. Therefore, neutralization of biologically generated radicals via antioxidants is very important to maintain a good health. Though, there is an enzymatic antioxidant defense inside the body, supply of antioxidant with food or drug is highly supportive to strength the antioxidant defense system of the body. *Chandraprabha vati* which was demonstrated different antioxidant actions, namely free radical scavenging, reducing power and oxygen radical absorption capacity in this study will support to neutralize the excess radicals inside the body. Antioxidant activity demonstrated by *Chandraprabha vati* may help to improve health condition and also might have association with its therapeutic potentials.

Conclusion

Chandraprabha vati possess potent antioxidant activity *in vitro* in terms of free radical scavenging, reducing power and oxygen radical absorption. The results of this study indicate that *Chandraprabha vati* has antioxidative principle/s with multiple actions and moderate strength which may play a role as a therapeutic herbo-mineral drug, which supports the beneficial effects claimed by ayurveda medicine.

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References

1. Sastri P. Saranghadhara Samhita by Pandit Saranghadharacharya with the commentary Adhamalla's Dipika and Kasirrama's Gudhartha Dipika (Sanskrit), Chawkhamba Orientalia, Varanasi, India 2002; 131.
2. Re R, Pellegrini N, Proteggente A, Pannala A, Young M. Antooxidant activity applying an improved ABST radical cationdecolorization assay. *Free Radical Biol. Med.* 1999; 26: 1231-7.
3. Benzine IFF And Szeto YT. Total antioxidant capacity of teas by the ferric reducing antioxidant power assay. *J. Agric, Food Chem* 1999; 47: 633-6.
4. Ou B, Hampsch-Woodill M, Prior RL. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric, Food Chem* 2001; 49: 4619-26.
5. Nagodavithana P, Sri Sharangadhara Samhita, Samayavardhana Book Shop, Colombo, 2001; 10: 155-6.
6. Halliwell B, Murcia MA, Chirico S, Aruoma OI. Free radicals and antioxidants in foods and in vivo: what they do and how they work. *Criticla Reviews in Food Science and Nutrition* 1995; 35: 7-20.
7. Huang D, Ou B, Prior RL. The Chemistry Behind Antioxidant Capacity Assays. *J. Agric. Food Chem* 2005; 53: 1841-56.
8. Halliwell B. Antioxidant in human diseases. *Annual Review of Nutrition* 1996; 16: 33-50.