In-vitro screening of DPPH free radical scavenging activity of freeze-dried extract of *Crateva adansonii* DC. stem bark decoction *(Lunuwarana pothu kalan 12 Kashaya)*

Nallaperuma D.M.* and Jayasiri A.P.A.

Abstract

Normal cellular metabolism in body produces highly reactive molecules called Reactive Oxygen Species (ROS). Excessive production of ROS can lead to oxidative stress causing damage to cells of body. Anti-oxidants are compounds that neutralize ROS and protect cells from oxidative damage. Natural anti-oxidants derived from plants are important due to their potential health benefits and minimal side effects. *Crateva adansonii* DC is an evergreen tree which is in family Capparidaceae. *Ayurveda* authentic texts have indicated the stem bark decoction of this plant (*Lunuwarana pothu kalan* 12 *kashaya*) for treatment of *Mutra ashmari* (urinary calculi). The objective of this study is in-vitro screening of the anti-oxidant activity of freeze-dried extract of *Crateva adansonii* DC stem bark decoction through DPPH free radical scavenging assay. The free radical used in this assay is 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) and Trolox was used as the standard. The results revealed that the extract was capable of neutralizing the DPPH free radicals by 8.06±0.39%, 12.59±0.86%, 21.69±0.17%, 35.21±0.88% and 56.73±0.35% at concentrations of 78.13, 156.25, 321.5, 625 and 1250µg/ml. IC_{50} was found to be 1161.51±18.12µg/ml (Trolox IC_{50} 10.06± 0.10µg/ml) for the extract. According to the previous studies the stem bark of this plant was positive for alkaloids, tannins, saponins flavonoids, steroids, terpenoides, glycosides and proteins. It has been proven that presence of phytochemicals like flavonoids, terpenoides, saponins and phenols can contribute towards the anti-oxidant activity. So, this DPPH free radical scavenging activity could be due to the presence of such phytochemicals in this plant. Therefore, it can be concluded that the therapeutic effect of this decoction could be due to its DPPH free radical scavenging property.

Keywords: *Crateva adansonii* DC, freeze-dried aqueous extract, DPPH, anti-oxidant, Trolox

Introduction

Crateva adansonii DC[1] or *Lunuwarana* which belongs to the family Capparidaceae is an evergreen tree indigenous to India and well grown in different climates of Sri Lanka. This tree grows up to a height about 10m and the bark of the stem is a greyish colour hard bark. Bark has white colour patches on the surface. Leaves are compound leaves made up of three leaflets (trifoliate) for each and the upper surface of them are soft and dark green in colour while the lower surface being light green in colour. Flowers are white or creamy yellow with long purple stamens and they are clustered at the ends of the branches. Fruits are globose berries which appear on long, woody stalks[2].

According to *Ayurveda* authentic texts pharmacodynamic properties of this plant are, *Tikta* (bitter) and *Kashaya* (astringent) in *Rasa* (Taste), *Laghu* (light) and *Ruksha* (un-unctuousness) in *Guna* (Properties), *Ushna* (hot) in *Virya* (Potency), *Katu* (pungent) in *Vipaka* (Post digestive effect) and *Ashmaribhedana* (anti-urolithic) in *Prabhava* (Therapeutic effect)[3]. When considering pharmacological actions, it has *Depana* (appetizer), *Bhedana* (purgative), *Anulomana* (laxative), *Krimighna* (anthelmintic), *Raktashodhaka* (blood

Faculty of Indigenous Medicine, University of Colombo, Rajagiriya, Sri Lanka.

**Correspondence: Nallaperuma D.M., Faculty of Indigenous Medicine, University of Colombo, Sri Lanka.*
Email: dinalimalindika1993@gmail.com

purifier), *Ashmaribhedana* (anti-urolithic) and *Mutrala* (diuretic) actions[4]. It is mainly indicated for treatment of urinary disorders like *Ashmari* (Urinary calculi), *Mutrakruchcha* (Difficulty in urination) and *Vastishula* (Pain in bladder). This plant is also useful in treating other diseases like *Agnimandya* (Mal digestion), *Shula* (Pain), *Gulma* (Tumors), *Yakrt roga* (Liver diseases), *Krimi* (worm infestations), *Vata rakta* (Gout) [4]. Stem bark is the main part used in medicines[5]and the stem bark decoction (*Lunuwarana pothu kalan 12 kashaya*) has been mainly indicated for *Mutra ashmari*.

Reactive Oxygen Species (ROS) are highly reactive molecules produced during normal cellular metabolism. Excessive production of ROS can lead to oxidative stress causing damage to cells of the body and contributing to various diseases[6]. Anti-oxidants are compounds that can neutralize ROS and protect cells from oxidative damage[7]. Anti-oxidant activity is the ability of molecules called as anti-oxidants to neutralize the damages caused by molecules called as free radicals in the body. Natural anti-oxidants derived from plants have gained significant attention due to their potential health benefits and minimal side effects. There are various methods like DPPH assay, FRAP assay, H_2O_2 assay, ABTS assay etc for the evaluation of the anti-oxidant capacity of the plants. The primary aim of this study is the in-vitro screening of the DPPH free radical scavenging activity of the freeze-dried extract of *Crateva adansonii* DC stem bark decoction (*Lunuwarana pothu kalan 12 kashaya*). This method uses a stable free radical called 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the assay is based on the measurement of the scavenging capacity of anti-oxidants towards it. The odd electron of Nitrogen atom in DPPH is reduced by receiving a Hydrogen atom from anti-oxidants[8]. DPPH shows a strong absorption band at 517nm due to its odd electron and solution appears a deep violet colour. When mixing DPPH solution with a substance that can donate a hydrogen atom, it gives Stembarks of *Crateva adansonii* DC. rise to the reduced form of DPPH, that is 2,2-diphenyl-1-picryl-hydrazine (DPPH-H) which is a colorless or yellow colour solution. This principle was used in this study to determine the DPPH free radical scavenging activity of freeze-dried extract of *Crateva adansonii* DC stem bark decoction.

Materials and Methods

Materials and Equipment

Stem barks of *Crateva adansonii* DC, Freeze dryer, 2,2-diphenyl-1-picryl-hydrazyl (DPPH) solution, Methanol, Trolox, Distilled water, Spectrophotometer

Collection of the plant material

The stem barks of *Crateva adansonii* DC (Figure 1) were collected in the month of July from Galle District, Sri Lanka and authenticated from the Department of Ayurveda Pharmacology, Pharmaceutics and Community Medicine, Faculty of Indigenous Medicine, University of Colombo.

Fig. 1: Stem bark of *Crateva adansonii* DC

Preparation of Crateva adansonii DC stem bark decoction

The collected stem barks were washed properly and shade dried for one day. Coarse powder of the barks were obtained by crushing them using a grinder and passing through the sieve no.80. Then 60g from the coarse powder was measured and it was mixed with 1920ml of water in a clay pot. Continuous mild heat was given until the volume of water reduced to 1/8th (240ml) of its initial quantity. The decoction was filtered using four folded cotton cloth and collected in a separate glass vessel (Figure 2).


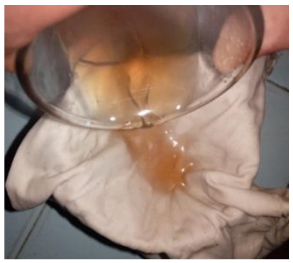

Fig. 2: Preparation of *Crateva adansonii* DC stem bark decoction

Preparation of the freeze-dried extract of Crateva adansonii DC stem bark decoction

The decoction was freeze-dried using the freeze dryer at Industrial Technology Institute, Colombo, Sri Lanka. Then the extract was stored in an air tight glass vial at 4^0C (Figure 3).

Fig. 3: Freeze-dried extract of *Crateva adansonii* DC stem bark decoction

DPPH Free Radical Scavenging Activity

The DPPH free radical scavenging assay was performed according to the method described by Blois[9]. Freeze-dried extract of *Crateva adansonii* DC stem bark decoction was diluted with distilled water and a stock solution was prepared. From the stock solution of the extract five samples (S1, S2, S3, S4 and S5) with five different concentrations were prepared by dissolving in methanol. Table 1 shows the concentrations of the five samples of the stem bark extract.

Table 1: Concentrations of the five samples of the freeze-dried extract

Sample	Concentration (µg/ml)
S1	78.13
S2	156.25
S3	321.5
S4	625
S5	1250

DPPH solution of concentration 0.5mg/ml was prepared with methanol. Then 150µl of the prepared DPPH solution was added to 200µl from each of the five samples. Each mixture was incubated in dark for 30 minutes and absorbance was measured using a spectrophotometer. As the control, only 3ml of DDPH was used and the absorbance of it was measured separately. Radical scavenging activity which was expressed as the percentage of inhibition was calculated using the below formula. The procedure was repeated for each mixture in triplicate and the average value for percentage of inhibition was calculated.

$$\text{Percentage of Inhibition} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100\%$$

A_{control} – Absorbance of the sample extracts in DPPH

A_{sample} – Absorbance of the control (3ml of DPPH)

Then the inhibition percentages (y) were plotted against the sample concentrations (x). By examining the graph, the concentration of the extract at which 50% inhibition occurred was determined and that value was founded as the IC_{50} (Half maximal inhibitory concentration) value of the extract.

The same procedure was repeated with Trolox which was used as the control sample. A concentration series (5, 10, 15, 20 and 25µg/ml) of trolox was prepared by diluting a stock solution of trolox in methanol. Each concentration was mixed with DPPH solution. The absorbance of each of the mixtures was measured and standard calibration curve for trolox anti-oxidant activity was plotted. The IC_{50} value of the standard Trolox was determined using the graph.

Results

When analysing the dose response relationship of freeze-dried extract of *Crateva adansonii* DC. stem bark decoction, it was revealed that when the concentration of the extract was increasing the percentage of inhibition was also increasing. Table 2 and Figure 4 shows the ability of the samples of different concentrations to scavenge the DPPH free radical from the obtained respective percentage inhibition values.

Table 2: Percentage of radical scavenging in the samples

Sample	Concentration (µg/ml)	% radical scavenging
S1	78.13	8.06± 0.39
S2	156.25	12.59± 0.86
S3	321.5	21.69± 0.17
S4	625	35.21± 0.88
S5	1250	56.73± 0.35

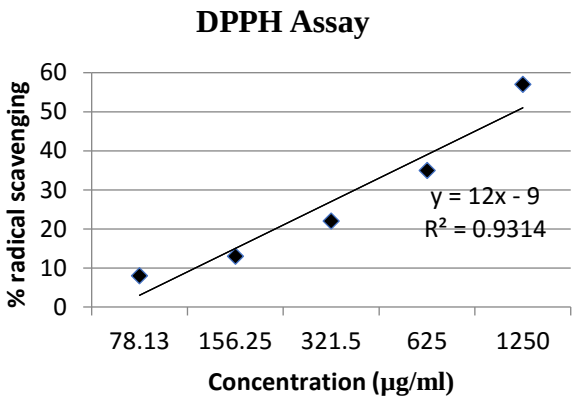

Fig. 4: Dose response relationship of free-dried extract *Crateva adansonii* DC stem bark decoction

Table 3 shows the IC_{50} values of the freeze-dried extract of *Crateva adansonii* DC stem bark decoction and standard Trolox.

Table 3: IC_{50} values of the sample extract and standard

Sample	IC_{50} value
Freeze-dried extract	1161.51 ± 18.12 µg/ml
Standard Trolox	10.06 ± 0.10 µg/ml

Discussion

DPPH assay is a simple, cheap, rapid and very sensitive assay. Multiple samples can be screened using DPPH assay. Anti-oxidants from complex biological systems can be quantified using this assay. The results from DPPH assays are reproducible and have a good sensitivity compared to other radical scavenging methods. Even weak anti-oxidants give good response in the assay with 30minutes of incubation. As the DPPH assay is performed in room temperature, even thermally unstable compounds can be measured efficiently. But the DPPH assay has some limitations. DPPH radical can only be dissolved in inorganic solvents. So, an organic media is essential to perform this assay. DPPH can react with other radicals present in the samples. DPPH assay should be performed in the dark as the absorbance decreases upon exposure to light[10].

Trolox is a water-soluble vitamin E analog often used as a standard anti-oxidant in assays such as DPPH assay, to measure the anti-oxidant capacity of samples[11]. In the DPPH assay, Trolox is used to create a standard curve to quantify the DPPH free radical scavenging capacity of the samples relative to Trolox.

In this study, based on the values obtained for the percentage of inhibition to scavenge the DPPH free radical, DPPH free radical scavenging activity of each concentration can be determined. It clearly shows that, when the concentration of the extract was increasing the DPPH free radical scavenging activity was also increasing. Previous research studies show that ethanol, ethyl acetate and dichloromethane extracts of *Crateva adansonii* DC have anti-oxidant activity[12]. According to the previous studies the stem bark of this plant is positive for alkaloids, tannins, saponins flavonoids, steroids, terpenoides, glycosides and proteins[13]. It has been proven that presence of phytochemicals like flavonoids, terpenoides, saponins and phenols can contribute towards the anti-oxidant activity[14].So this DPPH free radical scavenging activity could be due to the presence of such phytochemicals in this plant. *Crateva adansonii* DC is an ingredient in many decoctions which are prescribed by the *Ayurveda* physicians for urinary

disorders like urinary calculi. *Lunuwarana pothu kalan 12 kashaya* is one of such decoction which includes the stem barks of this plant. Anti-oxidant activity also can provide more protective or preventive effects from urinary disorders according to research studies[15]. Therefore, it shows that the therapeutic potential of this decoction could be due to its DPPH free radical scavenging activity.

Conclusion

The study concluded that there is a significant concentration dependent DPPH free radical scavenging activity in freeze-dried extract of *Crateva adansonii* stem bark decoction (*Lunuwarana pothu kalan 12 kashaya*) as the DPPH free radical scavenging activity was increasing when the concentration of the extract was increased.

Conflicts of Interest

Not declared.

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