Research Article

Evaluation of the In Vitro and In Vivo Antioxidant Potentials of Sudarshana Powder

Weerakoon Achchige Selvi Saroja Weerakoon,1 Pathirage Kamal Perera ,2 Dulani Gunasekera,3 and Thusharie Sugandhika Suresh4

1Department of Ayurveda Paediatrics, Institute of Indigenous Medicine, University of Colombo, Colombo, Sri Lanka
2Department of Ayurveda Pharmacology and Pharmaceutics, Institute of Indigenous Medicine, University of Colombo, Colombo, Sri Lanka
3Department of Paediatrics, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka
4Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Nugegoda, Sri Lanka

Correspondence should be addressed to Pathirage Kamal Perera; drkamalperera@yahoo.com

Received 1 December 2017; Revised 7 February 2018; Accepted 4 March 2018; Published 10 April 2018

Academic Editor: Gianni Sacchetti

Copyright © 2018 Weerakoon Achchige Selvi Saroja Weerakoon et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Sudarshana powder (SP) is one of the most effective Ayurveda powder preparations for paediatric febrile conditions. The objective of the present study was to evaluate the in vitro and in vivo antioxidant potentials of SP. The in vitro antioxidant effects were evaluated using ABTS radical cation decolourization assay where the TROLOX equivalent antioxidant capacity (TEAC) was determined. The in vivo antioxidant activity of SP was determined in Wistar rats using the Lipid Peroxidation (LPO) assay in serum. The in vitro assay was referred to as the TROLOX equivalent antioxidant capacity (TEAC) assay. For the in vivo assay, animals were dosed for 21 consecutive days and blood was drawn to evaluate the MDA level. The in vitro antioxidant activity of 0.5 μg of SP was equivalent to 14.45 μg of standard TROLOX. The percentage inhibition against the radical formation was 50.93 ± 0.53%. The SP showed a statistically significant (p < 0.01) decrease in the serum level of thiobarbituric acid-reactive substance in the test rats when compared with the control group. These findings suggest that the SP possesses potent antioxidant activity which may be responsible for some of its reported bioactivities.

1. Introduction

Sudarshana powder (SP) is the most effective antipyretic Ayurveda preparation, widely used in Sri Lanka as well as in India from the inception of Ayurveda treatment. SP is mentioned in the Sri Lankan Ayurvedic Pharmacopeia [1] compiled under Sec. 41(2) (c) of the Ayurveda Act no. 31 of 1961. The powder is prepared using different parts of plant materials and therefore is a 100% herbal product.

SP contains 53 [1] bitter ingredients and has the capability to treat fever associated symptoms such as dyspepsia, anorexia, fatigue, and nausea. It does not cause constipation and produces a mild laxative effect. It promotes the flow of bile and in gastrointestinal disorders it is used as a digestive.

Initially the main ingredient of the SP in Sri Lanka was Swertia chirata (Roxb. ex Fleming) which was later replaced by Andrographis paniculata (Burm. f.) Wall. ex Nees. Presently the SP contains Andrographis paniculata (Burm. f.) Wall. ex Nees. (50%) along with 52 other ingredients (50%). The other constituents too have different therapeutic uses [2].

Although literature suggests that SP is the most effective and popular Ayurvedic medicine, no scientific evidence is available for its antioxidant potential. Antipyretic effect [3–6], anti-inflammatory effect [7], analgesic effect [8–10], antihistamine effect [11], and long term administration effect [12–15] of the SP were established in Wistar rats in our recent experimental studies. Thus, in the present study, in vitro and in vivo antioxidant potentials of SP were evaluated.

2. Materials and Methods

2.1. Preparation of Sudarshana Powder. SP (Table 1) was prepared according to Ayurveda Pharmacopeia at the Pharmacy...
Table 1: Ingredients of Sudarshana powder.

| Botanical name | General user name in Sri Lanka | Sanskrit name | Parts used |
|----------------|-------------------------------|---------------|------------|
| (1) *Andrographis paniculata* (burm. f.) Wall. ex Nees. | Heen bincohomba | Chirayetah | Whole plant |
| (2) *Piper longum* Linn. | Thippili mul | Chapala | Root |
| (3) *Zingiber officinale* Rosc. | Inguru | Ardraka | Rhizome |
| (4) *Piper nigrum* Linn. | Gammiris | Katuka | Fruit |
| (5) *Piper longum* Linn. | Thippili | Chapala | Fruit |
| (6) *Santalum album* Linn. | Suduhandun | Chandana | Stem |
| (7) *Glycyrrhiza glabra* Linn. | Valmi | Yashtimadu | Stem |
| (8) *Picrorhiza kurroa* Royle ex Benth. | Katukarosana | Katurehini | Herb |
| (9) *Cedrus deodara* (Roxb.) Loud. | Devadara | Devodara | Timber |
| (10) *Hedychium spicatum* (Ham. ex Smith) | Hingurupiyali | Shati | Rhizome |
| (11) *Embelia ribes* Burm. F. | Valanghasal | Krimigna | Fruit |
| (12) *Trachyspermum roxburghianum* (DC.) Craib. | Asamodagam | Ugragandha | Whole herb |
| (13) *Holarrhena antidysenterica* Wall. | Kelinda | Kutaja | Bark and wood |
| (14) *Aconitum heterophyllum* Wall. | Athividayan | Athivida | Root |
| (15) *Terminalia chebula* Retz. | Aralu | Abaya | Dry fruit |
| (16) *Terminalia bellirica* (Gaertner) Roxb. | Bulu | Vibhitaka | Dry fruit |
| (17) *Phyllanthus emblica* Linn. | Nelli | Amalake | Dry fruit |
| (18) *Syzygium aromaticum* (L.) Merr. & Perry | Karabunati | Lavanga | Flower |
| (19) *Myristica fragrans* Linn. | Vasäväsi | Jathipala | Fruit cover |
| (20) *Cyperus rotundus* Linn. | Kaländuru ala | Granthi | Rhizome |
| (21) *Solanum melongena* Linn. | Elabatamul | Vruhathi | Plant |
| (22) *Alysicarpus vaginalis* (L.) DC. | Asvanna | - | Plant |
| (23) *Aerva lanata* (L.) Juss. ex Schult. | Polpala | - | Whole plant |
| (24) *Solanum xanthocarpum* Schrad. & Wendl. | Katuvelbatu | Brihati | Plant |
| (25) *Sauussurea lappa* Linn. | Suvandakottan | Kushta | Root |
| (26) *Azadirachta indica* A. Juss. | Kohambapothu | Nimba | Bark |
| (27) *Tinospora cordifolia* (Wild.) Miers ex Hook. f. & Thoms. | Rasakinda | Amurtha | Stem |
| (28) *Curcuma domestica* Valet. | Kaha | Haridra | Rhizome |
| (29) *Cinnamomum iners* Reinw. | Kollan kola | Thejapatra | Leaves |
| (30) *Trichosanthes cucumerina* Linn. | Dummalla | Patola | Leaves |
| (31) *Tragia involucrata* Linn. | Vaal khabiliia | Duralaba | Root |
| (32) *Marsdenia tenacissima* (Roxb.) Moon. | Murva | Tejowapi | Stem |
| (33) *Oldenlandia biflora* Linn. | Pathpadagam | Parpataka | Whole plant |
| (34) *Bacopa monniera* (L.) Wettst. | Lunuvila | Brahmi | Whole plant |
| (35) *Plectranthus zeylanicus* Benth. | Irivriya | Valakan | Whole plant |
| (36) *Holarrhena antidysenterica* (Linn.) Wall. | Kelinda hal | Kutaja | Seeds |
| (37) *Moringa oleifera* Lam. | Murunga eta | Shigruka | Whole plant |
| (38) *Cinnamomum zeylanicum* Blume. | Kurundupothu | Bahugandha | Bark |
| (39) *Abies webbiana* Lindl. | Thalispatara | Thalispatara | Leaves |
| (40) *Nelumbium speciosum* Willd. | Padma kashta | Padmakashta | Rhizome |
| (41) * Vetiveria zizanioides* (L.) Nash. | Savandará | Ushira | Root |
| (42) *Sida cordifolia* Linn. | Baviyum | Bala | Root |
| (43) *Aluminium sulphate* | Seenakkaran | Surashtaja | |
| (44) *Valeriana wallichii* DC. | warala | Tagar | Root |
| (45) *Plumbago indica* Linn. | Rathnitol | Chitraka | Root |
of the Institute of Indigenous Medicine, University of Colombo [2].

All the ingredients were purchased from Ayurveda Drug Corporation, Sri Lanka, and authentication of ingredients was done at the Institute of Indigenous Medicine, University of Colombo, Sri Lanka (Specimen No. 102), and SP was prepared according to Ayurveda Pharmacopeia [1] at the Pharmacy of the Institute of Indigenous Medicine.

All the 53 Ingredients were thoroughly cleaned to remove any contaminated materials using tap water. Washed ingredients were air-dried. Some herbal ingredients (i.e., Plumbago indica Linn.) were purified using purification methods, mentioned in Ayurveda authentic texts, prior to being powdered. All ingredients were powdered at the mesh size of 80 under the Ayurveda concepts. Andrographis paniculata (Burm. f.) Wall. ex Nees. (2600g) was mixed with 50g each of the rest of the ingredients (50×52) to obtain the Sudarshana powder.

2.2. Animals. Healthy adult male Wistar rats (200–250g) were used in the in vivo study. The animals were kept in plastic cages (two per cage) under standardized animal house conditions (temperature, 28–31°C; photoperiod, approximately 12 h natural light “per day”; relative humidity, 50–55%) at the Faculty of Medical Sciences, University of Sri Jayewardenepura, with continuous access to pelleted feed and tap water.

All experiments in rats were carried out in accordance with the guidelines for care and use of laboratory animals and the project proposal was approved (No. 591/11) by the Ethics Review Committee of the Faculty of Medical Sciences of the University of Sri Jayewardenepura, Sri Lanka (http://medical.sjp.ac.lk/index.php/ethics-review-committee-introduction).

2.3. In Vitro Antioxidant Activity. The in vitro antioxidant free radical scavenging activity of SP was determined by using ABTS radical cation decolourization assay [16].

In this assay, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) or ABTS (C₁₅H₁₁N₃O₆S₂) is converted to its radical cation. This radical cation is dark green in colour and absorbs light at 734 nm. [16]. The reaction was monitored spectrophotometrically. This assay is often referred to as the TROLOX equivalent antioxidant capacity (TEAC) assay.

2.3.1. Preparation of Extracts. The SP (5g) was extracted with 100ml ultrapure water, at 80°C for 20 min in a water bath shaker. After cooling, the extract was centrifuged at 5000 rpm for 10 min. The solution was filtered using No. 1 Whatman filter paper and used for ABTS analysis. The sample was diluted 1:10 (100μl sample + 900μl H₂O) [17].

2.3.2. Formation of ABTS Radical Solution. The ABTS solution (7 mmol) stock solution, 2.6ml, was mixed with 11.5ml of potassium persulfate (K₂S₂O₈) solution and kept in a dark place at room temperature (23°C) for 16 hours for free radical formation. The generated ABTS radical cations (ABTS⁺) solution was dark green in colour. The stock solution was diluted with ultrapure water until the absorbance reached 0.700 (±0.02) at 734 nm. The prepared sample (10μl) was added to ABTS⁺ solution (2990μl) with phosphate saline buffer until total volume was reached (3ml). The absorbance reading was taken 6 min after initial mixing. All determinations were performed in triplicate.

2.3.3. Standard Curve for ABTS Radical (ABTS⁺) Activity on TROLOX. TROLOX (C₁₅H₁₁N₃O₆) or (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) is equivalent antioxidant capacity (TEAC) which measures the antioxidant capacity of a given substance, as compared to the standard, TROLOX.

The stock solution of TROLOX was prepared by dissolving 0.0161g in 50 ml deionized water. From the prepared stock solution, 5μl (1.56μg TROLOX), 10μl (3.12μg TROLOX), 15μl (4.68μg TROLOX), 20μl (6.24μg TROLOX), and 25μl (7.8μg TROLOX) were taken and reacted with ABTS⁺ solution with initial absorbance of 0.700 at 734 nm against a phosphate saline buffer blank. Absorbance of reaction mixture was taken till the absorbance came to a plateau. The reduction of absorbance was calculated from the initial and final absorbance. The reduction of absorbance relevant to each TROLOX concentration was performed six times. Standard curve was drawn for mean reduction of absorbance versus quantity of TROLOX in μg. The antioxidant activity
was expressed as TROLOX equivalent antioxidant capacity (TEAC).

2.4. In Vivo Antioxidant Activity. In vivo antioxidant activity of SP was analysed using the method of determination of the Lipid Peroxidation (LPO) in serum. The level of thiobarbituric acid-reactive substance (TBARS) and malondialdehyde (MDA) production was measured in serum by the modified method by Draper and Hadly, 1990 [18].

Wistar rats were randomly divided into two groups of six animals each. On Day 0, blood samples were collected to assess the baseline serum malondialdehyde (MDA) level. The control group received distilled water and test group received hot water extraction of SP (0.5 g/kg). The animals were dosed for 21 consecutive days and were observed daily for signs of toxicity and death throughout the period of study. Body weights were recorded. Twenty-four hours after the last treatment, blood was obtained through direct cardiac puncture to evaluate the MDA level.

The serum (200 μL) was deproteinized by adding 1 mL of 14% trichloro acetic acid and 1 mL of 0.6% thiobarbituric acid. The mixture was heated in a water bath for 30 min at 95°C to complete the reaction and then cooled on ice for 5 min. Following centrifugation at 2000 rpm for 10 min, the absorbance of the coloured product (TBARS) was measured at 535 nm with a UV spectrophotometer.

The TBARS concentration was calculated using the following formula:

\[ A = \Sigma CL, \]

where

- \( A \) is absorbance,
- \( \Sigma \) is molar coefficient (1.56 × 10⁵ L/mol/cm),
- \( C \) is concentration of sample,
- \( L \) is path length (1 cm).

2.4.1. Statistical Analysis. The results were analysed using “Student’s t-test.” Values of \( p < 0.05 \) were considered statistically significant.

3. Results

3.1. In Vitro Antioxidant Activity. The standard curve equations \( y = 0.040x + 0.060 \) and \( R² = 0.9962 \) were obtained from the standard curve for TROLOX. The antioxidant activity of SP 0.5 μg was equivalent to 14.45 μg of standard TROLOX. The percentage inhibition against the radical formation was 50.93 ± 0.53%.

3.2. In Vivo Antioxidant Activity. Each value represents the mean ± SEM of MDA concentration. Values carrying different superscripts are significantly different (\(^* p < 0.05\), \(^** p < 0.01\), and \(^*** p < 0.001\)).

The findings of the study in the rats tested: the serum MDA levels of the control and test group (SP), respectively, were 3.9 ± 0.21 μmol/L and 2.07 ± 0.08 μmol/L \((p < 0.01)\) and significant reduction \((p < 0.001)\) of serum MDA level was observed on Day 21 when compared with Day 0 level of the test group; moreover there were no differences observed in the control group’s MDA concentration on Day 0 and Day 21 (Table 2).

| Rat group | MDA con. (μmol/L) |
|-----------|------------------|
|          | Day 0            | Day 21          |
| Control  | 3.8 ± 0.06       | 3.9 ± 0.21      |
| Test SP  | 3.7 ± 0.05       | 2.07 ± 0.08     |

Values are expressed as mean ± SEM; \( n = 6 \) per group.

4. Discussion

An antioxidant is a substance that is able to protect a substrate susceptible to oxidation, being itself present at fairly low concentrations in relation to the substrate. The SP possesses significant therapeutic effects but scientific evidence for these benefits is scarce. Therefore the in vitro and in vivo antioxidant activity of SP were evaluated in this study using ABTS and TBARS assays, respectively.

The ABTS radical method is one of the most frequently used assays for the determination of the concentration of free radicals. The method is applicable to the study of both water-soluble and lipid-soluble antioxidants, pure compounds, and food extracts. The results of the ABTS assay done with SP explained without doubt the potent ability to neutralize the radical spontaneously. The antioxidant activity of 0.5 μg of SP was equivalent to 14.45 μg of standard TROLOX. The percentage inhibition against the radical formation was 50.93 ± 0.53% and confirms the strong antioxidant power of the SP.

The thiobarbituric acid-reactive substances (TBARS) assay is a widely used method to quantify the concentration of MDA in serum, plasma, or tissue homogenates. At low pH and elevated temperature, MDA readily participates in a nucleophilic addition reaction with 2-thiobarbituric acid (TBA), generating a pink and fluorescent 1:2 MDA: TBA adduct. It is a simple, reliable, and a reproducible fluorometric method for measuring TBARS in samples [19]. In this study, the SP showed the significant scavenging activity against MDA formation in rats providing evidence for the potent antioxidant activity of the SP.

5. Conclusion

The present investigation suggests that poly herbal preparation of Sudarshana powder possesses good antioxidant potential and it can be a useful therapeutic agent for the diseases associated with oxidative stress.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this manuscript.
Acknowledgments

Financial assistance is provided by a grant from Human Resource Development Program-HETC project. CMB/IIM/N4 is gratefully acknowledged. The 9th International Research Conference at General Sir John Kotelawala Defence University, Sri Lanka, 8-9 September 2016, is acknowledged for the partial presentation of the abstract.

References

[1] Ayurveda Pharmacopia, Department of Ayurveda, Sri Lanka, 1976.
[2] P. Nagodavithana, Shri Sharangadara Samhita, Samayawardhana Book Shop (Pvt) Ltd., 1st edition, 2001.
[3] W. A. S. S. Weerakoon, P. K. Perera, D. Gunasekara, and T. S. Suresh, "Antipyretic activity of hot water extract of sudarshan powder, an ayurvedic formulation in wistar rats," in Proceedings of the 1st Anniversary Celebration of ISNHC World Congress on Pharmaceutical Sciences & Chemical Technology, 20 pages, Sri Lanka, 2013.
[4] K. Manivel, P. Rajangam, K. Muthusamy, and R. Somasundaram, "Evaluation of anti-pyretic effect of trichosanthestricuspidata pinn on albino rats," International Journal of Research in Pharmaceutical and Biomedical Sciences, vol. 2, no. 4, pp. 1718–1720.
[5] S. Chomchuen, C. Singharachai, N. Ruangrungsi, and P. Towiwat, "Antipyretic effect of the ethanolic extract of Ficus racemosa root in rats," Journal of Health Research, vol. 24, no. 1, pp. 23–29, 2010.
[6] S. Bhargava, P. Bhargava, S. Saraf, R. Pandey, S. S. Sukla, and A. Garg, "Evaluation of antipyretic activity of sudarshan churna: an ayurvedic formulation," Journal of Research Education in Indian Medicine, pp. 12–14, 2008.
[7] W. A. S. S. Weerakoon, P. K. Perera, D. Gunasekara, and T. S. Suresh, "Evaluation of the anti-inflammatory effect of the hot water extract of sudarshan powder; an ayurvedic formulation, in wistar rats," in Proceedings of the 1st International Conference on Unani, Ayurveda, Siddha and Traditional Medicine, 36 pages, IIM, University of Colombo, Sri Lanka, 2013.
[8] W. A. S. S. Weerakoon, P. K. Perera, D. Gunasekara, and T. S. Suresh, "Study the analgesic effect of Sudarshana Powder in animal model," in Proceedings of the 3rd International Conference on Ayurveda, Unani, Siddha and Traditional Medicine, 250 pages, IIM, University of Colombo, Sri Lanka, 2015.
[9] S. S. Bhadodariya, V. Mishra, S. Raut, A. Ganeshpursark, and S. K. Jain, "Anti-inflammatory and antinociceptive activities of a hydroethanolic extract of Tamarindus indica leaves," Scientia Pharmaceutica, vol. 80, no. 3, pp. 685–700, 2012.
[10] B. Adzu, S. Amos, S. D. Kapu, and K. S. Gamaniel, "Anti-inflammatory and anti-nociceptive effects of Sphaeranthus senegalensis," Journal of Ethnopharmacology, vol. 84, no. 2-3, pp. 169–173, 2003.
[11] W. D. Ratnasooriya, S. A. Deraniyagala, G. Galhena, S. S. P. Liyanage, S. D. N. K. Bathige, and J. R. A. C. Jayakody, "Anti-inflammatory activity of the aqueous leaf extract of Ixora coccinea," Pharmaceutical Biology, vol. 43, no. 2, pp. 147–152, 2005.
[12] D. M. Lembè, J. Domkaml, P. C. O. Oundou et al., "Acute and sub-acute toxicity of fagaraheitziin experimental animals," Molecular & Clinical Pharmacology, vol. 2, no.1, pp. 44–54, 2012.
[13] W. Panunto, K. Jaijoy, N. Lerdvuthisopon et al., “Acute and chronic toxicity studies of the water extract from dried fruits of Terminalia chebula Retz. in rats,” International Journal of Applied Research in Natural Products, vol. 3, no. 4, pp. 36–43, 2010.
[14] W. A. S. S. Weerakoon, K. Samarasinghe, P. K. Perera, D. Gunasekara, and T. S. Suresh, "Long term administration effects and antihistamine effect of sudarshana powder in animal model," in Proceedings of the International Research Sessions (iPURSE), vol. 18, 245 pages, University of Peradeniya, Sri Lanka, 2014.
[15] W. A. S. S. Weerakoon, P. K. Perera, D. Gunasekara, and T. S. Suresh, “Evaluation of the in-vitro and in-vivo antioxidant potentials of sudarshana powder," in Proceedings of the 9th International Research Conference General Sri Gohn Kothalawala Defence University, p. 257, Sri Lanka, 2016.
[16] R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans, "Antioxidant activity applying an improved ABTS radical cation decolorization assay," Free Radical Biology & Medicine, vol. 26, no. 9-10, pp. 1231–1237, 1999.
[17] Y. Cai, Q. Luo, M. Sun, and H. Corke, "Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer," Life Sciences, vol. 74, no. 17, pp. 2157–2184, 2004.
[18] S. O. Onoja, Y. N. Omeh, M. I. Ezeja, and M. N. Chukwu, "Evaluation of the in vitro and in vivo antioxidant potentials of aframomum melegueta methanolic seed extract," Journal of Tropical Medicine, vol. 2014, Article ID 519343, 2014.
[19] W. Wasowicz, N. Jean, and A. Peratz, "Optimized in fluorometric determination of thiobarbituric acid-reactive substances in serum. Importance of extraction pH and influence of sample, preservation and storage," Clinical Chemistry, vol. 39, no. 12, pp. 2522–2566, 1993.