Free Radicals and Antioxidants. 2021;11(2):35-37
A Multifaceted Journal in the field of Free Radicals and Antioxidants
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Original Article

In vitro Antioxidant Activities of a Poly Herbal Preparation: *Sarasvatha choorna*

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ABSTRACT

Objectives: *Sarasvatha choorna* is one of the polyherbal preparations used for the management of dementia and contains 12 medicinal plants and rock salt. It is well known that free radicals attack the brain tissue and cause dementia which lead to impair the memory performance. Therefore, an attempt was taken to investigate the antioxidant activities of the water extract and the ethanol extract of *Sarasvatha choorna*. **Methods:** *Via* ferric reducing antioxidant power (FRAP), oxygen radical absorbance capacity (ORAC), 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis (3 ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS) assays. **Results:** FRAP (295.67 ± 5.48mg Trolox equivalents/g of extract), ORAC (45.09 ± 0.79mg Trolox equivalents/g of extract) and ABTS (99.22 ± 4.96mg Trolox equivalents/g of extract) values of the ethanol extract was significantly higher than those of the water extract (FRAP: 170.01 ± 1.17 mg Trolox equivalents/g of extract; ORAC: 29.73 ± 2.60 mg Trolox equivalents/g of extract and ABTS: 47.17 ± 1.24 mg Trolox equivalents/g of extract). In contrast, DPPH scavenging ability of the water extract (9.89 ± 0.32mg Trolox equivalents/g of extract) was significantly higher than that of the ethanol extract (6.69 ± 0.0mg Trolox equivalents/g of extract). **Conclusion:** *Sarasvatha choorna* has potent antioxidant activities and may play a major role to attack free radicals which is one of causes for dementia.

Key words: Antioxidants, Dementia, *Sarasvatha choorna.*

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History
- Submission Date: 16-10-2021;
- Review completed: 13-11-2021;
- Accepted Date: 02-12-2021.

DOI : 10.5530/fra.2021.2.8

Article Available online
http://www.antiox.org

INTRODUCTION

Dementia is characterized by progressive impairment of memory performance along with impairments in cognitive skills, learning ability and behavioral activities. Approximately 50 million people are believed to be suffering with dementia symptoms and nearly 7.7 million new cases are added annually.[1] Brain ischemia, toxin exposure, oxidative stress and aging are some of the factors involve in the pathogenesis of dementia.[2] The age group in between 40 to 60 years rises rapidly as victims for dementia even though it is usually considered as a disease for elderly people.[3] As conventional treatments choline esterase inhibitors, N-methyl-D-aspartate (NMDA) antagonists (memantine) and calcium channel blockers are given to dementia patients. However, due to the many side effects associate with conventional therapies[4] research have been focused on herbal medicine as a new source to treat dementia. *Sarasvatha choorna*[5] is one of the polyherbal preparations used for the management of dementia and contains 12 medicinal plants [(stems of *Saussurea lappa* Sch.Bip), (roots of *Withania somnifera* Linn), (seeds of *Apium graveolens* Linn), (seeds of *Cuminum cyminum* Linn), (seeds of *Carum carvi* Linn), (rhizomes of *Zigiber officinale* Linn), (fruits of *Piper nigrum* Linn), (fruits of *Piper longum* Linn), (whole plant of *Cissampelos Pereira* Linn), (whole plant of *Evolvulus alsinoides* Linn), (rhizomes of *Acorus calamus* Linn), (leaves of *Bacopa monieri* Linn)] and rock salt.

Oxidative stress is defined as the loss of cell homeostasis provoked by excess amounts of free radicals. Brain tissue is especially susceptible to oxidative stress due to its high aerobic metabolic activity and high lipid content.[6] According to the literature,[7,8] free radicals attack the brain tissue and cause dementia which lead to impair the memory performance. Therefore, an attempt was taken to investigate the antioxidant activity of *Sarasvatha choorna* which is given as a remedy for dementia.

MATERIALS AND METHODS

Preparation of *Sarasvatha choorna*

S. leppa (stems), *W. somnifera* (roots), *A. graveolens* (seeds), *C. cyminum* (seeds), *C. carvi* (seeds), *Z. officinale* (rhizomes), *P. nigrum* (fruits), *P. longum* (fruits), *C. pereira* (whole plant) and *E. alsinoides* (whole plant) were purchased from Colombo district, Sri Lanka and powdered. Above powders and rock salt were taken in equal quantities and mixed with

Cite this article: Karunaratne TDN, Arawwawala LDAM, Sugatharatana K, Ariyawansa HS, Silva HAD. *In vitro* Antioxidant Activities of a Poly Herbal Preparation: *Sarasvatha choorna*. Free Radicals and Antioxidants. 2021;11(2):35-7.

powdered *A. calamus* rhizomes. Finally, the whole mixture was soaked in the juice of *B. monieri* and the pulp was dried completely.

Preparation of hot water extract

Sarasvatha choorna (50 g) was refluxed with water (150 mL) for 4 hr and filtered. The filtrate was evaporated to dryness using a rotary evaporator and kept at 4°C.

Preparation of hot ethanol extract

Sarasvatha choorna (50 g) was refluxed with ethanol (150 mL) for 4 hr and filtered. The filtrate was evaporated to dryness using a rotary evaporator and kept at 4°C.

Evaluation of antioxidant properties of the hot water and the hot ethanol extracts of *Sarasvatha choorna*

Four *in vitro* antioxidant assays were used to investigate the antioxidant potential of the water and the ethanol extracts of *Sarasvatha choorna.*

Ferric reducing antioxidant power (FRAP) assay

In brief, FRAP reagent (150 µl), 30 µL of acetate buffer and 20 µL of *Sarasvatha choorna* [hot water extract (n = 6) or hot ethanol extract (n=6)] were mixed and incubated at room temperature (30 ± 2°C) for 8 min. Absorbance was recorded at 600 nm. Results were expressed as mg Trolox equivalents per gram of extract on dry weight basis.[9]

Oxygen radical absorbance capacity (ORAC) assay

The ORAC radical scavenging assay was performed in 96-well microplates according to the method described by Ou[10] with some modifications. In brief, 100 μL of 4.8 Mm fluorescein and 50 μL *Sarasvatha choorna* [hot water extract; n=6 (15.62 and 7.81 μg/mL) or hot ethanol extract; n=6 (15.62 and 7.81 μg/mL)] were mixed and preincubated at 37 °C for 10 min followed by addition of 50 μL of AAPH (40 mg/mL) to each well to initiate the reaction. The plate was placed on the fluorescent microplate reader set with excitation and emission at 494 nm and 535 nm and decay of fluorescein was recorded in 1-min intervals for 60 min. Results were expressed mg Trolox equivalents per gram of extract on dry weight basis.

1,1-diphenyl-2-picrylhydrazyl (DPPH) assay

In brief, DPPH solution (125 µM, 200 µL) and 50 µL of different concentrations of *Sarasvatha choorna* (hot water extract; n= 6 or hot ethanol extract; n=6) were mixed and incubated at 25 ± 2°C for 15 min. Absorbance was recorded at 517 nm. Results were expressed as mg Trolox equivalents per gram of extract on dry weight basis.[11]

2,2-azino-bis (3 ethylbenzothiazoline-6-sulfonicacid) diammonium salt (ABTS) assay

A stable stock solution of ABTS radical cation was produced by reacting 10 mM of ABTS in potassium persulfate at 37°C for 16 h in dark. $ABTS^+$ radical (40 µM) and 50 µl of *Sarasvatha choorna* (hot water extract; n=6 or hot ethanol extract; n=6) were mixed and incubated at 25 ± 2°C for 10 min. and the absorbance was recorded at 734 nm. Results were expressed as mg Trolox equivalents per gram of extract on dry weight basis.[12]

Statistical analysis

Statistical analysis was performed using statistical software origin pro 8. All data were expressed as Mean ± SEM. All statistical comparison compared through one-way analysis of variance (ANOVA), using Tukey's HSD post hoc test ($p \leq 0.05$).

RESULTS AND DISCUSSION

Sarasvatha choorna is consisting of 12 medicinal plants and rock salt. Bioactive compounds from medicinal and aromatic plants and herbal medicines known to have potent effect on dementia[2,12,13] and also exhibit potent antioxidant activities.[14,15] *Sarasvatha choorna* was standardized previously in terms of physico-chemical parameters and Thin Layer Chromatography fingerprint profile.[16] Standardization of medicines is very important before conducting bio-assays or clinical trials. Single antioxidant assay will not truly reflect the total antioxidant capacity of a plant or medicine. Therefore, four *in vitro* antioxidant assays including FRAP, ORAC, DPPH and ABTS were used in the present study in order to fully reflect both hydrophilic and lipophilic antioxidants. Antioxidant potential of the ethanol extract of *Sarasvatha choorna* was significantly higher than that of the water extract in terms of Trolox equivalents in one gram of extract against FRAP, ORAC and ABTS assays except DPPH assay (Table 1). Antioxidant compounds may vary with the solvent which use to extract the compounds from the plants or medicines. More polar compounds will extract towater than that of ethanol.

FRAP assay is based on the reduction of Fe^{3+} TPTZ complex (colorless complex) to Fe^{2+}-tripyridyltriazine (blue colored complex) by the action of electron donating antioxidants at low pH.[17] Similar to the present study, ethanol extracts of *Xanthium brasilicum* Vell, *Colutea cilicica* Boiss, *Achillea vermicularis* Trin, *Baucus muricatus* L, *Thalictrum minus* L[18] and *Dendropanax morbifera* H. Lev[19] have shown significantly higher FRAP values than that of water extract. In contrast,water extracts of *Nepheliummutabile*Blume[20] and *Mangifera indica*[21] L have shown significantly higher FRAP values compared to that of ethanol extract. The ORAC test measures the splitting ability of the radical chain reaction by antioxidants through monitoring the inhibition of the oxidation of the peroxyl radical. Peroxyl radicals are characterized as free radicals that predominate in lipid oxidation in biological systems and also in foodstuffs, under physiological conditions.[22] Similarly, ORAC activities were significantly higher in ethanol extracts of *Asparagus officinalis* L.[23] and a Sri Lankan herbal medicine[24] than that of water extracts.

DPPH scavenging assay is one of the popular antioxidant assays due to its simplicity, speed and low cost.[25] DPPH is a stable radical which gives a dark purple color at 517 nm and when it react with a antioxidant, its purple color is disappeared and gives pale yellow color at 517 nm.[26] In ABTS assay, ABTS and potassium per-sulphate produce ABTS radical cation which gives blue green color at 734 nm and this characteristic color of ABTS radical will be converted to colorless when presence of antioxidants.[27,28] Trolox equivalents per gram of extract was significantly higher in ABTS assay than that of DPPH assay for the both water and ethanol extracts of *Sarasvatha choorna*. This may be due to the capability of measuring both hydrophilic and lipophilic antioxidants in ABTS

Table 1: Antioxidant properties of *Sarasvatha choorna*.

Type of extract	Antioxidant Activity in mg Trolox equivalents/g of extract			
	FRAP	ORAC	DPPH	ABTS
Water extract	170.01± 1.17	29.73 ± 2.60	9.89 ± 0.32*	47.17± 1.24
Ethanol extract	295.67 ± 5.48*	45.09 ± 0.79*	6.69 ± 0.09	99.22± 4.96*

Data represented as mean ± SEM; n=6;

*Significant at $p \leq 0.05$ when compared the each respective antioxidant value of water extract that of ethanol extract

FRAP: Ferric reducing antioxidant power; ORAC: Oxygen radical absorbance capacity; DPPH: 1,1-diphenyl-2-picrylhydrazyl; ABTS:2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonicacid) diammonium salt

assay where as DPPH assay is capable of measuring only the hydrophilic antioxidants.[11] Similar observations were reported with other research work also.[24,29-31]

An *in vivo* study has been conducted in India to confirm the memory enhancing properties of *Saraswatarishta*[32] which similar to *Sarasvatha choorna*. *Saraswatarishta* consist of 7 major and 16 minor ingredients. According to the experiments five groups of mice (6 mice in each) were used for this study. Control group (group I) received distilled water, Group II received *Saraswatarishta* (2.5mL/kg) single dose and Group III received *Saraswatarishta* 2.5 mL/kg) for 2 weeks. Group IV was given Diazepam (1mg/kg) to produce amnesia. For Group V, *Saraswatarishta* (2.5 mL/kg) was given for 2 weeks followed by Diazepam (1mg/kg). Effect of *Saraswatarishta* on learning and memory of mice was studied using elevated plus maze model (EPM). Reduction in TL (Transfer Latency) indicates improvement in learning or memory and prolongation indicates impairment. Therefore, similar *in vivo* study can be conducted for *Sarasvatha choorna*.

CONCLUSION

Sarasvatha choorna exhibits potent antioxidant activities which may responsible to attack free radicals.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Cite this article: Karunaratne TDN, Arawwawala LDAM, Sugatharatana K, Ariyawansa HS, Silva HAD. *In vitro* Antioxidant Activities of a Poly Herbal Preparation: *Sarasvatha choorna*. Free Radicals and Antioxidants. 2021;11(2):35-7.